Edited by
Karl Esser

THE MYCOTA

A Comprehensive Treatise on Fungi
as Experimental Systems for Basic and Applied Research

Physiology and Genetics

Selected Basic and Applied Aspects

XV

Second Edition

Timm Anke
Anja Schüffler
Volume Editors

Springer

The Mycota

Edited by
K. Esser

The Mycota

I *Growth, Differentiation and Sexuality*
1st edition ed. by J.G.H. Wessels and F. Meinhardt
2nd edition ed. by U. Kües and R. Fischer
3rd edition ed. by J. Wendland

II *Genetics and Biotechnology*
1st and 2nd edition ed. by U. Kück

III *Biochemistry and Molecular Biology*
1st and 2nd edition ed. by R. Brambl and G. Marzluf
3rd edition ed. by D. Hoffmeister

IV *Environmental and Microbial Relationships*
1st edition ed. by D. Wicklow and B. Söderström
2nd edition ed. by C.P. Kubicek and I.S. Druzhinina
3rd edition ed. by I.S. Druzhinina and C.P. Kubicek

V *Plant Relationships*
1st edition ed. by G. Carroll and P. Tudzynski
2nd edition ed. by H.B. Deising

VI *Human and Animal Relationships*
1st edition ed. by D.H. Howard and J.D. Miller
2nd edition ed. by A.A. Brakhage and P.F. Zipfel

VII *Systematics and Evolution*
1st edition ed. by D.J. McLaughlin, E.G. McLaughlin, and P.A. Lemke†
2nd edition ed. by D.J. McLaughlin and J.W. Spatafora

VIII *Biology of the Fungal Cell*
1st and 2nd edition ed. by R.J. Howard and N.A.R. Gow

IX *Fungal Associations*
1st and 2nd edition ed. by B. Hock

X *Industrial Applications*
1st edition ed. by H.D. Osiewacz
2nd edition ed. by M. Hofrichter

XI *Agricultural Applications*
1st and 2nd edition ed. by F. Kempken

XII *Human Fungal Pathogens*
1st edition ed. by J.E. Domer and G.S. Kobayashi
2nd edition ed. by O. Kurzai

XIII *Fungal Genomics*
1st edition ed. by A.J.P. Brown
2nd edition ed. by M. Nowrousian

XIV *Evolution of Fungi and Fungal-Like Organisms*
Ed. by S. Pöggeler and J. Wöstemeyer

XV *Physiology and Genetics: Selected Basic and Applied Aspects*
1st edition ed. by T. Anke and D. Weber
2nd edition ed. by T. Anke and A. Schüffler

The Mycota

A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research

Edited by K. Esser

XV

Physiology and Genetics
Selected Basic and Applied Aspects
2nd Edition

Volume Editors:
Timm Anke and Anja Schüffler

Springer

Series Editor

Professor Dr. Dr. h.c. mult. Karl Esser
Allgemeine Botanik
Ruhr-Universität
44780 Bochum, Germany

Tel.: +49 (234)32-22211
Fax.: +49 (234)32-14211
e-mail: Karl.Esser@rub.de

Volume Editors

Professor Dr. Timm Anke
Institut für Biotechnologie und Wirkstoff-Forschung gGmbH (IBWF)
Erwin-Schrödinger-Str. 56
67663 Kaiserslautern, Germany

e-mail: anke@rhrk.uni-kl.de

Dr. Anja Schüffler
Institut für Biotechnologie und Wirkstoff-Forschung gGmbH (IBWF)
Erwin-Schrödinger-Str. 56
67663 Kaiserslautern, Germany

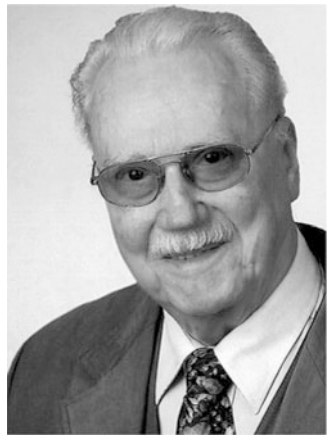
Tel.: +49 (631)31672-19
Fax: +49 (631)31672-15
e-mail: schueffler@ibwf.de

ISBN 978-3-319-71739-5 ISBN 978-3-319-71740-1 (eBook)
https://doi.org/10.1007/978-3-319-71740-1

Library of Congress Control Number: 2017964284

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland



Karl Esser
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

Timm Anke
(born 1944) studied biochemistry at the University of Tuebingen where he got his PhD with a dissertation on the biosynthesis of fungal siderophores. In 1973, he joined the group of Fritz Lipmann at the Rockefeller University in New York City where he was investigating the biosynthesis of valinomycin, a streptomycete ionophore. After his return to Tuebingen in 1975, he started to build up a group searching for new antibiotics from basidiomycetes within the framework of Hans Zaehners Collaborative Research Center (SFB 76) focusing on the chemistry and biology of microorganisms. In 1981, he became full professor of biotechnology at the University of Kaiserslautern from which he retired in 2010. In addition, he headed the Institute of Biotechnology and Drug Research IBWF e. V. in Kaiserslautern from 1998 to 2010. One of his outstanding achievements in the field of antibiotic research is the discovery of the strobilurins, a major class of agricultural fungicides for which he was awarded the Karl Heinz Beckurts Prize in 1996.

Anja Schüffler
(born 1981) studied biology at the University of Kaiserslautern. Her PhD focused on the characterization of fungal natural products with antimicrobial activity. In 2010, she received a DFG fellowship and joined Prof. Fenical's group at the Scripps Institute for Oceanography in San Diego, where she studied antibacterial natural products from streptomycetes. In 2011, she started a research fellowship at the Institute of Biotechnology and Drug Research (IBWF, Kaiserslautern). Her scientific work focuses on bioactive natural products and assay development, the biosynthesis of secondary metabolites as well as the isolation and taxonomy of fungi of diverse habitats.

Series Preface

Mycology, the study of fungi, originated as a sub discipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for self-incompatibility, termed "heterothallism," and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus, began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with Neurospora crassa, led to the one gene:one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to *Saccharomyces cerevisiae*, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title The Mycota. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this series of volumes, the names adopted for major groups of fungi (representative genera in parentheses) are as follows:

Pseudomycota

Division:	Oomycota (*Achlya, Phytophthora, Pythium*)
Division:	Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (*Allomyces*)
Division:	Zygomycota (*Mucor, Phycomyces, Blakeslea*)
Division:	Dikaryomycota
Subdivision:	Ascomycotina
Class:	Saccharomycetes (*Saccharomyces, Schizosaccharomyces*)
Class:	Ascomycetes (*Neurospora, Podospora, Aspergillus*)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (*Ustilago, Tremella*)
Class:	Homobasidiomycetes (*Schizophyllum, Coprinus*)

We have made the decision to exclude from The Mycota the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The series throughout will address three basic questions: what are the fungi, what do they do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather than ingest, nutrients and they have rigid cell walls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is a multitude of spore forms, fungal spores are basically only of two types: (1) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (2) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy. The metabolic capacities of fungi have attracted much interest in natural products

chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid, and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insect associated fungi, and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important. There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission, or inconsistency in this series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine volumes.

Bochum, Germany — Karl Esser
Auburn, AL, USA — Paul A. Lemke
April 1994 — *Series Editors*

Volume Preface

More than 120,000 different fungal species have been described, and it is estimated that there exist more than 1.5×10^6 species. Fungi have adopted many different ways of living in very diverse habitats as saprophytes, pathogens, symbionts or endophytes. Fungi and their products are used for the fermentation and processing of food and feeds, for biological control and for the production of vitamins and amino acids. Some of their secondary metabolites are used in medicine, e.g. as antibiotics, immunosuppressants, cholesterol-lowering drugs or agrochemical fungicides. Recently, progress in the field of mycology has been substantial due to new methodological approaches and technologies, many of them DNA-based, strongly adding to the motivation to compile a new volume of *Mycota XV Physiology and Genetics: Selected Basic and Applied Aspects*.

Chapter "Fruiting Body Development in Ascomycetes" provides a general overview about the morphology and development of fruiting bodies in ascomycetes with discussion of regulatory networks such as signal transduction pathways, protein degradation mechanisms as well as transcriptional regulators and chromatin modifiers. Chapter "Fungal Inteins: Distribution, Evolution and Applications" summarizes the current knowledge of inteins, their occurrence, evolution and application. Inteins are internal protein sequences which are excised from a precursor protein (extein) whose N- and C-termini are subsequently ligated to yield two stable proteins, the mature protein and the intein. In the interaction of yeasts, killer toxins play an important role. Their structures, modes of action and resistance as well as possible applications are discussed in chapter "Yeast Killer Toxins: Fundamentals and Applications". Chapter "The Fungal MCC/Eisosome Complex: An Unfolding Story" deals with the fungal MCC/eisosome complex which plays an important role in plasma membrane organization and diverse plasma membrane-associated processes. In chapter "The Genus Periglandula and Its Symbiotum with Morning Glory Plants (Convolvulaceae)", the enigma of why ergot alkaloids are equally present in fungi (*Clavicipitaceae*) and higher plants (Convolvulaceae) is addressed and solved. Chapter "Volatiles in Communication of Agaricomycetes" presents a comprehensive survey on communication activities of *Agaricomycetes* on all organismal scales and community levels in which signalling by fungal volatile organic compounds (VOCs) is recognized. The substantial progress in elucidating the lifestyle, metabolism and genetics of endophytic fungi is addressed in the chapter "Endophytic Fungi, Occurrence, and Metabolites". Basidiomycetes are a rich source of unique secondary metabolites in most cases not found in other fungi. The chapter "Secondary Metabolites of Basidiomycetes" offers a survey of new compounds isolated since 2008. Chapter "Identification of Fungicide Targets in Pathogenic Fungi"

covers the current molecular targets of antifungal compounds and discusses future directions of fungicide research. Helminths can pose serious problems to animal and human health. It is therefore quite remarkable that fungi produce low molecular weight compounds specifically interfering with reactions not present in mammalian hosts, thus paving the way for selective nontoxic medications or agrochemicals. For the discovery of avermectin, successfully used in the therapy of roundworm parasites, Prof. Satoshi Omura, author of chapter "Helminth Electron Transport Inhibitors Produced by Fungi", was awarded the Nobel Prize in Physiology or Medicine in 2015. Chapter "Cyclic Peptides and Depsipeptides from Fungi" describes the occurrence, structures and biological activities of peptides and depsipeptides produced by fungi and discusses the importance of these compounds as lead compounds for agricultural and pharmaceutical applications.

In chapter "Polyketide Synthase–Nonribosomal Peptide Synthetase Hybrid Enzymes of Fungi", recent efforts in engineering-selected fungal species to make them amenable to efficient genetic modifications for identifying genes responsible for the biosynthesis of secondary metabolites are addressed. This review also discusses how the engineered fungi are used in deciphering the mechanism of natural product biosynthesis, primarily through heterologous reconstitution of biosynthetic pathways of interest. Fungal polyketides are among the prominent fungal metabolites. As addressed in chapter "Biosynthesis of Fungal Polyketides", their biosynthesis is increasingly well understood at chemical, biochemical and genetic levels, thus offering a chance to obtain sufficient quantities of complex but potentially valuable therapeutics. The mycotoxins ochratoxin, citrinin and patulin are often found as contaminants of foods. New results concerning the regulation and the simultaneous occurrence of ochratoxin, citrinin and patulin producing penicillia in certain habitats are presented in chapter "Aspects of the Occurrence, Genetics and Regulation of Biosynthesis of the Three Food Relevant Penicillium Mycotoxins: Ochratoxin A, Citrinin and Patulin".

We do hope that readers enjoy reading this volume of *The Mycota*. We are very grateful to the contributing authors, whose expertise and efforts have made this project possible. We thank Dr. Andrea Schlitzberger of Springer Verlag for her support and engagement during the preparation of this volume.

Kaiserslautern and Mannheim, Germany — Timm Anke
October 2017 — Anja Schüffler

Contents

List of Contributors

Heidrun Anke
Institute for Biotechnology and Drug Research, IBWF gGmbH, Kaiserslautern, Germany

Anna Beier
Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany

Russell J. Cox
Institute for Organic Chemistry and Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover, Hannover, Germany

Bastian Dörnte
Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Göttingen, Germany

Skander Elleuche
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Andrew J. Foster
University of Exeter, Biosciences, Exeter, UK

Rolf Geisen
Max Rubner-Institut, Karlsruhe, Germany

Yuichiro Hirayama
Department of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Frank Kempken
Botanisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Weeradej Khonsuntia
Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Göttingen, Germany

Shinji Kishimoto
Department of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Roland Klassen
Fachgebiet Mikrobiologie, Institut für Biologie, Universität Kassel, Kassel, Germany

Krisztina Kolláth-Leiß
Botanisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Ulrich Kück
Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany

Ursula Kües
Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Göttingen, Germany

Hartmut Laatsch
University of Goettingen, Institute of Organic and Biomolecular Chemistry, Goettingen, Germany

Eckhard Leistner
Institut für Pharmazeutische Biologie, Rheinische Friedrich Wilhelm-Universität Bonn, Bonn, Germany

Rokuro Masuma
The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Friedhelm Meinhardt
Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität Münster, Münster, Germany

Mihoko Mori
The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Kenichi Nonaka
The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Minou Nowrousian
Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany

Satoshi Ōmura
The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Stefanie Pöggeler
Abteilung Genetik eukaryotischer Mikroorganismen, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Göttingen, Germany

Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University Göttingen, Göttingen, Germany

Anna Maria Pirttilä
Genetics and Physiology, University of Oulu, Oulu, Finland

Anja Schüffler
Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany

Raffael Schaffrath
Fachgebiet Mikrobiologie, Institut für Biologie, Universität Kassel, Kassel, Germany

Markus Schmidt-Heydt
Max Rubner-Institut, Karlsruhe, Germany

Kazuro Shiomi
The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Elizabeth Skellam
Institute for Organic Chemistry and Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover, Hannover, Germany

Ulrike Steiner
Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES), Rheinische Friedrich Wilhelm-Universität Bonn, Bonn, Germany

Dominic Stoll
Max Rubner-Institut, Karlsruhe, Germany

Shanta Subba
Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Göttingen, Germany

Ines Teichert
Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany

Mysore V. Tejesvi
Genetics and Physiology, University of Oulu, Oulu, Finland

Najim Touhami
Max Rubner-Institut, Karlsruhe, Germany

Kenji Watanabe
Department of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Katherine Williams
Institute for Organic Chemistry and Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover, Hannover, Germany

Fruiting-Body Development in Ascomycetes

S. Pöggeler[1], M. Nowrousian[2], I. Teichert[2], A. Beier[2], U. Kück[2]

CONTENTS

[1] Institut für Mikrobiologie und Genetik, Genetik eukaryotischer Mikroorganismen, Universität Göttingen, Göttingen, Germany; e-mail: spoegge@gwdg.de
[2] Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, Bochum, Bochum, Germany; e-mail: ulrich.kueck@rub.de

I. Introduction

Fruiting bodies are multicellular structures, which protect the products of meiosis, the sexual spores. They occur during the sexual life cycle of the *Dikarya*, a group that encompasses the ascomycetes and basidiomycetes (Hibbett et al. 2007; Peraza-Reyes and Malagnac 2016) (Fig. 1). However, only filamentous species show the development of fruiting bodies, while yeasts never exhibit comparable structures.

In this chapter, which is an extension and update of a previous review in this series (Pöggeler et al. 2006b), we will give an overview of the development of fruiting bodies in ascomycetes, including an outline of some model ascomycetes, which have been used to study fruiting-body development at the molecular level. Further, we will summarize factors that can either be environmental or endogenous, which control this process. Finally, regulatory networks will be mentioned that govern fruiting-body development. This includes signal transduction pathways, protein degradation mechanisms, and transcriptional regulatory networks. Ultimately, we observe that novel experimental approaches such as quantitative mass spectrometry, functional genomics, or super resolution microscopy have begun to improve our knowledge about the mechanistic

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

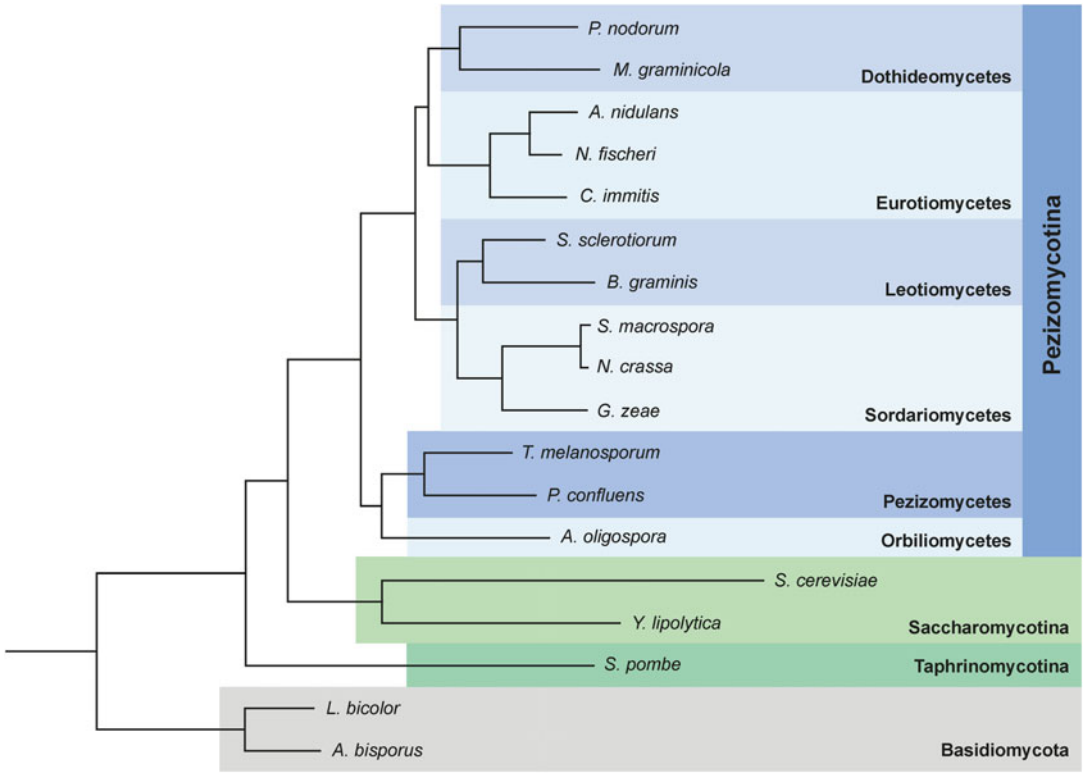


Fig. 1 Phylogenetic tree of *Ascomycota*. Characteristic species are given as examples. Branch lengths are proportional to genetic distances [adapted from Traeger et al. (2013)]. Species used to construct the phylogenetic tree: *Agaricus bisporus*, *Arthrobotrys oligospora*, *Blumeria graminis*, *Coccidioides immitis*, *Aspergillus nidulans*, *Gibberella zeae*, *Laccaria bicolor*, *Mycosphaerella graminicola*, *Neosartorya fischeri*, *Neurospora crassa*, *Phaeosphaeria nodorum*, *Pyronema confluens*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Sclerotinia sclerotiorum*, *Sordaria macrospora*, *Tuber melanosporum*, *Yarrowia lipolytica*. *S. pombe*, *L. bicolor*, and *A. bisporus* served as outgroups

processes that lead to the formation of multicellular structures.

A. Fungal Sexual Development

Fungi propagate either asexually or sexually. Asexual propagation is characterized by mitotic divisions, and as a result, endospores within sporangia or exospores like conidia are generated. In contrast, sexual propagation is characterized by karyogamy and meiotic divisions, and fungi share this feature with most other eukaryotes. Generally, sexual reproduction is thought to be the source of genetic diversity. During meiotic divisions, recombination occurs between chromosomes of two heterogenetic mating partners (Peraza-Reyes and Malagnac 2016). As a result of meiotic divisions, fungi produce four haploid spores, which may be doubled or multiplied by one or several postmeiotic mitoses.

The ascus is the meiosporangium of the Ascomycota. These sac-like sporangia carry the ascospores, the products of meiosis. In mycelial ascomycetes, asci are usually formed inside developmentally complex fruiting bodies that are called the ascomata or ascocarps. In contrast to filamentous ascomycetes, ascospores of unicellular ascomycetes (yeasts) are never found in fruiting bodies. The development of fruiting bodies is a rather complex cellular process that requires special environmental and genetic conditions, which control

the expression of developmentally regulated genes. Fruiting bodies are highly complex structures, which contain several different tissues protecting the asci. For example, 15 different cell types were recognized in fruiting bodies of the Sordariomycete *Neurospora crassa* (Bistis et al. 2003). For a coordinated fruiting-body development, enzymes involved in cell wall biogenesis and metabolism are required, as well as genes responsible for the cytoskeleton structure and organization. Here we will mention some representative examples, and a more detailed description on this subject can be found in our previous review (Pöggeler et al. 2006a). The *ami1* gene from *Podospora anserina*, for example, is necessary for nuclear positioning, most likely by regulating components of the dynein pathway. This gene was shown to be responsible for male fertility, and deletion results in a delayed formation of fruiting bodies in the corresponding mutants (Bouhouche et al. 2004). The outer shell of the fruiting body, the peridium, is an essential structure to protect the meiosporangia with the ascospores. The peridium consists of bundles of filamentous cells, and their cell walls have three main constituents, namely, chitin, mannan, and β-glucan. Though the related biosynthetic pathways have intensively been investigated, it has not been demonstrated with certainty that the corresponding genes are preferentially expressed in fruiting-body tissues. For *Sordaria macrospora* functional analysis of the class VII (division III) chitin synthase gene (*chs7*) has shown that it is dispensable for fruiting-body formation, but the corresponding mutant displayed sensitivity toward cell wall stress (Traeger and Nowrousian 2015). Another result comes from *Tuber borchii*, where three genes for chitin synthesis were investigated. Albeit they are constitutively expressed in vegetative mycelium, they show a differential expression in sporogenic or vegetative tissue of the fruiting bodies (Balestrini et al. 2000). In contrast, several chs mutants from *N. crassa* and *Aspergillus nidulans* show severe defects in perithecial development (Fajardo-Somera et al. 2015).

Important pigments of the cell walls are melanins. They are synthesized either through the DHN (1,8-dihydroxynaphthalene) or the DOPA (L-3,4-dihydroxyphenylalanine) pathways. Some can also be generated by the L-tyrosine degradation pathway (Langfelder et al. 2003). One of the best-characterized melanin biosynthetic pathways is the DHN melanin pathway, which has been verified for many members of the Pezizomycotina. Melanins stabilize the cell wall and provide protection against UV light-induced DNA damage. An investigation with *S. macrospora* showed that expression of melanin biosynthesis genes is correlated with fruiting-body development. For example, melanin gene expression is highly repressed in submerged cultures, where no sexual development occurs. Similarly, sterile mutants of *S. macrospora* showed only reduced transcript levels of melanin biosynthesis genes (Engh et al. 2007). Finally, mutants with a defect in melanin biosynthesis from *Ophiostoma piliferum* and *Podospora anserina* showed defects in the formation of fruiting bodies. These observations are consistent with early reports for *N. crassa*, *P. anserina*, and *Tuber* species, where correlation between melanin biosynthesis and the reproductive cycle was suggested (Hirsch 1954; Esser 1966; Prade et al. 1984; Ragnelli et al. 1992; Teichert and Nowrousian 2011).

Very important proteins of the cell wall are the hydrophobins and lectins. Although they have mainly been characterized in higher basidiomycetes, where they are implied in mushroom formation, they have also a function in the Pezizomycotina. Cryparin, a class II hydrophobin, was found mainly in the cell walls of fruiting bodies from the chestnut blight pathogen, *Cryphonectria parasitica*. Deletion mutants lacking the cryparin gene were unable to generate wild-type-like fruiting bodies. Thus, this pathogen needs hydrophobins for its fitness under natural conditions (Kazmierczak et al. 2005).

On the genetic level, there are many genes regulating the sexual cycle of ascomycetes. Important master genes involved in the general control of sexual development are part of the mating-type loci. They have been found so far in all ascomycetes, irrespective of whether they produce fruiting bodies or not. Their regulatory role during the sexual cycle has been thoroughly

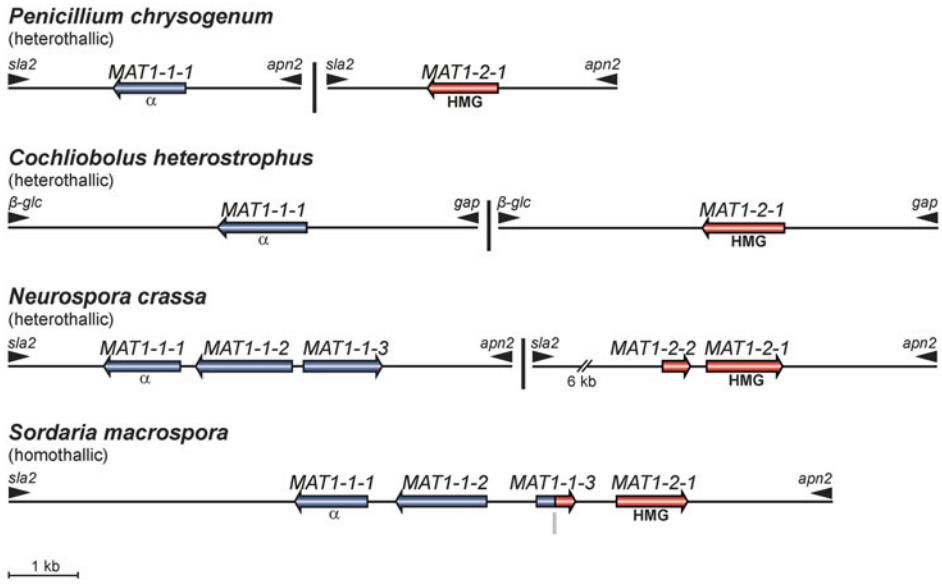


Fig. 2 Examples of mating-type loci of heterothallic members of the Dothideomycetes (*Cochliobolus heterostrophus*) (Wirsel et al. 1998), Eurotiomycetes (*Penicillium chrysogenum*) (Böhm et al. 2013, 2015), and Sordariomycetes (*N. crassa*). For comparison the mating-type locus encoding four open reading frames from the homothallic fungus *S. macrospora* (Sordariomycetes) is shown. The flanking regions often carry conserved genes, such as *sla2* (cytoskeleton assembly control factor) and *apn2* (DNA lyase). An exception is the MAT locus from *C. heterostrophus* with the following flanking genes: GAP, GTPase-activating protein; ß-Glc, ß-glucosidase. Abbreviations: "α" and "HMG" indicate genes encoding transcription factors with conserved DNA-binding domains

studied in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which produce asci, but no fruiting bodies. The role of mating-type genes during fruiting-body development is by far less well understood, although some studies have shown that mating-type genes are directly involved in fruiting-body development (Nelson and Metzenberg 1992; Pöggeler et al. 1997) (see also Sect. IV.C.1). In general, two types of fungal breeding systems are distinguished. Heterothallism involves two individuals with opposing mating types, while homothallism refers to sexual reproduction by selfing. In the latter case, individual strains do not need a mating partner to propagate sexually. Pseudohomothallism finally can be considered to be an exceptional type of heterothallism. The term was used for species that contain asci with four ascospores, each carrying two nuclei with opposite mating-type genes. Thus, after germination, these resulting heterokaryotic mycelia can undergo selfing. This type of breeding system is found, e.g., in *P. anserina* or *Neurospora tetrasperma*.

Usually the mating-type loci of heterothallic species contain dissimilar sequences, albeit they are located at identical chromosomal positions. Thus, mating-type loci do not represent alleles of a given gene but rather dissimilar DNA sequences which are called idiomorphs. MAT loci from the Pezizomycotina carry one or more open reading frames of which at least one codes for a mating-type transcription factor (TF). In general, the MAT1-1 locus of heterothallic species contains one to three open reading frames, while only a single gene is found in MAT1-2 loci. In contrast to baker's yeast, species of the Pezizomycotina carry no silent mating-type loci. Thus, mating-type switching as observed in yeast does usually not occur in heterothallic filamentous ascomycetes.

Mating-type loci encode TFs that are directly involved in the sexual life cycle. Figure 2 displays the general structure of mating-type

loci from members of the Eurotiomycetes, the *Dothideomycetes*, and the *Sordariomycetes*. The *MAT1-1-1* gene encodes a TF that is characterized by an α DNA-binding domain, while the *MAT1-2-1* gene codes for TFs with a high-mobility group (HMG) DNA-binding domain. A detailed description of mating-type locus-encoded TFs is given in Sect. IV.C.1.

B. Fruiting-Body Morphology

During their sexual life cycle, filamentous fungi of subdivision Pezizomycotina generate fruiting bodies that were historically used for their taxonomic classification. Current classification systems that rely on molecular data show that these conventional classifications contain non-monophyletic groups (Schoch et al. 2009; Ebersberger et al. 2012). However, different fruiting-body morphologies are important traits in fungal ecology, and the foremost common types of fruiting bodies (Esser 1982) are described below (Figs. 3 and 4).

1. **Cleistothecia** are closed, spherical fruiting bodies that distribute the ascospores after disintegration of the peridium of the fruiting bodies. Typically, members of the Eurotiomycetes such as *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Penicillium chrysogenum* generate cleistothecia.
2. **Pseudothecia** are spherical fruiting bodies that contain cavities (loculi) that contain the gametangia. Spores are actively discharged through openings which arise from local lysis of the peridium. Pseudothecia are, for example, found in the Dothideomycetes, e.g., *Venturia inaequalis*.
3. **Perithecia** are closed flask-like fruiting bodies that look similar to the pseudothecia. Within perithecia, sterile hyphae are found that enclose the generative tissue (hymenium). The hymenium generates asci with usually eight ascospores, which are actively discharged from the perithecium through a preformed opening, the ostiole. Perithecia are typical fruiting bodies of members of the Sordariomycetes, such as *N. crassa*, *P. anserina*, and *S. macrospora*.
4. **Apothecia** are open to cup-shaped fruiting bodies that have a hymenium layer on their surface carrying the asci. The spores are actively discharged, and examples of species that have apothecia are *Ascobolus immersus*, *Pyronema confluens*, and *Morchella* sp. within the Pezizomycetes and *Botrytis cinerea* within the Leotiomycetes.

II. Systems to Study Fruiting-Body Development

Fruiting-body development has been studied in a wide range of different ascomycetous species. Here we describe four model systems, which were used intensively for investigations on fruiting-body development. Further, we will mention some emerging model ascomycetes that were used recently for studying specific aspects of the sexual life cycle, including fruiting-body formation.

A. *Neurospora crassa*

The model fungus *Neurospora crassa* is a heterothallic species of the *Sordariaceae* and has a rather complex sexual life cycle. In general, two mating types can be distinguished, which are called “A” (MAT1-1) and “a” (MAT1-2). Both strains generate macro- and microconidia, which can be considered as male gametangia. In addition, both strains form female gametangia that are called ascogonia. Female gametangia are surrounded by supporting hyphae, which after 2–3 days generate a protoperithecium (young fruiting body). During fertilization, the female gametangium generates a distinct uptake hypha called trichogyne. This trichogyne will fuse with male gametes, which can be macro- or microconidia as described above. Alternatively, a fusion with vegetative hyphae has also been described. However, self-fertilization of each strain is prevented by an incompatibility mechanism. Thus, trichogynes can only fuse with male gametes from an opposite mating-type partner. In summary, the protoperithecium with a trichogyne from

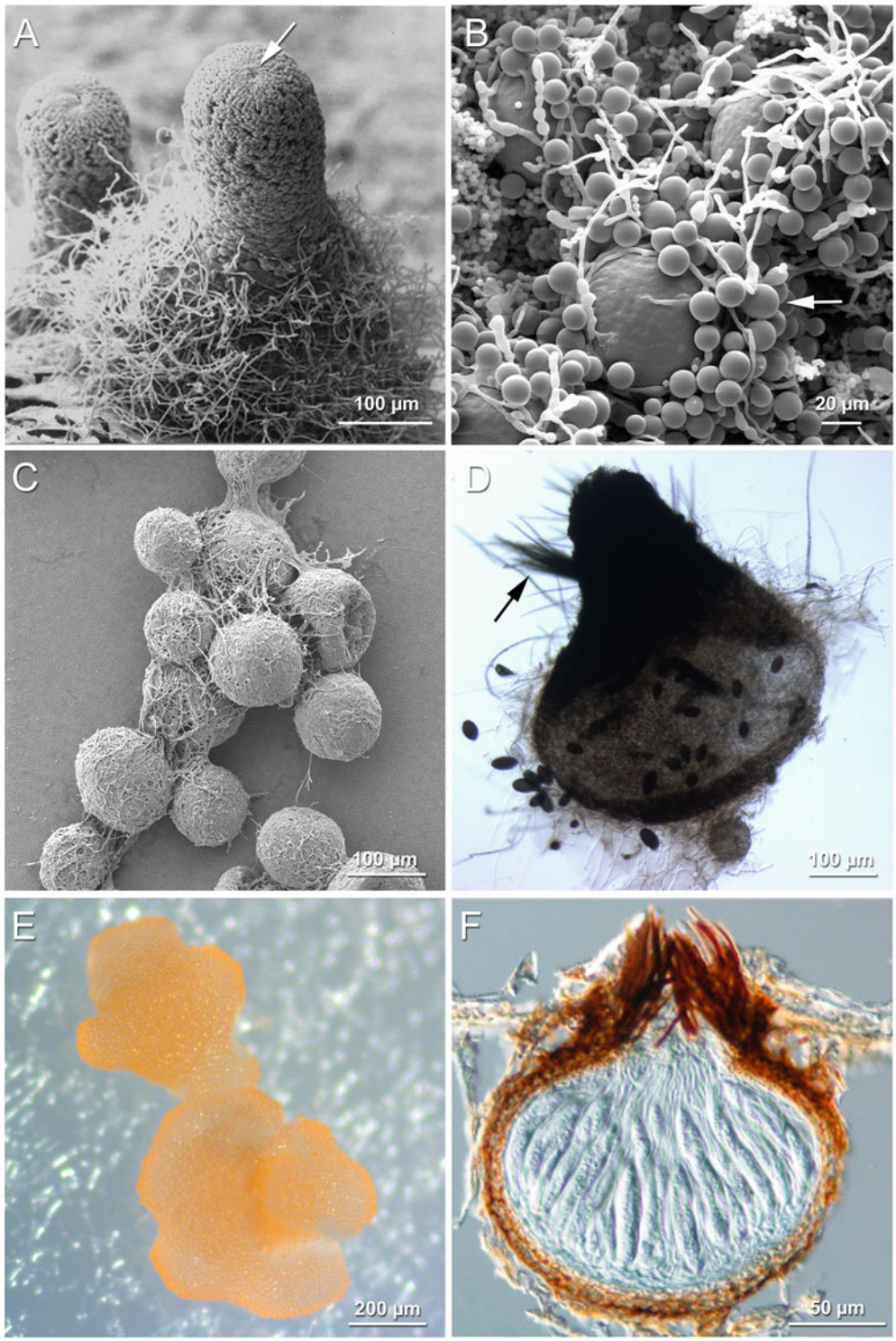


Fig. 3 Typical fruiting bodies of ascomycetes. (**a**) Perithecia of the homothallic Sordariomycete *S. macrospora*; the arrow points to the ostiolum that is used to discharge the eight-spored asci. (**b**) Cleistothecia from

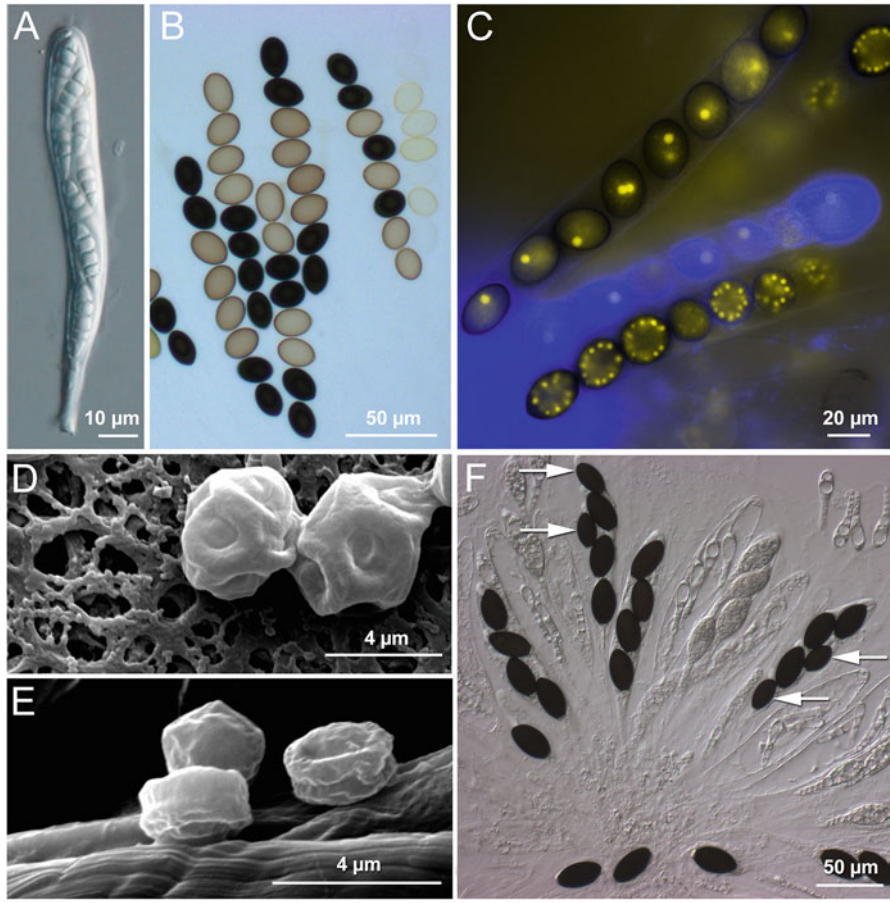


Fig. 4 Asci from different fungi of the Pezizomycotina. (**a**) Ascus from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**b**) Asci from *Sordaria macrospora*, obtained from a cross between a wild type (black spores) and a spore color mutant (lu with yellow spores). (**c**) Fluorescence microscopy of *S. macrospora* asci and ascospores. YFP-tagged histones label nuclei. In the upper ascus, clearly one to two nuclei are visible in each ascospore, while the lower one shows ascospores with several nuclei, which appear after several mitotic divisions. (**d**) Asci from the fungus *E. crustaceum*. (**e**) Ascospores from *E. crustaceum*. (**f**) Asci from *Podospora anserina* contain four spores or five spores. Usually, each ascospore carries two nuclei; however, in rare cases, asci contain smaller spores with only a single nucleus. Arrows indicate small spores with only a single nucleus compared to the regular ascospores with two nuclei

an "A" strain can only be fertilized by a nucleus of an "a" strain and vice versa. The fusion of the male gamete with the trichogyne will lead to the induction of the dikaryotic phase. During this phase, two genetically different nuclei exist within one cell. After several conjugated divisions, ascus development will start with the formation of ascogenous hyphae, which generate the so-called crozier cell, which undergoes conjugated divisions resulting in three cells,

Fig. 3 (continued) A. nidulans with small surrounding Hülle cells. (**c**) Cleistothecia from the homothallic fungus *Eupenicillium crustaceum*. (**d**) Perithecia from *Podospora anserina* show typical hairs (arrow) at the neck of the perithecia. (**e**) Apothecia from *P. confluens*. (**f**) Pseudothecium from the Dothideomycete *Keissleriella quadriseptata* [from Tanaka et al. (2015)]. (**a**), (**b**), and (**c**) are scanning electron micrographs and (**d**)–(**f**) light microscopy; (**b**) courtesy of G. Braus (Göttingen, Germany); (**e**) from Traeger et al. (2013); (**f**) copyright from Elsevier Press

two basal and one upper cell. The two nuclei in the upper dikaryotic cell undergo karyogamy followed by meiosis (Peraza-Reyes and Malagnac 2016). In *N. crassa*, a postmeiotic mitosis follows before spore formation starts. Thus, each ascus contains eight linearly ordered ascospores. After maturation, perithecia have a size of about 300 μm, while ascospores have a size between 15 and 30 μm. Importantly, ascospore germination occurs only after a heat shock. Fruiting-body formation in *N. crassa* was investigated in diverse genetic, biochemical, and molecular studies (Davis 1995).

B. *Podospora anserina*

P. anserina is a coprophilic fungus with a pseudohomothallic mating system, which shows similarities to the life cycle of *N. crassa*. The mating-type strains are designated "+" (MAT1-2) and "−" (MAT1-1). However, there are some distinct differences compared to *N. crassa*. As male gametes, microconidia, but no macroconidia, are generated that germinate under specific physiological conditions. Secondly, the asci usually contain only four spores, which are generated as a result of specific nuclear distribution mechanisms. After meiosis and postmeiotic mitosis, spore-wall formation covers two genetically distinct nuclei. Usually one nucleus carries the "+" and the other the "−" mating type. With a frequency of about 3%, five- or six-spored asci are generated. They carry either two or four smaller spores that carry only a single nucleus. These spores can be used to generate haploid mycelial isolates (Scheckhuber and Osiewacz 2008; Peraza-Reyes and Malagnac 2016).

C. *Sordaria macrospora*

S. macrospora is a coprophilic fungus that is taxonomically closely related to the above-described species *N. crassa* and *P. anserina*. The life cycles of all these ascomycetes are very similar, although *S. macrospora* has a homothallic mating system. In contrast to *N. crassa* however, *S. macrospora* does not generate macro- or microconidia, and thus, only the sexual cycle contributes to the propagation of this fungus. The sexual cycle can be completed in the laboratory within 1 week, since ascospores require no heat shock or resting period for germination (Esser and Straub 1958). The sexual cycle starts with the formation of ascogonia. However, so far the molecular mechanisms leading to the formation of the dikaryotic hyphae are not understood. After karyogamy of two nuclei in the abovementioned crozier cells, meiosis will follow to generate the meiotic products as a source for ascospore formation. Similar to ascus formation in *N. crassa*, meiosis is followed by a postmeiotic mitosis. As a result, eight ascospores within a single ascus are derived from a single dikaryotic mother cell.

As mentioned above, sexual reproduction is a source of genetic diversity. Usually strains of opposite mating types from heterothallic species (e.g., *N. crassa* or *P. anserina*) are used for conventional genetic recombination studies. However, it has been shown for many species that recombination can also occur between two strains of a homothallic species. In these cases, the strains are distinguished by at least a single mutation. Homothallic species such as *S. macrospora* and other *Sordaria* species are used for conventional genetic analysis (Teichert et al. 2014a).

D. *Aspergillus nidulans*

A. nidulans, which is like *S. macrospora* a homothallic species, was used extensively to study genetic recombination and fruiting-body formation. The sexual cycle starts with the formation of ascogonia and later dikaryotic hyphae, a process, which is probably very similar to the life cycle of *S. macrospora*. Within cleistothecia, spherical asci are generated containing eight ascospores. These octades are unordered and thus distinguished from the ordered asci of the abovementioned species. In recent years, several factors controlling cleistothecia formation were studied extensively, such as the velvet complex (Bayram and Braus 2012) (see Sect. IV.C).

E. Emerging Model Systems

Here we mention fungal genera or species, which were used recently to investigate fruiting-body development.

1. *Aspergillus fumigatus*, *A. flavus*, and *A. parasiticus*

The genus *Aspergillus* comprises about 340 species and was traditionally believed to contain species that generally propagate only asexually. Species of this genus having a sexual life cycle were grouped in the teleomorphic genera *Eurotium*, *Emericella*, *Neosartorya*, or *Petromyces*. Recently evidence emerged that heterothallic *Aspergillus* species, which were for long believed to propagate exclusively asexually, have also the potential to undergo sexual reproduction. These are, for example, the human pathogen *A. fumigatus* and the mycotoxin-producing fungi *A. flavus* and *A. parasiticus* (Dyer and O'Gorman 2011, 2012; Kück and Pöggeler 2009; Dyer and Kück 2017).

2. *Botrytis cinerea*

The gray mold *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana)* is a notorious plant pathogenic fungus with a wide host range and has become an important model in molecular plant pathology. This fungus has two dissimilar mating types (MAT1-1 and MAT1-2) and is therefore heterothallic (Amselem et al. 2011). Asexual propagation occurs through microconidia in the light or sclerotia in the dark. The latter represent survival structures, which also serve the fertilization process during the sexual life cycle. When microconidia of the opposite mating type are available, the sclerotia are fertilized and generate apothecia. This process is induced by light, and fruiting bodies grow in the early stages toward the light source (Schumacher 2017). However, in the laboratory, induction of the sexual life cycle is a rather time-consuming process that takes 4 to 6 months until fully developed apothecia are obtained (Faretra and Antonacci 1987).

3. *Trichoderma reesei*

Trichoderma reesei, the anamorph of *Hypocrea jecorina*, is a major industrial enzyme producer, particularly of cellulases and hemicellulases, which are used for applications in food, feed, and biorefinery businesses. Until recently, it was believed that industrial strains propagate exclusively asexually. The industrial strains are derived from a single isolate that carries the MAT1-2 mating-type locus. Crossing experiments of *T. reesei* with *H. jecorina* MAT1-1 wild-type isolates led to fertilized fruiting bodies and the production of mature ascospores. However, the industrial *T. reesei* strain, which was used for mating experiments, can be used only as male partner in crossings but is unable to produce fruiting bodies and thus has to be considered to be female sterile (Seidl et al. 2009; Linke et al. 2015; Schmoll and Wang 2016).

4. *Penicillium* species

Like *Aspergillus* species, *Penicillium* species belong to the order of *Eurotiales*. Most species were for long believed to represent the asexual teleomorphs of the genera *Eupenicillium* or *Talaromyces*. However, the recent discovery of a sexual cycle with cleistothecia in the industrial penicillin producer *P. chrysogenum* was further evidence that supposedly asexual fungi can undergo a heterothallic life cycle. Genetic analysis has provided evidence that even industrial strains have conserved their potential to undergo a recombinant genetic cycle (Böhm et al. 2013, 2015). Recently, also the cheese fungus *Penicillium roqueforti* was shown to have a sexual cycle with ascogonia, cleistothecia, and ascospores (Ropars et al. 2014). These investigations suggest that the life cycle of sexually propagating *Penicillium* species is very similar to the one of heterothallic *Aspergillus* species.

5. *Fusarium graminearum* and *F. verticillioides*

Fusarium species belong to the *Sordariomycetes*, which are characterized by perithecia formation. *F. graminearum* (syn. *Gibberella zeae*)

and *F. verticillioides* (syn. *Gibberella moniliformis*) are plant pathogenic fungi, which are responsible for high losses in the harvest of cereals. *F. graminearum* is homothallic, and its sexual spores are responsible for disease initiation. In contrast, *F. verticillioides* is heterothallic and produces only modest numbers of fruiting bodies. Both fungi have been used for comparative expression studies, using six developmental stages of perithecia (Sikhakolli et al. 2012; Geng et al. 2014). Recently, *F. graminearum* served as source in several genome-wide large-scale functional analyses to decipher developmental genes and events related to sexual development. These attempts identified targets of the mating-type locus-encoded TFs, RNA interference (RNAi) mechanisms, and perithecium-specific RNA-editing events (Kim et al. 2015; Liu et al. 2016; Son et al. 2017)

6. *Pyronema confluens*

P. confluens is a homothallic soil-living saprophytic Pezizomycete, which forms apothecia in a light-dependent manner. The eight-spored asci are generated under laboratory conditions within 6 days. In the early twentieth century, this fungus was one of the first examples to elucidate the dikaryotic phase during sexual development in filamentous ascomycetes. Extensive transcriptome analysis was recently performed to investigate the light-dependent fruiting-body formation (Traeger et al. 2013).

III. Factors Influencing Fruiting-Body Development

A. Environmental Factors

Ascomycetes generate fruiting bodies in certain environmental conditions that are species-specific. Among others, light, nutrients, temperature, physical properties of growth substrates, and atmospheric conditions are relevant for fruiting-body formation. In this review, we will focus on the influence of light and nutrients, highlighting recent advances in our understanding of how these factors integrate into developmental signaling.

1. Light

Fungi respond to light in various ways, including phototropic growth of reproductive structures, modification of (circadian) rhythms, and changes in gene expression as well as primary and secondary metabolism, among others (Casas-Flores and Herrera-Estrella 2016). Here, we will focus on the influence of light on sexual reproduction of ascomycetes.

Light-dependent fruiting-body formation, phototropism of perithecial necks, and light-dependent ascospore discharge have been described early on for a number of ascomycetes (reviewed in Moore-Landecker 1992). For example, light is required for apothecia formation in *Ascobolus magnificus*, *Pyronema confluens*, and *P. domesticum* and for perithecia formation in *Pleurage setosa* (syn. *Podospora setosa*) (Yu 1954; Carlile and Friend 1956; Callaghan 1962). In *T. reesei*, formation of stromata, harboring the perithecia, occurs only in the presence of light (Seidl et al. 2009), while light inhibits formation of *B. cinerea* sclerotia, which serve as survival structures and female mating partners (Schumacher 2017). Light-dependent positioning of the perithecial neck has been described, e.g., for *Neurospora crassa* and *P. setosa* (Callaghan 1962; Harding and Melles 1983). In *Aspergillus glaucus* and *A. nidulans*, light favors asexual reproduction, while darkness favors sexual reproduction (Chona 1932; Mooney and Yager 1990; Blumenstein et al. 2005).

Ascomycetes possess several **photoreceptors**, sensing near-UV/blue, green, and red light (Idnurm and Heitman 2005; Casas-Flores and Herrera-Estrella 2016). All of these photoreceptors sense light by physical interaction with a chromophore: flavin for near-UV/blue and blue-light receptors, retinal for green-light receptors, and linear tetrapyrroles for red-light receptors. Upon photon absorption, structural changes in the chromophore induce conformational changes in the photoreceptors, leading to changes in protein-protein interactions or

signaling via certain output domains (see below). The repertoire of photoreceptors sensing diverse light qualities differs between species, and, e.g., *A. nidulans* and *B. cinerea* encode 3 and 11 photoreceptors, respectively (Schumacher 2017).

Blue-light receptors include cryptochromes and LOV (light oxygen voltage) domain proteins. Cryptochromes sense near-UV/blue light by binding flavin dinucleotide (FAD) and pterins/folates. Most cryptochromes show strongly reduced or no photolyase activity, one exception being *A. nidulans* (Bayram et al. 2008a). The LOV domain is a specialized PAS (found in Per, Arnt, Sim) domain, which binds the flavin chromophore. LOV domain proteins may contain additional output domains, e.g., zinc finger or RGS (regulator of G-protein signaling) domains. The white collar 1 homologs are light-activated transcription factors with three PAS domains, one of which is a FAD-binding LOV domain, and a GATA-type zinc finger DNA-binding domain. WC-1 of *N. crassa* interacts with a second white collar protein, WC-2, via its PAS domains, and both control expression of light-regulated genes by binding to promoter sequences as a heterodimer (Cheng et al. 2002; Froehlich et al. 2002; He et al. 2002; Smith et al. 2010). An example for a LOV domain protein without further output domains is *N. crassa* Vivid (VVD-1) involved in photoadaptation (Chen et al. 2010).

Green-light receptors have not been extensively characterized in filamentous ascomycetes so far. These receptors are related to rhodopsins, composed of a seven-transmembrane domain opsin bound to retinal via a conserved lysine. Retinal binding in opsins leads to the formation of green-light responsive ion pumps as demonstrated for the CarO protein of *F. fujikuroi* (Garcia-Martinez et al. 2015). However, other opsins like *N. crassa* NOP-1 lack proton pump activity, indicating a putative regulatory role (Bieszke et al. 1999; Brown et al. 2001).

Fungal **phytochromes** are red-light sensors and contain multiple domains, with an N-terminal photosensory domain binding to a bilin-type linear tetrapyrrole, a histidine kinase domain, and a response regulator domain. The photosensory domain combines PAS, GAF (for vertebrate cGMP-specific phosphodiesterases, cyanobacterial adenylate cyclases, transcription activator FhlA), and PHY (phytochrome-specific PAS-related) domains. Absorption of red light leads to reversible conformational changes of the chromophore, shifting the absorption maximum to far-red light. Thus, phytochromes are able to sense red/far-red ratios (Rockwell and Lagarias 2010).

Blue- and red-light photoreceptors have roles in sexual development in various fungi (for a review, see Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016). Besides photoreceptors, chromatin modifiers (see Sect. IV.C.3), MAPK pathways (see Sect. IV.A.1), and transcription factors (see Sect. IV.C.1) are required for light regulation of sexual development. Light perception and light signal transduction have been analyzed in great detail in few model systems, including *A. nidulans*, *N. crassa*, and *T. reesei* (Seidl et al. 2009; Bayram et al. 2010; Schmoll et al. 2010a; Dasgupta et al. 2016). An overview of photoreceptors of these three model fungi and their role in sexual development is shown in Fig. 5.

As mentioned above, *A. nidulans* reproduces mainly sexually in the dark and mainly asexually in light, and blue together with red light is the responsible light quality. Molecular genetic analysis has shown that cryptochrome CryA, the white collar proteins LreA (light response A) and LreB, as well as the phytochrome FphA are required for this light-dependent behavior (Blumenstein et al. 2005; Bayram et al. 2008a; Purschwitz et al. 2008). The cryptochrome CryA still has photolyase activity and functions in DNA repair. Notably, *A. nidulans* lacks a vivid and a functional, i.e., retinal-binding opsin homolog and has only one phytochrome, FphA, which binds biliverdin and absorbs red as well as far-red light (Bayram et al. 2008a; Idnurm et al. 2010; Fischer et al. 2016). CryA and FphA repress sexual development in light conditions, while LreA and LreB stimulate sexual development, and *lreA* as well as *lreB* deletion mutants generate no perithecia in the light (Blumenstein et al. 2005; Bayram et al. 2008a; Purschwitz et al. 2008).

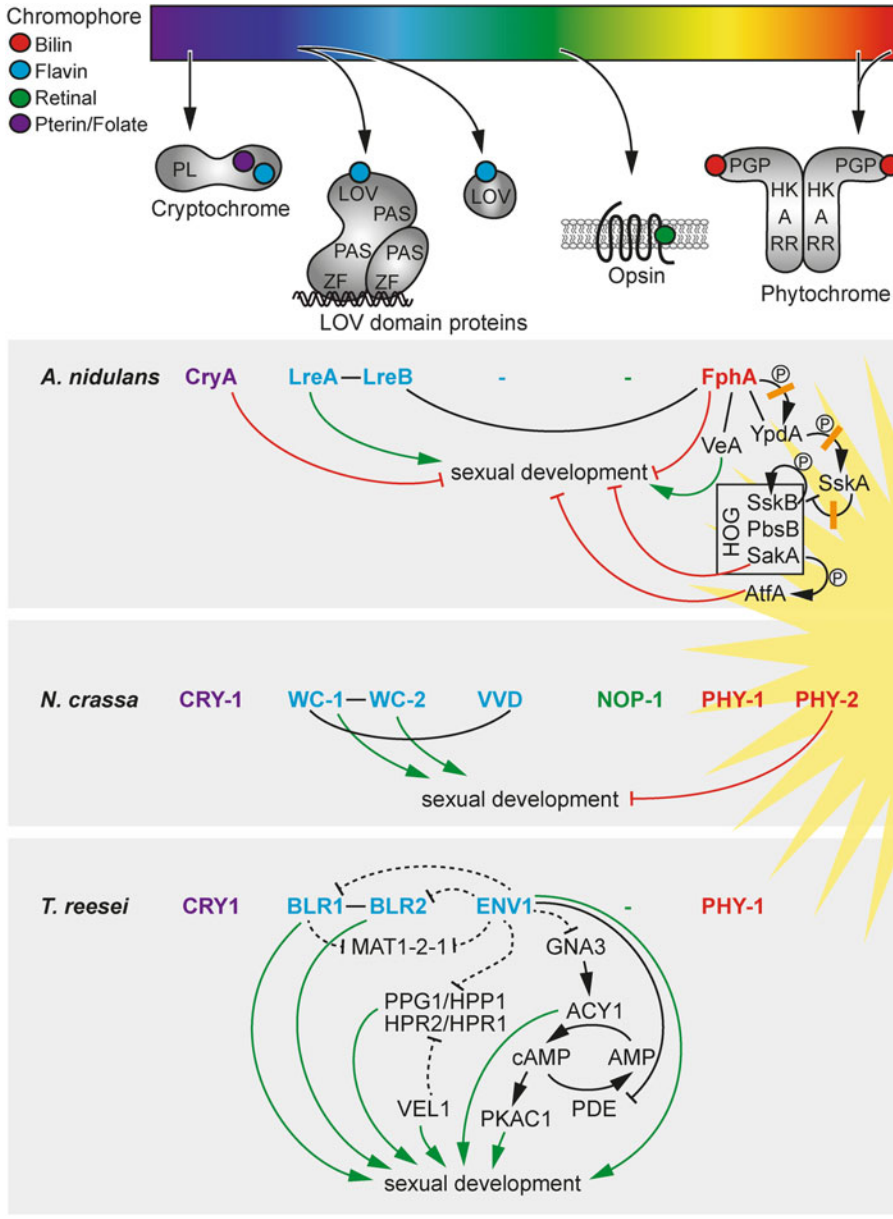


Fig. 5 Regulatory role of photoreceptors in sexual development of model ascomycetes. Data are summarized from recent reviews and research articles (Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016; Schmoll and Wang 2016; Bazafkan et al. 2017). The upper part of the figure summarizes photoreceptor types found in fungi and their respective chromophors. The lower part of the figure summarizes the occurrence of photoreceptors and current knowledge on signal transduction in light conditions in the three model fungi *A. nidulans*, *N. crassa*, and *T. reesei*. Dashed lines indicate transcriptional regulation, while continuous lines indicate physical interaction. An encircled P symbolizes phosphorylation. Bold orange lines indicate light-induced interruption of signal transduction. For details, see main text. A, ATPase domain; HK, histidine kinase; LOV, light oxygen voltage domain; PAS, found in Per, Arnt, Sim; PGP, photosensory domain of phytochromes combining PAS, GAF (for vertebrate cGMP-specific phosphodiesterases, cyanobacterial adenylate cyclases, transcription activator FhlA), and PHY (phytochrome-specific PAS-related) domains; PL, photolyase; RR, response regulator domain; ZF, GATA-type zinc finger DNA-binding domain

Light signal transduction in *A. nidulans* involves several key regulators of development, namely, the velvet complex and the high-osmolarity glycerol (HOG) MAPK pathway (Purschwitz et al. 2008; Yu et al. 2016). FphA interacts with both, LreB and the velvet protein VeA, in the nucleus, and the latter interaction is dependent on light. Furthermore, VeA nuclear accumulation is partially dependent on FphA, and VeA is required for binding of LreA to promoter sequences (Purschwitz et al. 2008; Hedtke et al. 2015). In concert, LreA and FphA modulate gene expression by inducing histone acetylation, linking light signaling and chromatin remodeling (see Sect. IV.C.3). Recently, a regulatory function of FphA in the cytoplasm was elucidated (Yu et al. 2016). FphA is the upstream component of a phosphorelay system consisting of phosphotransfer protein YpdA and response regulator SskA (Fig. 5). In the dark, phosphorylation inhibits interaction of SskA with the downstream HOG pathway, consisting of MAPKKK SskB, MAPKK PbsB, and MAK SakA (see Sect. IV.A.1). In the light, phosphorylation of FphA, YpdA, and SskA decreases, leading to activation of the HOG pathway, light-dependent translocation of the MAPK SakA to the nucleus, and activation of the transcription factor AtfA (Yu et al. 2016). SakA and AtfA are activated during asexual development and repress cleistothecia formation (Kawasaki et al. 2002), explaining why red light represses sexual development in *A. nidulans*.

In *N. crassa*, the blue-light response is best characterized. In this ascomycete, blue light regulates perithecial neck positioning and neck orientation, and protoperithecia production is induced by blue light (Harding and Melles 1983; Innocenti et al. 1983; Oda and Hasunuma 1997). All of these light responses are mediated by the white collar proteins WC-1 and WC-2, but not cryptochrome CRY-1 (Harding and Melles 1983; Degli-Innocenti and Russo 1984; Oda and Hasunuma 1997; Froehlich et al. 2010) (Fig. 5). The role of the WC complex in circadian rhythm has been studied in great detail (reviewed in Hurley et al. 2015; Montenegro-Montero et al. 2015), but its regulatory function in sexual development remains largely elusive. As for *A. nidulans*, an interplay of the WC complex (WCC) and histone acetylation in the activation of light-induced genes has been described (Grimaldi et al. 2006). Further, it has been suggested that WCC activity is modulated by other photoreceptors, namely, CRY-1, opsin NOP-1, and one of the two phytochromes, PHY-2, and that some light responses require the velvet homolog VE-1 (Olmedo et al. 2010). Recently, phytochrome PHY-2 was described to regulate proper timing of sexual development, since deletion of *phy-2* led to early induction of protoperithecia formation in red light (Wang et al. 2016b) (Fig. 5). Taken together, regulation of sexual development by light may use the same protein machinery in *N. crassa* as in *A. nidulans*, but the wiring of light signaling needs to be analyzed further.

Unlike *A. nidulans*, *N. crassa*, and most other ascomycetes, *T. reesei* requires light for timely sexual reproduction (Seidl et al. 2009; Chen et al. 2012). *T. reesei* exhibits all photoreceptor types but an opsin homolog, and an influence on sexual development has been described for the blue-light receptors BLR1 and BLR2 and the Vivid homolog ENVOY (ENV1) (reviewed in Schmoll et al. 2010a, 2016) (Fig. 5). While ENV1 is required for female fertility in light, BLR1 and BLR2 are not, but mutants lacking these receptors produce fewer and larger fruiting bodies (Chen et al. 2012; Seibel et al. 2012a). ENV1 effects transcription of several light-regulated genes, including pheromone receptor and peptide pheromone precursor genes as well as genes for G-protein α subunits *gna1* and *gna3* (Chen et al. 2012; Seibel et al. 2012a; Tisch and Schmoll 2013). GNA3 acts in the cAMP pathway by activating adenylyl cyclase ACY1, which generates cAMP, which in turn activates protein kinase PKAC1. Both ACY1 and PKAC1 are required for sexual development in *T. reesei* (Schuster et al. 2012) (Fig. 5). ENV1 further intervenes with the cAMP pathway by inhibiting phosphodiesterase PDE, which is required for cAMP degradation (Tisch et al. 2011) (Fig. 5). As in *A. nidulans*, velvet plays a role in light signaling. Like ENV1, the *T. reesei* VEL1 protein is required for female fertility in light,

and ENV1 and VEL1 act in concert to regulate pheromone response genes in a mating-type-dependent manner (Bazafkan et al. 2015, 2017).

Since this section focused on the influence of light on fruiting-body formation, we would like to refer the interested reader to several recent reviews that focused on other aspects of fungal life affected by light (Casas-Flores and Herrera-Estrella 2016; Dasgupta et al. 2016; Fischer et al. 2016; Schumacher 2017).

2. Nutrients

Nutrients play an important role in the induction and completion of fruiting-body formation in filamentous ascomycetes, as has been reviewed before (Moore-Landecker 1992; Pöggeler et al. 2006a; Debuchy et al. 2010). In short, most fungi generate fruiting bodies at low-nutrient conditions, although there are exceptions, e.g., *A. nidulans* and *S. macrospora* (Molowitz et al. 1976; Han et al. 2003). A certain C/N ratio seems to be required for induction of fruiting-body formation, and this ratio is in the range of 1:3 to 1:10 (Moore-Landecker 1992). Several fungi need additional nutrients for fruiting-body formation, e.g., vitamins or certain amino acids. *S. fimicola* and *S. macrospora* require biotin for perithecia formation, and arginine is required by *S. macrospora* for timely completion of the sexual cycle (Barnett and Lilly 1947; Molowitz et al. 1976).

In recent years, research has focused on signaling pathways sensing nutrient status and on transport routes. In general, fungi sense nutrients using plasma membrane proteins, among them **G-protein-coupled receptors** (GPCRs) (Van Dijck et al. 2017). The *A. nidulans* GPCRs GprD and GprH are required for repression of sexual development in low-nutrient conditions. GprD probably represses sexual development via regulation of glucose and amino acid metabolism, while GprH senses glucose and tryptophan and acts upstream of the cAMP-PKA pathway (de Souza et al. 2013b; Brown et al. 2015). Proton-coupled dipeptide transporters (PTR2s) support sexual development in *F. graminearum*, since mutants lacking FgDPTR2A, FgDPTR2C, or FgDPTR2D formed fewer perithecia than wild type (Droce et al. 2017). In *T. reesei*, the gene encoding a homolog of yeast ABC transporter Ste6p is strongly upregulated on lactose. Since Ste6p exports the **a** pheromone in yeast in an ATP-dependent manner, this upregulation indicates an effect of carbon sources on fruiting-body formation in *T. reesei* (Ivanova et al. 2013; Schmoll et al. 2016).

Signaling proteins have also been shown to simultaneously influence nutrient utilization and fruiting-body formation. The *F. graminearum* sucrose nonfermenting 1 (GzSNF1) protein kinase is involved in utilization of alternative carbon sources and sexual development. A GzSNF1 mutant generated 30% less perithecia than wild type, and asci were abnormal in containing one to eight ascospores that were abnormally shaped (Lee et al. 2009b). In *N. crassa*, the kinase IME-2 (inducer of meiosis 2) supposedly downregulates protoperithecia formation by inhibiting the transcription factor VIB-1 (see Table 2) in the presence of nitrogen (reviewed in Irniger 2011). Ime2 kinases are conserved S/T kinases that function in meiotic control in *S. cerevisiae*. Ime2 homologs from various fungi are involved in repression of fruiting-body formation in response to environmental signals. As mentioned above (Sect. III.A.1), the small LOV domain protein ENV1 is a central regulator of sexual development in *T. reesei*. ENV1 and the phosducin-like protein PhLP1 involved in G-protein signaling were found in a mutual interrelationship to downregulate nutrient signaling as an early response to light, supposedly to enable protective measures prior to metabolic adaptation (Tisch et al. 2014). How exactly ENV1 and other signaling proteins integrate diverse environmental signals to control fruiting-body formation remains to be determined. Transcription factors might be the targets of these pathways (see Sect. IV.C.1).

B. Endogenous Factors

In addition to environmental factors described above, fungi need endogenous substances to reproduce sexually. Nutrients are processed

via metabolic processes, and primary as well as secondary metabolites are required for or modulate fruiting-body formation. Moreover, pheromones are necessary at distinct stages of fruiting-body formation.

1. Primary and Secondary Metabolites

The influence of metabolites on fruiting-body formation has been documented by the sterility of mutants defective in diverse metabolic pathways, as reviewed before (Pöggeler et al. 2006a). The occurrence of these mutants indicates a requirement for certain metabolites during sexual reproduction. An example is sterile mutant pro4 from *S. macrospora* with a defect in *leu1*, encoding β-isopropylmalate dehydrogenase involved in leucine biosynthesis (Kück 2005). Similarly, mutants defective in mitochondrial respiration have been reported as (female) sterile in different ascomycetes, supposedly because of the massive energy demand during sexual reproduction, as also discussed for protein degradation processes and autophagy (see Sect. IV.B) (Videira and Duarte 2002). Here, we will review recent progress concerning the role of **primary and secondary metabolites** in fruiting-body formation.

As mentioned above, many ascomycetes produce fruiting bodies upon nutrient starvation and thus rely on reserve compounds such as carbohydrate or lipid reserves. Mobilization of storage lipids requires peroxisomal functions, namely, β-oxidation and the glyoxylate pathway. Several peroxisomal import and peroxisomal metabolism mutants have been described as defective in fruiting-body formation (reviewed in Peraza-Reyes and Berteaux-Lecellier 2013). For example, in *A. nidulans*, induction of cleistothecium formation by oleic acid is abolished in mutants lacking PexF, a recycling factor for a peroxisomal import receptor (Hynes et al. 2008). In *P. anserina*, mutants of RING finger complex components, required for peroxisomal matrix protein import and subsequent export of the import receptor, are blocked in sexual development. This block occurs prior to karyogamy, leading to fruiting bodies containing no ascospores (Bonnet et al. 2006; Peraza-Reyes et al. 2008). Mutants lacking the glyoxylate cycle enzyme isocitrate lyase (ICL1) have sexual developmental defects in *F. graminearum*. Transcription of *icl1* ceases in the late sexual stage, indicating a shutdown of the glyoxylate cycle or other peroxisomal metabolic processes for fruiting-body formation in this fungus (Lee et al. 2009a). Interestingly, linoleic acid has been shown to accumulate during *Nectria haematococca* perithecium formation, and exogenous linoleic, linolenic, oleic, and palmitoleic acid stimulated perithecia formation (Dyer et al. 1993).

Nitric oxide (NO) has recently been shown to affect fruiting-body formation (Canovas et al. 2016). *A. nidulans* shows elevated levels of NO immediately after switching from vegetative growth to sexual or asexual development. Thus, NO, together with reactive oxygen species (see Sect. IV.A.3), may be an early signal triggered by or triggering development. The balance of CO_2 and HCO_3^- is another factor affecting sexual development. In *A. nidulans*, increasing CO_2 levels by sealing agar plates promotes sexual reproduction (Dyer and O'Gorman 2012). Carbonic anhydrases (CAS) catalyze the reversible interconversion of CO_2 and bicarbonate (HCO_3^-). Deletion of all four *cas* genes in *S. macrospora* led to immature perithecia that were embedded in the agar and devoid of ascospores (Lehneck et al. 2014).

A metabolic enzyme affecting fruiting-body formation is ATP citrate lyase (ACL). This enzyme generates cytoplasmic acetyl-CoA from mitochondria-derived citrate and has been shown to function in sexual development in *A. nidulans*, *F. graminearum*, and *S. macrospora*, as have *F. graminearum* acetyl-CoA synthase ACS and components of carnitine-dependent acetyl-CoA transport (Nowrousian et al. 1999; Hynes and Murray 2010; Lee et al. 2011; Son et al. 2011a, 2012). Citric acid itself may regulate sexual development. Cleistothecia formation in *A. glaucus* is strongly enhanced by citric acid, and deletion of the citrate synthase gene *citA* in *A. nidulans* abolished meiosis, leading to cleistothecia without any ascospores (Cai et al. 2010; Murray and Hynes 2010). ACL-generated acetyl-CoA is commonly used as a precursor for fatty acid and sterol biosynthesis.

Interestingly, ACL function is required for increasing histone acetylation during mammalian differentiation and has also been linked to histone acetylation in fungi (Wellen et al. 2009; Son et al. 2011a). If the sexual defects in all fungal ACL mutants are caused by defects in chromatin modification (see Sect. IV.C.2) remains to be determined.

Secondary metabolites have recently emerged as central regulators of sexual development, although a connection of secondary metabolite production and developmental processes has been known for a long time (reviewed in Calvo et al. 2002). In many ascomycetes, fruiting bodies and/or ascospores are pigmented, and often this pigment is the polyketide melanin (see Introduction). Supposedly, melanin protects ascospores from environmental damage and may also structurally reinforce three-dimensional structures by strengthening the cell wall (Langfelder et al. 2003). However, melanin per se is not required for fruiting-body formation, since mutants unable to synthesize melanin still are fertile (e.g., Engh et al. 2007; Nowrousian et al. 2012). Other secondary metabolites may function as regulators of sexual development. Loss of the polyketide synthase *pks4* gene in *S. macrospora* leads to a block of perithecia formation, while overexpression of *pks4* results in large aberrant perithecia (Schindler and Nowrousian 2014). The above-described PTR2 transporters of *F. graminearum* are required for fusarielin H production, and the gene cluster responsible for its production is upregulated during perithecia formation (Sorensen et al. 2013; Droce et al. 2017).

Oxylipins are secondary metabolites derived from peroxidation of fatty acids, and they occur in mammals, plants, and fungi (Brodhun and Feussner 2011). The function of oxylipins has been mainly analyzed in *A. nidulans*. Here, the so-called psi (precocious sexual inducer) factors regulate the balance between sexual and asexual development. Psi factor is a mixture of PsiA, PsiB, and PsiC, consisting mainly of hydroxylated oleic and linoleic acid, and generated by psi-factor producing oxygenases PpoA, PpoB, and PpoC (Tsitsigiannis et al. 2005). The role of oxylipins in sexual development of other ascomycetes remains obscure, although lipids have been known to induce sexual development for a long time (see above).

Velvet has been shown to be a common regulator of (sexual) development and secondary metabolism (reviewed in Bayram and Braus 2012). It was shown recently that *T. reesei* VEL1 promotes the production of specific secondary metabolites during encounter of a mating partner. The Δvel1 mutant showed a secondary metabolite profile different from wild type in these conditions, and the wild type exhibited different secondary metabolite profiles when confronted with another wild type or a Δvel1 mating partner (Bazafkan et al. 2015). Velvet also controls fruiting-body development in response to light (Sect. III.A.1) and via regulation of gene expression (Sect. IV.C.1).

2. Pheromones

Sexual reproduction of filamentous ascomycetes involves the establishment of a dikaryotic stage with two compatible nuclei synchronously dividing in the same hyphal compartment (see Introduction). Often, heterothallic filamentous ascomycetes send out a specialized hypha (trichogyne) from the female prefruiting body, which senses a male cell of the opposite mating type, grows toward it, and fuses with the male cell. Uninucleate spermatia and microconidia, multinucleate macroconidia, or even vegetative hyphae can act as male cells. Similar to *S. cerevisiae*, diffusible peptide pheromones activate cognate G-protein-coupled receptors (GPCRs) at the surface, which activate signaling to control chemoattraction and fusion of male and female cells (Alvaro and Thorner 2016; Bennett and Turgeon 2016).

Already in the 1980s, diffusible **pheromones** have been suggested to be involved in the mating process of *N. crassa* and to be the cause for the directional growth of trichogynes toward the male fertilizing cells of the opposite mating type (Bistis 1981, 1983). In *N. crassa*, this directional growth of the trichogynes did not occur when the recipient male cells harbored mutations at the mating-type locus,

suggesting that the mating-type locus regulates the pheromone production (Bistis 1981).

Meanwhile, two different types of pheromone precursor genes have been isolated from various heterothallic and homothallic filamentous ascomycetes. These include *A. fumigatus*, *Cryphonectria parasitica*, *F. graminearum*, *H. jecorina*, *Magnaporthe grisea*, *N. crassa*, *P. chrysogenum*, *P. anserina*, and *S. macrospora* (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002; Coppin et al. 2005; Paoletti et al. 2005; Hoff et al. 2008; Kim et al. 2008; Schmoll et al. 2010b; Böhm et al. 2013).

Filamentous ascomycetes also encode two types of pheromone receptors, which are related to the Ste2 and Ste3 GPCRs of *S. cerevisiae* (Pöggeler 2011). Unlike in basidiomycetes, which encode only Ste3-like receptors, the two types of pheromone precursor and receptor genes are present in the same nucleus and are not part of the mating-type locus (Raudaskoski and Kothe 2010; Kües et al. 2011; Pöggeler 2011). In many heterothallic ascomycetes, mating-type encoded transcription factors directly control expression of pheromone precursor and receptor genes (Herskowitz 1989; Zhang et al. 1998; Shen et al. 1999; Bobrowicz et al. 2002; Coppin et al. 2005; Kim et al. 2012), while in others such as *H. jecorina* expression of pheromone genes does not depend on mating-type genes. However, these fungi showed enhanced expression of pheromone and receptor genes in the cognate mating type (Schmoll et al. 2010b; Seibel et al. 2012b).

In self-fertile, homothallic ascomycetes, spermatia and trichogynes are absent, and conidia are often missing. Therefore, recognition between a female and male cell of opposite mating type and a **pheromone/receptor system** do not seem to be necessary for sensing a mating partner or initializing fertilization events in these fungi. Nevertheless, they transcriptionally express pheromone and receptor genes (Pöggeler 2000; Pöggeler and Kück 2001; Paoletti et al. 2007; Kim et al. 2008; Lee et al. 2008).

Similar to the *S. cerevisiae* α-factor, one of the precursor genes, termed *ppg1* or *ppgA* in filamentous ascomycetes, encodes a polypeptide containing a signal sequence for secretion and multiple repeats of a putative pheromone sequence bordered by protease processing sites (Fig. 6a). A Ste2-like pheromone receptor termed PRE2 or PREB is able to sense the mature peptide pheromone. The other pheromone gene, *ppg2* or *ppgB*, encodes a short polypeptide similar to the *S. cerevisiae* **a**-factor precursor with a C-terminal CAAX (C = cysteine, a = aliphatic, and X = any amino acid residue) motif expected to produce a mature pheromone with a C-terminal carboxymethyl isoprenylated cysteine (Fig. 6b) (Jones and Bennett 2011). The mature lipopeptide is sensed by the Ste3-like pheromone receptor PRE1 (PREA).

Common to all α-like pheromone precursors are 2–15 repeats of a presumed mature pheromone. Within a given precursor, the length of the repeated sequence bordered by the processing sites varies between 9 and 12 aa with an 8-aa consensus motif (CR[RW]PGQPC). Three-dimensional structure determination of the ten-amino acid pheromone from *Fusarium oxysporum* revealed the presence of a central β-turn similar to its *S. cerevisiae* counterpart (Fig. 7). Structure-activity relationship of the α-like *F. oxysporum* pheromone demonstrated that the conserved central GQ is crucial for its chemoattractive activity (Naider and Becker 2004; Vitale et al. 2017).

Usually, the length of the repeated sequence is constant within a peptide pheromone precursor. However, some positions may vary in the repeated sequence (Martin et al. 2011; Pöggeler 2011). An N-terminal signal sequence is predicted for all of the PPG1/PPGA precursors. Usually maturation signals for endopeptidase (KR for KEX2), carboxypeptidases (KEX1 for removal of basic residues, e.g., KR, KE, KV, or KA after KEX2 cleavage), and aminopeptidases (XA, XP) surround the repeated sequences of the pheromone precursors (Fig. 6a). Phylogenetic analysis of repeats from PPG1/A pheromones revealed that repeated sequences undergo a rapid evolution that might contribute to speciation in ascomycetes (Martin et al. 2011).

Similar to **a**-factor-like precursors of ascomycete yeasts and basidiomycetes, the PPG2/PPGB precursors from filamentous ascomycetes are short with only 21–32 aa and harbor

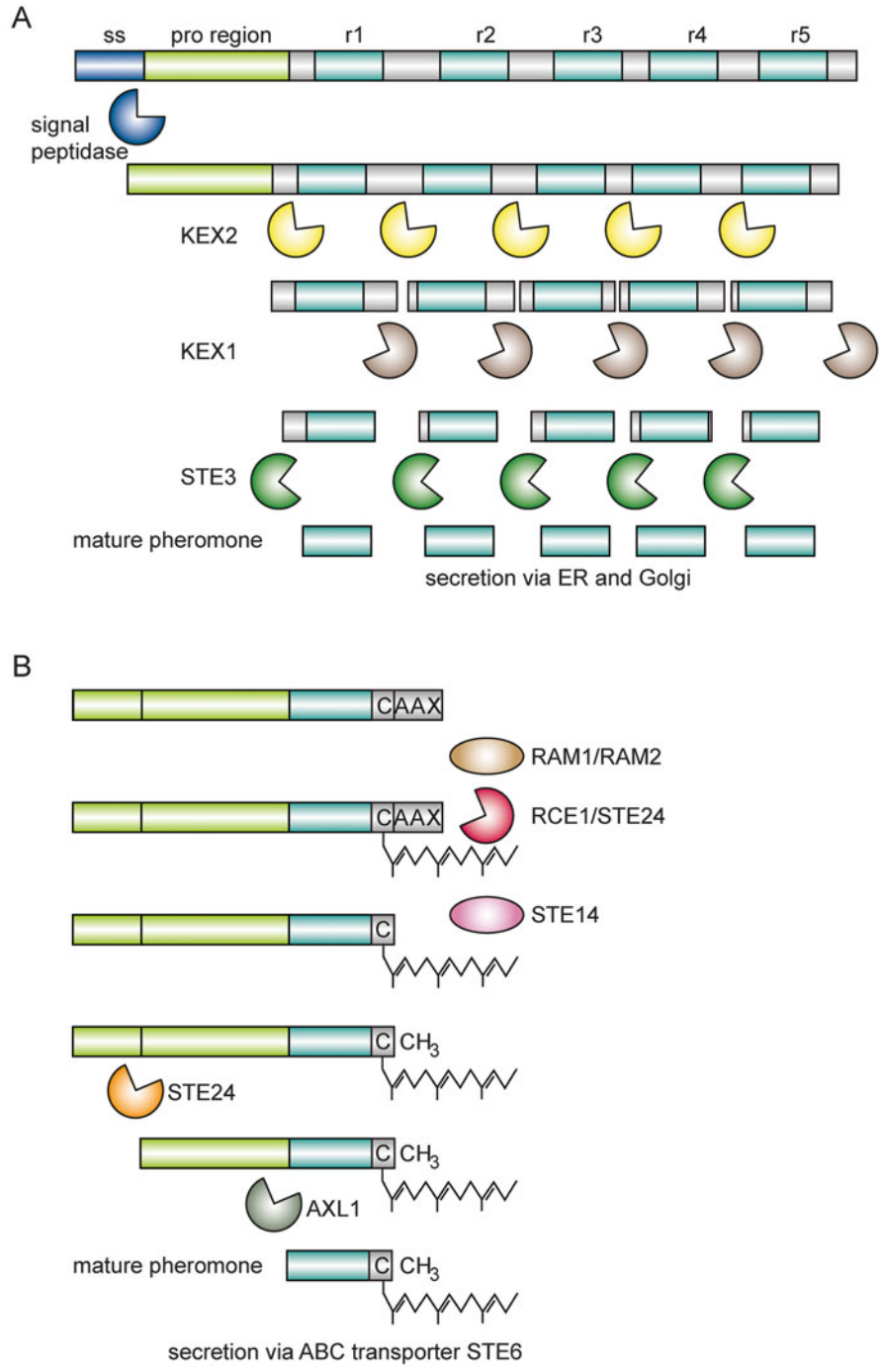


Fig. 6 Processing of PPG1/PPGA and PPG2/PPGB mating pheromones in analogy to α-factor and **a**-factor processing of *S. cerevisiae*. (**a**) Processing of the pre-pro peptide pheromone precursor PPG1/PPGA. The secretion signal (ss, blue) is cleaved by the signal peptidase, KEX2 endopeptidase removes the pro-region (green), and carboxypeptidase KEX1 and aminopeptidase STE13 digest the connecting regions of the repeated copies (r1–r5). The mature peptide pheromone is exported via the ER/Golgi pathway. (**b**) Processing of the lipopeptide pheromone precursor PPG2/PPGB. Farnesylation at the conserved cysteine residue

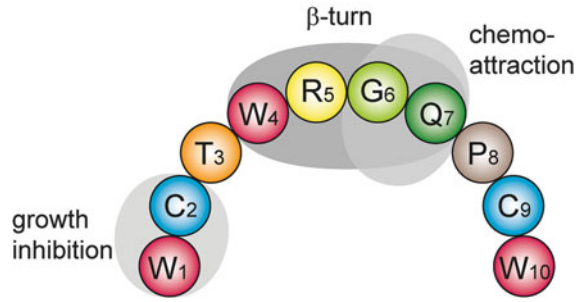


Fig. 7 Cartoon representing functional and structural segmentation of the *F. oxysporum* PPG1 pheromone according to Vitale et al. (2017). Shaded in light gray are those residues that are functional in growth inhibition and chemoattraction, while the β-turn is indicated by dark gray shading

no signal sequence for secretion. They have a 9-aa conserved motif comprising the CAAX motif for farnesylation by farnesyltransferase RAM1/RAM2 and five preceding residues. In addition to the invariant C four amino acids from the end, in filamentous ascomycetes, the last residue of the CAAX motif is an invariant methionine and the penultimate residue an aliphatic amino acid (Pöggeler 2011). After farnesylation, methylation, and N-terminal processing, the mature lipopeptide pheromone is predicted to be exported from the hyphae using an ATB-binding cassette (ABC) transporter (Fig. 6b).

TBLASTN searches of genomes from many filamentous ascomycetes, e.g., from species of the genera *Aspergillus*, *Paracoccidioides*, and *Penicillium*, identified only the α-factor-like pheromone gene *ppg1/ppgA*, but failed to identify a *ppg2/ppgB* homolog (Dyer et al. 2003; Hoff et al. 2008; Gomes-Rezende et al. 2012). This may be explained by the small size and poor conservation of **a**-factor-like pheromone precursors or by the absence of a hydrophobic pheromone gene in these species (Pöggeler 2011). However, the conserved coding capacity for the Ste3-like receptor PRE1/PREA argues against the absence of **a**-like pheromones in these species.

Species of the order *Hypocreales*, including *F. oxysporum*, *Fusarium verticillioides*, *H. jecorina*, and *N. haematococca*, encode a novel class of pheromone precursors (Schmoll et al. 2010b). This new type of pheromone precursor has characteristics of both PPG1/PPGA and PPG2/PPGB pheromone precursors. It was therefore termed h-type (hybrid) pheromone precursor. H-type pheromone precursors contain the CPAX motif at the C-terminus (with a proline replacing one aliphatic residue of the CAAX motif) or copies of this motif and putative KEX2 processing sites. Similar to PPG2/PPGB pheromone precursors, h-type pheromone precursors are predicted to contain no signal sequence and are therefore suggested to be secreted via a nonclassical ABC-transporter-mediated secretion pathway (Schmoll et al. 2010b). Martin et al. (2011) considered this gene structure to be a variation of the **a**-class precursor, as opposed to an entirely distinct precursor class.

In filamentous ascomycetes, pheromones and their cognate pheromone receptors have two main functions: (1) recognition between male and female cells of opposite mating types in heterothallic filamentous ascomycetes and (2) the regulation of postfertilization events, which are equally important for homothallic and heterothallic fungi. These latter include processes such as nuclear migration, maintenance of the dikaryotic state, cell fusion, and meiosis (Spellig et al. 1994; Willer et al. 1995; Casselton 2002).

Male and female fertility of heterothallic ascomycetes depend on the specific interaction

Fig. 6 (continued) of the CAAX motif by farnesyltransferases RAM1/RAM2 is followed by proteolysis of the three most C-terminal amino acids AAX by RCE1 and STE24. Carboxymethylation is performed by the methyltransferase STE14. Further proteolytic events remove amino acids from the N-terminus. The mature lipophilic pheromone is exported by an ABC transporter

of pheromones with their cognate receptors. Deletion of pheromone genes results in male sterility, but does not lead to defects in vegetative development and female fertility. Spermatia or macroconidia of pheromone mutants are unable to attract their female partners, and heterologous expression of a pheromone gene enables male cells to direct the chemotropic growth of trichogynes from an otherwise incompatible mating-type background. However, pheromone-deleted mutants are not affected in fusion of vegetative hyphae (Kim et al. 2002a, 2012; Turina et al. 2003; Coppin et al. 2005; Kim and Borkovich 2006). In *N. crassa*, co-expression of the Ste2-like receptor gene *pre2 and* the cognate pheromone gene *ppg1* in a MAT1-1 instead of a MAT1-2- background leads to self-attraction and development of barren perithecia without ascospores. Forced heterokaryons of opposite mating-type strains expressing one receptor gene and the compatible pheromone gene are able to form mature perithecia. Thus, the presence of one receptor and its compatible pheromone is necessary and sufficient for perithecial development and ascospore production (Kim et al. 2012).

In the heterothallic *N. crassa* and the pseudohomothallic *P. anserina*, **pheromone precursor genes** are highly expressed under conditions that favor sexual development (Bobrowicz et al. 2002; Kim et al. 2002a; Coppin et al. 2005; Bidard et al. 2011; Wang et al. 2014b). Recently, ChIP-seq and in vivo binding assays verified direct binding of the MAT1-1-1 a domain transcription factor to the *ppg1* gene promoter and *pre1* receptor gene promoter in *P. chrysogenum* (Becker et al. 2015).

In the homothallic *S. macrospora*, both pheromone genes are positively controlled by MAT1-1-1, while *ppg2* is controlled by MAT1-2-1 (Pöggeler et al. 2006b; Klix et al. 2010). Moreover, laser microdissection and RNA-seq analysis revealed that pheromone genes *ppg1* and *ppg2* are strongly upregulated in prefruiting bodies of *S. macrospora* (Teichert et al. 2012).

The expression of pheromone receptors is not significantly altered in *MAT* deletion strains of *S. macrospora* (Pöggeler et al. 2006b; Klix et al. 2010), while in the homothallic *Sclerotinia sclerotiorum*, all *MAT* genes are involved in the expression of both pheromone and receptor genes (Doughan and Rollins 2016). In *F. graminearum*, expression of *ppg2* is under control of the MAT1-2 locus, and *pre1* is regulated by the MAT1-1 locus (Kim et al. 2015), whereas in *A. nidulans* expression of *ppgA* and the two pheromone receptor genes is not regulated by *MAT* genes, although upregulated during sexual development (Paoletti et al. 2007).

In homothallic filamentous ascomycetes, deletion of any single pheromone or receptor gene does not impair vegetative growth or fruiting-body development (Seo et al. 2004; Mayrhofer and Pöggeler 2005; Mayrhofer et al. 2006; Kim et al. 2008; Lee et al. 2008). However, double-deletion strains without any compatible pheromone receptor pair showed a reduced number of fruiting bodies and ascospores. The most drastic negative effects occurred in receptor double-deletion mutants. In *A. nidulans* and *S. macrospora*, these mutants are unable to form fruiting bodies and ascospores (Seo et al. 2004; Mayrhofer et al. 2006). In *F. graminearum*, only deletion combinations that include either the *ppg1* pheromone gene or the *pre2* receptor gene cause increased numbers of immature perithecia and display reduced fertility in self-fertilization tests (Kim et al. 2008; Lee et al. 2008). However, even a quadruple mutant of *F. graminearum* without any pheromone and receptor genes produces fruiting bodies and ascospores. The pheromone/receptor system seems to play a nonessential role in the sexual development in *F. graminearum* (Kim et al. 2008). Thus, similar to heterothallic ascomycetes, in some homothallic species, at least one pheromone/receptor system is required for later stages of the sexual development such as nuclear migration, crozier, and ascogenous hyphae formation, while in others these processes seem to proceed independently of a functional pheromone/receptor system. Differences in the regulation of the expression and functions of the pheromone/receptor system might be the consequence of an independent adaptation to the homothallic lifestyle.

Studies in the asexual root-infecting ascomycete *F. oxysporum* recently revealed that

germinating conidiospores use the peptide pheromone receptor PRE2 to sense gradients of sugars, amino acids, pheromones, and plant root exudates to redirect their growth toward these chemoattractants. Interestingly, synthetic peptide pheromones from *S. cerevisiae* and *F. oxysporum* as well as root peroxidase can induce polarized growth of the germ tubes equally well (Turrà et al. 2015, 2016). Moreover, the *F. oxysporum* PPG1 pheromone inhibits cell division of germ tubes in a STE2-independent way (Vitale et al. 2017).

These examples show that during adaptation to different lifestyles, the fungal pheromone/receptor system can be used or converted to sense and respond to various environmental cues. Therefore, pheromones and receptors might have more functions than so far assumed.

IV. Regulatory Networks

The formation of three-dimensional fruiting bodies is a highly controlled and complex process. Development in filamentous ascomycetes is orchestrated by signal transduction pathways, protein degradation systems, as well as transcriptional regulation.

A. Signal Transduction Pathways

Sexual development in ascomycetes requires a tight regulation of sending, responding, and processing signals. These signals contribute to, e.g., mating partner recognition, cell-to-cell communication, induction of sexual development, and finally fruiting-body formation. The communication processes are conducted by several signal transduction pathways, in which reversible protein phosphorylation is often the key signal. Remarkably, many of the signaling components are highly conserved in eukaryotes, and a detailed knowledge on their molecular mechanisms not only contributes to our understanding of fungal fruiting-body formation but also to our understanding of signal transduction pathways in general.

1. MAP Kinase Signaling

Many **signal transduction pathways** rely on the transfer of protein phosphorylation on mainly S, T, or Y amino acid residues. The enzymes catalyzing the transfer of a phosphate to one of these residues are protein kinases. Fungal kinomes, the entirety of all protein kinases encoded in a genome, contain approximately 90 protein kinases (Park et al. 2011; De Souza et al. 2013a). In *N. crassa*, 77 viable deletion mutants of these kinase genes are available, out of which 33 exhibit defects in sexual development (Park et al. 2011). Strikingly, all kinases involved in mitogen-activated protein kinase (MAPK) cascades are necessary for sexual development in *N. crassa* (Park et al. 2011; Lichius et al. 2012). MAPK cascades are highly conserved signaling modules that transmit signals from the cell surface to nuclei and consist of three kinases. The MAPKKK phosphorylates the MAPKK, which phosphorylates the MAPK that activates downstream targets. Many of these targets are nuclear proteins and regulate transcription. In most filamentous ascomycetes, three different MAPK cascades are present (Fig. 8), which regulate **cell wall integrity (CWI)**, **pheromone signaling (PR)**, and **osmotic stress (HOG)** (Irniger et al. 2016). The corresponding subunits in several model ascomycetes are given in Table 1.

The **CWI pathway** in filamentous ascomycetes is homologous to the *S. cerevisiae* Bck1p, Mkk1/Mkk2, and Slt2 MAP kinase pathway (Borkovich et al. 2004). This kinase module is crucial for fruiting-body development, since the corresponding *N. crassa* and *S. macrospora* deletion strains are unable to generate mature fruiting bodies, exhibit defects in hyphal fusion, and are sensitive to cell wall stress (Maerz et al. 2008; Park et al. 2008; Teichert et al. 2014b). In *S. macrospora*, the kinases of the CWI pathway, MIK1, MEK1, and MAK1 are crucial for sexual development, hyphal growth, and vegetative cell fusion. Extensive mass spectrometry, yeast two-hybrid, and phosphorylation studies showed that the developmental protein PRO40, the homolog of *N. crassa* SOFT (SO), is the scaffold protein for the CWI pathway (Teichert et al. 2014b). Further CWI components were

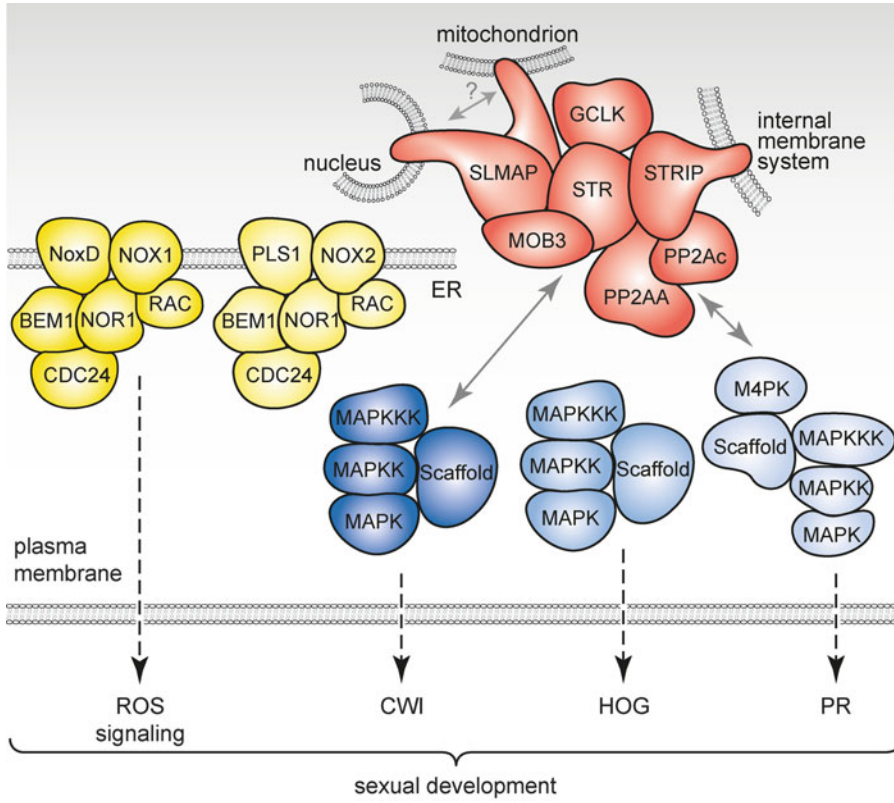


Fig. 8 Schematic overview on signal transduction pathways controlling fruiting-body development in filamentous ascomycetes. The NOX complex exists in two different compositions with either NoxD (synonymous to PRO41) and NOX1 or PLS1 and NOX2. NOX1 is the NADPH oxidase involved in sexual development. The three MAPK cascades are equally important for fruiting-body development with a high likelihood of interdependence. Presumably, the connection to other signaling pathways has also a fundamental impact on sexual development. The STRIPAK complex is a major complex of developmental proteins, which are connected to the CWI and the PR pathway (double arrows). This interconnection might be the key link for controlled development

Table 1 Overview of MAPK cascade components in *S. cerevisiae* (*Sc*), *N. crassa (Nc)*, *S. macrospora (Sm)*, *P. anserina (Pa)*, *A. nidulans (An)*, *and B. cinerea (Bc)*

		Sc	Nc	Sm	Pa	An	Bc
PR	MAPKKK	Ste11	NRC-1	MIK2	PaTLK2	SteC	BcSte11
	MAPKK	Ste7	MEK-2	MEK2	PaMKK2	Ste7	BcSte7
	MAPK	Fus3	MAK-2	MAK2	PaMPK2	MpkB	BcBmp1
	Scaffold	Ste5	HAM-5	HAM5	IDC1	hypoth.	Ste50
CWI	MAPKKK	Bck1	MIK-1	MIK1	PaASK1	BckA	BcBCK1
	MAPKK	Mkk1/2	MEK-1	MEK1	PaMKK1	MkkA	BcMKK1
	MAPK	Slt2	MAK-1	MAK1	PaMPK1	MpkA	Bmp3
	Scaffold	–	SOFT	PRO40	PaSO	hypoth.	hypoth.
HOG	MAPKKK	Ste11	OS-4	OS4	hypoth.	SskB	Bos4
	MAPKK	Pbs2	OS-5	OS5	hypoth.	PbsB	Bos5
	MAPK	Hog1	OS-2	OS2	hypoth.	HogA/MpkC	BcSak1
	Scaffold	Pbs2	–	–	–	–	–

CWI cell wall integrity pathway, *HOG* high-osmolarity glycerol pathway, *PR* pheromone response pathway

identified in *S. macrospora*, namely, the essential upstream activator protein kinase C (PKC1) and the small GTPase RHO1, which has been functionally described in *N. crassa* (Richthammer et al. 2012; Teichert et al. 2014b). The deletion strains of the CWI cascade components are female sterile and are involved in germling fusion in *N. crassa*. This process is highly regulated and requires communication between the tips of two germlings (Fleissner et al. 2009). Especially, the scaffold protein SO is required for communication between germling tips, and SO and the MAPK of the PR pathway, MAK-2, oscillate to opposing germling tips during fusion (Fleissner et al. 2009).

Accumulating evidence points toward a genetic and direct interaction of the PR and the CWI pathway during the formation of fruiting bodies and the regulation of cell wall integrity (Maerz et al. 2008; Dettmann et al. 2013; Kamei et al. 2016). The **PR pathway** in filamentous ascomycetes is homologous to the *S. cerevisiae* PR pathway Ste11-Ste7-Fus3, which has been the paradigm for understanding signaling upon pheromone response in ascomycetes. In *N. crassa*, the PR kinases NRC-1, MEK-2, and MAK-2 and their scaffold protein HAM-5 are important for early colony development, all types of cell fusion, female fertility, and cell-cell communication (Dettmann et al. 2014; Jonkers et al. 2014, 2016). The *A. nidulans* PR pathway is also a major regulator of sexual development, while it is also crucial for proper formation and germination of conidia (Kang et al. 2013; Irniger et al. 2016).

A conidiation defect is present in some deletion strains of another developmental protein complex, the multi-subunit velvet complex, which is a major regulator of secondary metabolism (Bayram and Braus 2012). One subunit, the velvet protein VeA discussed later, is phosphorylated by the PR MAPK FUS3, which is mandatory for the proper ratios of different proteins in the velvet complex (Bayram et al. 2012). Thus, the PR pathway has an indirect impact on secondary metabolism by influencing the phosphorylation status of the velvet component VeA. Further, the PR pathway most likely includes the upstream G proteins and the aforementioned pheromone receptors (Li et al. 2007). Even though the actual inducing signal of the PR pathway, such as pheromones, remains to be determined, it seems likely that GPCRs are involved in sensing in filamentous fungi, as described in the pheromone section above (Sect. III.B.2). GPCRs transmit signals via heterotrimeric G proteins that consists of Gα and Gβγ. Fungal genomes mostly encode for three Gα subunits, and single Gβ and Gγ subunits (Mayrhofer and Pöggeler 2005; Li et al. 2007; Kamerewerd et al. 2008). Mostly, the Gα subunit determines the target pathway, and in *N. crassa* and *S. macrospora*, the Gα subunits GNA-1 and GSA-1 are major regulators of sexual development (Ivey et al. 1996; Kamerewerd et al. 2008).

There are strong links that both other subunits, Gβ and Gγ, contribute to fruiting-body development, but their many functions are also related to other developmental processes, such as asexual development and carbon sensing (Deka et al. 2016). Like the *N. crassa gna-1* deletion strain, the deletion strains of Gβ and Gγ are female sterile but male fertile (Krystofova and Borkovich 2005). Strikingly, none of the *A. nidulans* Gα subunits has been linked to sexual development; however, deletion strains of Gβ and Gγ are sterile (Rosen et al. 1999; Seo et al. 2005). Besides the aforementioned downstream signaling through MAPK cascades, stimulated G proteins activate cyclic AMP (cAMP)-dependent signaling by inducing soluble adenylyl cyclases. These enzymes produce cAMP from ATP as a second messenger, which is involved in several important developmental processes ranging from nutrient sensing, stress response, metabolism, and pathogenicity to sexual development (Lengeler et al. 2000; D'Souza and Heitman 2001). A possible outcome of second messenger signaling via cAMP is also a stimulation of MAPK cascade signaling.

The third MAPK pathway in filamentous ascomycetes is homologous to the yeast **HOG MAPK pathway**, which is required for the adaptation to stress (Hohmann 2009). A plethora of stressors can activate this pathway ranging from heat stress to high osmolarity and oxidative stress. In *N. crassa*, the HOG kinases OS-2,OS-4, and OS-5 influence the development of protoperithecia and the generation of fruiting

bodies (Park et al. 2011; Lichius et al. 2012). In contrast to other filamentous ascomycetes, the aspergilli genomes encode two HOG MAPKs, SakA and MpkC (May et al. 2005). Mutants lacking SakA show premature cleistothecia formation, and SakA is involved in light signaling (see Sect. III.A.1) and represses expression of *noxA*, encoding a NOX pathway component (see Sect. IV.A.3) (Kawasaki et al. 2002; Lara-Ortiz et al. 2003).

2. STRIPAK

The **striatin-interacting phosphatase and kinase (STRIPAK) complex** is conserved from yeast to human (Kück et al. 2016), while only a few subunits were detected in plants so far (Rahikainen et al. 2016). The mammalian STRIPAK encompasses at least the protein phosphatase 2A (PP2A) scaffolding and catalytic subunits, striatins, striatin-interacting proteins STRIP1 and STRIP2, the monopolar spindle one-binder (Mob) protein Mob3, the cerebral cavernous malformation 3 protein (CCM3), and associated germinal center kinases (Hwang and Pallas 2014). In yeasts, the STRIPAK-like *S. cerevisiae* FAR (factor arrest) complex regulates pheromone-induced cell cycle arrest and antagonizes TORC2 signaling (Kemp and Sprague 2003; Pracheil and Liu 2013), while the *S. pombe* SIP (septation initiation network (SIN) inhibitory protein complex) controls coordination of mitosis and septation (Singh et al. 2011). The first hint of a highly conserved signaling complex in filamentous fungi, as depicted in Fig. 8, came from the discovery that the sterile phenotype of the *S. macrospora* mutant pro11 was restored to wild type by the mouse striatin cDNA (Pöggeler and Kück 2004). Concomitant studies identified several developmental proteins that are homologous to human STRIPAK complex subunits, namely, the STRIP1/2 homolog PRO22, the MOB3 homolog SmMOB3, PRO45, PP2AA, PP2Ac1, as well as the kinases SmKin24 and SmKin3 (Kück et al. 2016). The characterization of the mutant pro22 led to the initial discovery of the STRIPAK in ascomycetes (Bloemendal et al. 2010, 2012). The fungal STRIPAK complex is a regulator of fruiting-body development, vegetative growth, hyphal fusion, and asexual development (Kück et al. 2016). In *S. macrospora*, the STRIPAK complex is a key factor of sexual development, and all available deletion mutants show similar defects, producing only nonpigmented protoperithecia (Bernhards and Pöggeler 2011; Bloemendal et al. 2012; Nordzieke et al. 2015). Further, the strains are impaired in hyphal growth and fusion in vegetative mycelium. Strikingly, PRO22 and PP2Ac1 appear to regulate septation of the ascogonial coil, which might interfere with proper coordination of meiosis (Bloemendal et al. 2010; Beier et al. 2016). In *N. crassa*, most deletion mutants lacking genes for STRIPAK are female sterile and exhibit a defect in germling fusion (Fu et al. 2011; Dettmann et al. 2013). In both, *S. macrospora* and *N. crassa*, most mutants lacking MAPK subunits of CWI and PR resemble the STRIPAK deletion mutant phenotype. This similarity and data from protein-protein interaction studies indicate a functional relationship between MAPK cascade and STRIPAK signaling during the formation of fruiting bodies (Dettmann et al. 2013; Kück et al. 2016). Even the nuclear localization of MAK-1 is partially affected by STRIPAK (Dettmann et al. 2013). Many STRIPAK subunits are localized to the ER and the nuclear envelope, while some evidence indicates additional localizations at mitochondria and tubular vacuoles in *S. macrospora* (Bloemendal et al. 2012; Nordzieke et al. 2015). Interestingly, data from *S. cerevisiae* indicate that the PRO22 homolog Far11 is a target of MAPKs, while PP2A might negatively regulate the CWI pathway (Junttila et al. 2007; Lisa-Santamaría et al. 2012; Sacristán-Reviriego et al. 2015). Homologs of STRIPAK subunits are present in all ascomycetes and have well-conserved functions. For example, striatin homologs in *A. nidulans*, *F. graminearum*, and *F. verticillioides* are key regulators of polar growth, sexual development, and conidiation (Shim et al. 2006; Wang et al. 2010a). Besides sexual development, the STRIPAK complex appears to influence pathogenic and symbiotic interactions with plants in diverse fungi (Shim et al. 2006; Green et al. 2016).

3. NOX Complexes

The production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, was long believed to be an inevitable and damaging byproduct of several metabolic processes (Halliwell and Gutteridge 2015). Accumulating data from plant, animal, and fungal species revealed the importance of the controlled production of **ROS by NADPH oxidases (NOX)** for multiple signaling pathways (Kaur et al. 2014; Marschall and Tudzynski 2016; Gao and Schöttker 2017). The mechanisms of ROS perception and signaling functions remain to be determined in all species.

In filamentous ascomycetes, the NOX enzymes regulate several developmental processes like fruiting-body formation, hyphal fusion, and ascospore germination. In fungi, three enzymes of the NOX family have been described. NOX1 and NOX2 (also referred to as NOXA and NOXB) are present in most ascomycetes, while NOX3 has been identified only in *M. grisea*, *P. anserina*, and several *Fusarium* species (Aguirre et al. 2005; Scott and Eaton 2008; Brun et al. 2009; Dirschnabel et al. 2014). As depicted in Fig. 8, two fungal NOX complexes exist, containing the common regulator NOR1 (NOX regulating), associated with the small GTPase RAC1, CDC24, and BEM1. This complex can be either associated with NOX1 and NoxD (PRO41) or with NOX2 and PLS1 (Marschall and Tudzynski 2016).

Especially NOX1 and NOR1 are indispensable for the formation of proper fruiting bodies in filamentous ascomycetes. The *A. nidulans noxA* deletion strain is unable to form mature cleistothecia, the *S. macrospora nox1 and nor1* deletion strains form only protoperithecia, and *P. anserina* and *N. crassa nox1* deletion strains are female sterile (Lara-Ortiz et al. 2003; Malagnac et al. 2004; Cano-Domínguez et al. 2008; Dirschnabel et al. 2014). Strikingly, sterility of *P. anserina* and *S. macrospora nox1* mutants can be surpassed by serial passaging to nutrient-rich medium (Malagnac et al. 2004; Dirschnabel et al. 2014). This result indicates a link between sterility and availability of nutritional factors or ROS scavenging and signaling molecules (Malagnac et al. 2004; Dirschnabel et al. 2014). Cytochemical analysis in *A. nidulans* revealed that NoxA generates ROS in young primordial, peridial, and Hülle cells. Presumably, these ROS function as a second messenger in the regulation of developing ascogenous and peridial tissues (Lara-Ortiz et al. 2003). Besides regulating fruiting-body formation, NOX1 and NOR1 are major regulators of conidial anastomosis tube (CAT) fusion and hyphal fusion in vegetative mycelium in *N. crassa*, *B. cinerea*, *E. festucae*, and *S. macrospora* (Read et al. 2012; Roca et al. 2012; Kayano et al. 2013; Dirschnabel et al. 2014). Often, sexual differentiation and hyphal fusion seem to be co-regulated, although this is not always the case (Dirschnabel et al. 2014; Lichius and Lord 2014). Additionally, transcriptional data from *S. macrospora* and phenotypic analysis from *N. crassa* indicate a functional relationship between F-actin organization and regulation through NOX1 (Roca et al. 2012; Dirschnabel et al. 2014).

B. Protein Degradation

Development of the multicellular fruiting bodies relies on ongoing changes and remodeling of the proteome. Disused proteins have to be removed when specialized cells are formed. As in all other eukaryotes, two major degradation systems handle protein degradation in fungi, autophagy and the ubiquitin-proteasome system (UPS).

Autophagy ("self-eating") delivers cytoplasm and whole organelles to the vacuole for their degradation. In addition, it protects the cell from harm by dangerous protein aggregates or dysfunctional and superfluous organelles (Yin et al. 2016). The UPS is primarily responsible for proteolytic degradation of short-lived, misfolded, and damaged proteins which are marked for degradation via the 26S proteasome by ubiquitination (Ciechanover 1994; Doherty et al. 2002). Protein degradation by the UPS is highly specific and precisely regulated by E3 ubiquitin ligases (Buetow and Huang 2016).

1. Autophagy

Autophagy is conserved in all eukaryotes and essential for the delivery of cytosolic cargoes to the vacuole for their degradation (Feng et al. 2014). The recycled building blocks can be reused to survive starvation or stress conditions and to drive cellular remodeling during development. Proteins involved in autophagy are termed autophagy-related (ATG) proteins (Klionsky et al. 2003). At least 41 ATG proteins have been identified by genetic experiments in yeasts, and most of them are conserved in filamentous ascomycetes (Araki et al. 2013; Reggiori and Klionsky 2013; Voigt and Pöggeler 2013b; Nazarko et al. 2014; Mochida et al. 2015; Yao et al. 2015). About half of the proteins (core ATG proteins) are required for all autophagy-related pathways, while others are only involved in selective types of autophagy.

There are three distinct types of **autophagy: macroautophagy**, **microautophagy**, and **chaperone-mediated autophagy (CMA)**. Macro- and microautophagy take place in all eukaryotes, while CMA is mammalian specific and does not rely on *atg* genes (Okamoto 2014). Microautophagy describes the direct engulfment by the vacuolar membrane (Li et al. 2012). However, this type has not yet been described in filamentous ascomycetes (Voigt and Pöggeler 2013b).

Macroautophagy (hereafter autophagy) is the best-characterized and most prominent type and can be either selective or nonselective. It involves sequestering of cytosolic cargoes by a double-membrane phagophore that by expansion and closure becomes a double-layered autophagosome. By fusion of the outer membrane of the autophagosomes with the vacuole, the inner vesicle, termed autophagic body, is released into the lumen of the vacuole. Vacuolar hydrolases degrade the membrane of the vesicle and the cargo. The resulting macromolecules are delivered into the cytoplasm via permeases (Fig. 9).

The phagophore assembly site (PAS) is the initiation site for the forming of autophagosomes. The majority of ATG proteins are recruited in a hierarchical manner to the PAS (Suzuki et al. 2007). The ATG1 kinase complex consisting of the serine/threonine kinase ATG1, its regulatory subunit ATG13, and other associated proteins is the autophagy initiation complex. Upon starvation this complex is required for recruitment of further ATG proteins and membrane sources such as ATG9 vesicles (Stanley et al. 2014). The origin of the autophagosomal membranes is still discussed. Membranes originating from different sources seem to be involved (Ge et al. 2014). The phosphatidylinositol (PtdIns) 3-kinase complex produces phosphatidylinositol 3-phosphate (PI3P) at the PAS, which is necessary to recruit proteins that function in autophagosomes formation (Reggiori and Klionsky 2013). Expansion of the phagophore requires the ubiquitin-like (UBL) proteins ATG8 and ATG12, which are conjugated by a UBL machinery. ATG8 conjugated to the lipid phosphatidylethanolamine (PE) is a major component of autophagosomal membranes. ATG8-PE covers both sides of the phagophore. During later stages, the protease ATG4 acts as deconjugating enzyme to release ATG8 from PE on the outer membrane for reuse. ATG8-PE conjugates from the inner membrane are not cleaved off and are therefore degraded by hydrolytic enzymes in the vacuole together with the autophagic body and the cargos (Reggiori and Klionsky 2013). Permeases release the degradation products back into the cytoplasm for reuse (Yin et al. 2016) (Fig. 9).

Autophagy can be further divided into nonselective and selective processes. The latter are specific for the degradation of superfluous or damaged organelles like peroxisomes (pexophagy), mitochondria (mitophagy), nuclei (nucleophagy), endoplasmatic reticulum (reticulophagy), and ribosomes (ribophagy) (Anding and Baehrecke 2017).

In *S. cerevisiae*, a specific type of selective autophagy is the cytoplasm to vacuole targeting (Cvt) pathway. This pathway utilizes the core machinery of autophagy and specific receptor proteins to deliver hydrolases such as peptidases and mannosidases the vacuole (Yamasaki and Noda 2017).

The detailed mechanisms of selective autophagy remain to be characterized; however, common principles are arising. A receptor able to interact with ATG8 at the convex site of

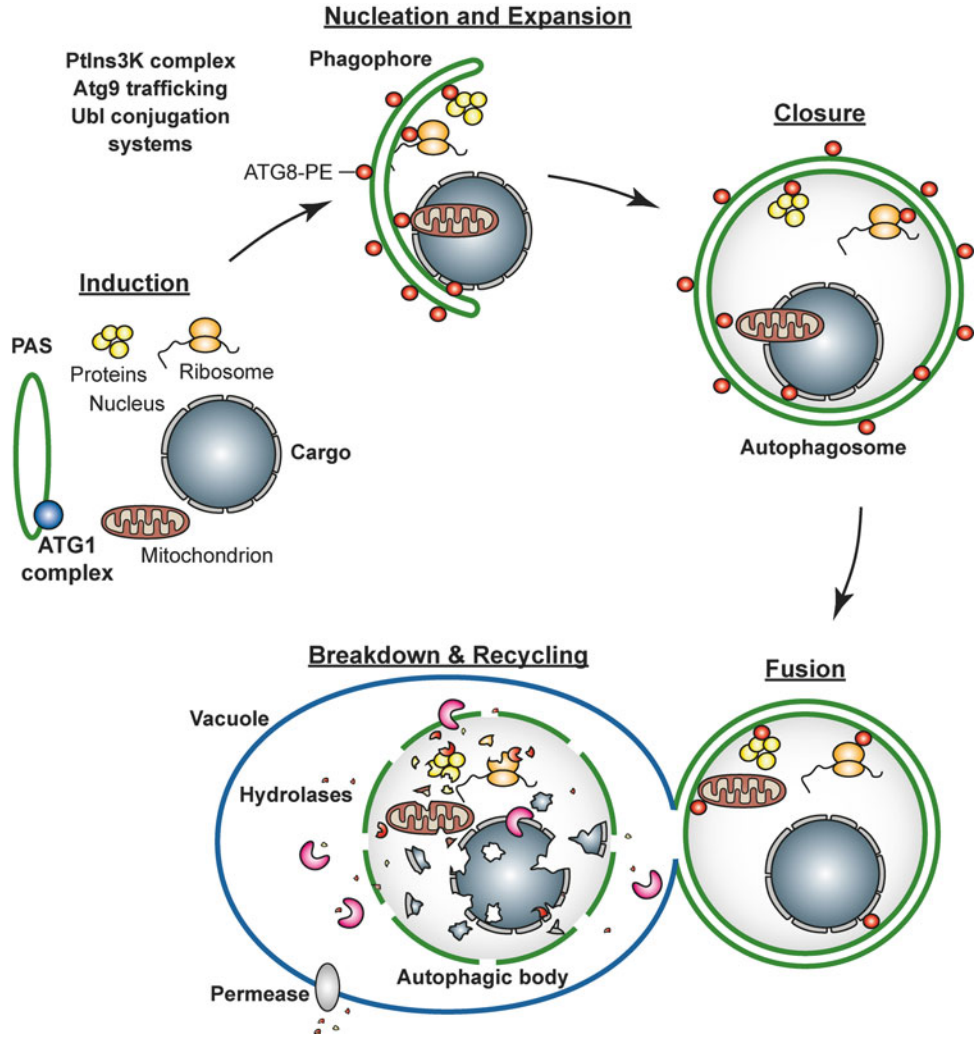


Fig. 9 Sequential steps of autophagy in *S. cerevisiae*. The initiation of autophagy requires the ATG1 complex at the PAS, which recruits other ATG proteins. Expansion and curvature of the phagophore relies on the PtdIns 3-kinase complex, the transmembrane protein ATG9, and two UBL conjugation systems. ATG8-PE participates in cargo recognition during selective autophagy and is important for expansion of the phagophore. After vesicle closure, the protease ATG4 deconjugates ATG8 from the outer membrane of the autophagosomes. The resulting vesicle fuses with the vacuolar membrane resulting in the release of an autophagic body surrounded by the inner autophagosomal membrane. The membrane of the autophagic body is lysed by the lipase ATG15, and the sequestered cargoes are degraded by hydrolases. Finally, the breakdown products are exported into the cytoplasm via permeases

the growing phagophore recognizes and recruits specific cargoes. During selective autophagy, core ATG proteins are required and often ubiquitin acts as a signaling molecule (Khaminets et al. 2016).

Molecular analyses of autophagy have been mostly performed in the unicellular budding yeast *S. cerevisiae* and in *Pichia pastoris* (Ohsumi 2014; Harnett et al. 2017). The main physiological role for autophagy in yeasts is to maximize survival under stress and starvation conditions, to generate nutrients for ascospore formation, and to deliver hydrolytic enzymes into the vacuole via the Cvt pathway (Reggiori

and Klionsky 2013; Yamasaki and Noda 2017). However, in filamentous ascomycetes, autophagy is involved in pathogenicity, production of secondary metabolites, and asexual and sexual development even under non-starvation conditions (Palmer et al. 2008; Pollack et al. 2009; Bartoszewska and Kiel 2010; Khan et al. 2012; Voigt and Pöggeler 2013b).

With regard to fruiting-body development, autophagy has been investigated in *Podospora anserina*, *S. macrospora*, and *N. crassa* as well as in the plant pathogens *Magnaporthe oryzae* and *Fusarium graminearum*.

In *P. anserina*, *atg* genes (*idi* genes) have been initially identified as genes induced during heterokaryon incompatibility, a cell death reaction after fusion of cells of dissimilar genotype (Pinan-Lucarré et al. 2003). Among the genes upregulated during heterokaryon incompatibility were the vacuolar protease gene *idi-6*/*pspA* and *idi-7*/*atg8* encoding the UBL autophagosomal membrane protein ATG8. Deletion of both genes causes differentiation defects such as a lower density of aerial hyphae, decreased pigmentation of the mycelium, and the absence of female reproductive structures (Pinan-Lucarré et al. 2003). Similarly, deletion of the serine threonine kinase gene *atg1*, the UBL genes *atg8* and *atg12*, and the protease gene *atg4* resulted in sterility in the homothallic *F. graminearum* and *S. macrospora*, in the heterothallic *N. crassa*, and in the plant pathogen *M. oryzae*. In addition to defects in sexual development, autophagy mutants of all fungi displayed a reduced vegetative growth rate (Pinan-Lucarré et al. 2005; Liu et al. 2010; Park et al. 2011; Josefsen et al. 2012; Voigt and Pöggeler 2013a; Chinnici et al. 2014; Werner et al. 2016). In *S. macrospora*, cross-species microarray experiments revealed that the bZIP transcription factor gene *Smjlb1* was downregulated in the sterile fruiting-body mutants pro1, pro11, and pro22, as well as in the mating-type mutant ΔSmta-1 (Nowrousian et al. 2005; Pöggeler et al. 2006b) (see Sects. IV.A.2 and IV.C.1). In *P. anserina*, expression of the *Smjlb1* ortholog *idi-4* is assumed to be involved in the regulation of *atg* genes (Dementhon et al. 2004; Dementhon and Saupe 2005). Generation of the homokaryotic *S. macrospora* deletion mutant demonstrated that *Smjlb1* is required for fruiting-body development and proper vegetative growth. Quantitative real-time PCR experiments suggest that SmJLB1 acts as a repressor on gene expression of *atg8* and *atg4* (Voigt et al. 2013).

Careful microscopic examination of *N. crassa* and *S. macrospora* autophagy mutants revealed that they initiate female development and are able to produce ascogonia and small protoperithecia, indicating that they are unable to fully support perithecia development (Voigt and Pöggeler 2013a; Chinnici et al. 2014; Werner et al. 2016). Protoperithecia grafting experiments in *N. crassa* have demonstrated that autophagy is required within the vegetative hyphal network, as fertilized autophagy mutant perithecia are able to complete perithecia development and to produce ascospores when grafted onto a wild-type host (Chinnici et al. 2014). In filamentous ascomycetes, autophagy seems to be an essential and constitutively active process to sustain high energy levels for filamentous growth and multicellular development. Autophagy mutants of filamentous fungi seem to be affected in fruiting-body development because the underlying vegetative mycelium is unable to provide an adequate supply of nutrients to the developing fruiting body.

In contrast to *N. crassa*, where it has been shown that a homokaryotic knockout mutant of *atg7*, encoding the common E1-like enzyme of the ATG2 and ATG8 UBL conjugation systems, is female sterile, the ortholog of *S. macrospora*, *Smatg7*, was shown to be required for viability (Nolting et al. 2009; Chinnici et al. 2014). Interestingly, a heterokaryotic ΔSmatg7/*Smatg7 S. macrospora* strain and transformants generated by RNA interference showed considerable morphological phenotypes during fruiting-body development and an increased number of double-neck perithecia. In addition, these mutants displayed a significantly reduced vegetative growth rate and ascospore germination efficiency (Nolting et al. 2009). These results indicate that core autophagic genes might have species-specific relevance for vegetative growth, sexual development, and viability.

2. Proteasomal Degradation

Besides protein degradation via autophagy, the **UPS protein degradation pathway** is essential for the degradation of a broad array of intracellular proteins (Sontag et al. 2014; Cohen-Kaplan et al. 2016). Among the proteins tagged for degradation by ubiquitin are damaged, misfolded, and regulatory proteins such as transcription factors or time-limited cell cycle proteins (Geng et al. 2012; Genschik et al. 2014). Therefore, the UPS plays important roles during developmental processes, and misregulation of the UPS degradation pathway is associated with defects in development (Chung and Dellaire 2015). Two reversible posttranslation modifications are important for the UPS pathway: ubiquitination and neddylation. Ubiquitin is conserved in all eukaryotes and consists of 76 amino acids. It is attached to target proteins in an enzymatic cascade, which involves three successive enzymatic steps by E1, E2, and E3 enzymes (Fig. 10). Successive rounds of E1-E2-E3 cascades assemble polyubiquitin chains at the substrate protein (Glickman and Ciechanover 2002). Proteins covalently linked to at last four ubiquitin molecules are recognized and degraded by the 26S proteasome, and ubiquitin is recycled from the target protein (Bhattacharyya et al. 2014).

E1 and E2 enzymes are characterized by a conserved domain containing a cysteine residue as an acceptor for ubiquitin, whereas two types of E3 ligases are present in eukaryotes. They are defined by a HECT domain (homologous to E6-AP carboxyl terminus) or a RING (really interesting new gene) motif. RING E3 ligases are the largest class of E3 ligases. They facilitate the direct transfer of ubiquitin from an E2-ubiquitin conjugate to a substrate protein (Vittal et al. 2015). The best-characterized group of RING E3 ligases is the cullin-based RING ligases (CRLs), which are multi-protein complexes with cullin as a central scaffold (Fig. 10b). Fungi possess the minimal eukaryotic set of cullins which are the three cullins CUL1, CUL3, and CUL4 (Braus et al. 2010). The prototype of CRL is termed SCF (SKP1/CUL1/F-box) ligase. In SCFs, cullin binds at its C-terminal domain the RING-domain protein RBX1 (Ring box protein 1) that functions as an adaptor for the E2 enzyme and at its N-terminal domain the adaptor protein SKP1 (S-phase kinase-associated protein 1) and an F-box protein (Lee and Diehl 2014). The F-box proteins, carrying an F-box domain and variable protein-protein interaction domains, are the substrate-specifying factors that recruit the substrate to be ubiquitinated to SCF ligases.

Approximately 70 different proteins with F-box domains and variable protein interaction domains were identified in *A. nidulans* (Draht et al. 2007), whereas in plants around 700 F-box domain proteins are described (Gagne et al. 2002). Despite their central role in protein turnover, only few F-box proteins have been characterized in filamentous fungi (Jonkers and Rep 2009). The F-box gene *grrA* was identified among genes that are transcriptionally upregulated during fruiting-body formation in *A. nidulans*. *A. nidulans* Δ*grrA* mutants resemble the wild type in hyphal growth, asexual sporulation, Hülle cell formation, and development of asci-containing cleistothecia, but they are unable to produce mature ascospores due to a block in meiosis (Krappmann et al. 2006). Deletion of the F-Box gene *fbx15* in *A. nidulans* results in reduced asexual and sexual development, whereas a *fbx23* mutant forms cleistothecia during conditions favoring asexual development (von Zeska Kress et al. 2012). Recently, it was demonstrated that FBX15 is required for an appropriate oxidative stress response in *A. fumigatus* (Jöhnk et al. 2016).

The covalent attachment of another UBL protein, the NEDD8 protein, to a conserved lysine residue of cullin is essential for the function of CRLs, because neddylation stabilizes the CRL complex (Bornstein and Grossman 2015).

Among all UBLs, NEDD8 is the most identical to ubiquitin with 60% amino acid and high structural identity. It consists of 81 amino acids including a lysine at position K48R (Kumar et al. 1993; Jones et al. 2008). As in most eukaryotes, NEDD8 is essential in *A. nidulans* (Kerscher et al. 2006; Rabut and Peter 2008; von Zeska Kress et al. 2012).

Removal of NEDD8 from cullins results in the disassembly of the CRL complex and dissociation of the substrate recognition unit SKP1/F-Box. Since F-box proteins determine the specificity of CRLs, repeating

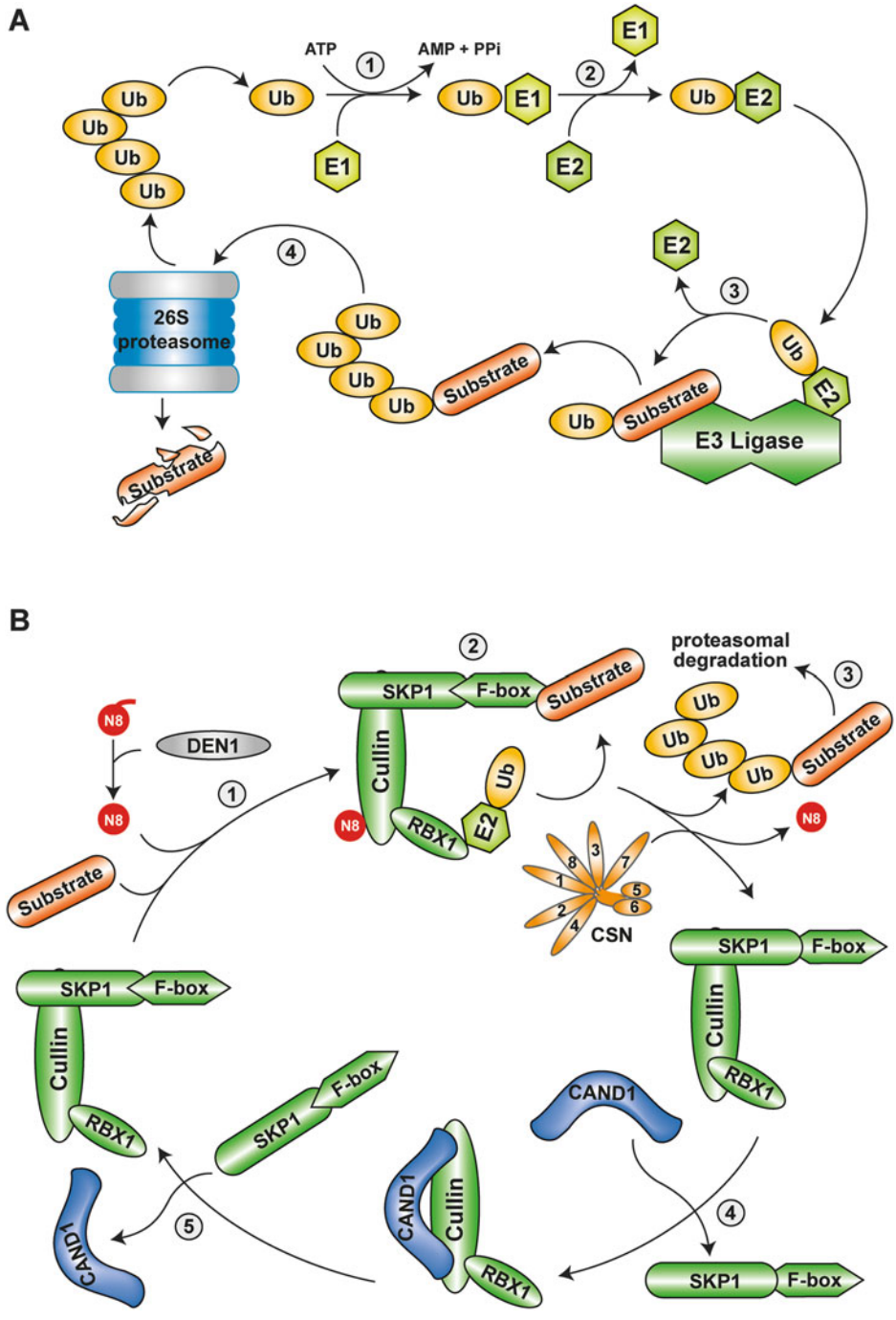


Fig. 10 The ubiquitin-proteasome system (UPS). (**a**) Ubiquitin-dependent proteasomal degradation of a substrate protein. (1) Free monomeric ubiquitin (Ub) is activated and bound to the E1 ubiquitin-activating enzyme in an ATP-dependent manner. (2) Ubiquitin is transferred to an internal cysteine of the ubiquitin-conjugating enzyme E2. (3) The substrate protein and the E2 with the activated ubiquitin are bound by the E3 ubiquitin ligase, which catalyzes the transfer of ubiquitin to a lysine residue of the substrate. (4) Repeated rounds of ubiquitination create a polyubiquitin chain that labels the substrate for degradation via the 26S proteasome, which degrades the substrate and recycles the ubiquitin. (**b**) Regulation of E3 cullin-RING ligase

cycles of neddylation and deneddylation allow binding of new F-box proteins.

The *deneddylase COP9* (constitutive photomorphogenesis 9) signalosome (CSN) hydrolyzes cullin-NEDD8 conjugates. The CSN is conserved from fungi to human, and the prototype harbors eight subunits (CSN1–CSN8). In filamentous ascomycetes, the CSN has been intensively studied in *A. nidulans* and *N. crassa*. As plants and animals, *A. nidulans* possess an eight-subunit CSN, whereas the CSN of *N. crassa* consists of seven subunits lacking CSN8 (Busch et al. 2003, 2007; He et al. 2005; Braus et al. 2010; Wang et al. 2010b). The CSN complex shares structural similarities with the lid of the 26S proteasome and with the eukaryotic translation initiation factor eIF3. Subunit CSN5 is a metalloprotease and acts as a deneddylase (Zhou et al. 2012; Beckmann et al. 2015; Meister et al. 2016). In higher eukaryotes, deletion of CSN subunits results in embryonic lethality. *A. nidulans* and *N. crassa* CSN mutants are viable but show defects in sexual fruiting-body development, a misregulated secondary metabolism, and defects in vegetative growth, in conidiation, as well as in circadian rhythm (Busch et al. 2003, 2007; He et al. 2005; Wang et al. 2010b; Gerke et al. 2012; Beckmann et al. 2015). Unexpectedly, a *csn3* deletion mutant of *N. crassa* had a wild-type-like phenotype (Wang et al. 2010b).

Combined transcriptome, proteome, and metabolome analysis of *A. nidulans* Δcsn5 mutants revealed that the CSN affects transcription of at least 15% of genes during development, including numerous oxidoreductases, and leads to changes in the fungal proteome indicating impaired redox regulation and hypersensitivity to oxidative stress. More than 100 metabolites, including orsellinic acid derivatives, accumulate in the Δcsn5 mutant. These results suggest different roles for CSN during development. During early development, the CSN is required for protection against oxidative stress and later essential for control of the secondary metabolism and cell wall rearrangement (Nahlik et al. 2010). A comprehensive genome-wide analysis of gene expression throughout the first 144 h of perithecial development of *N. crassa* revealed that expression of *csn* genes is low at early stages during fruiting-body development and increases dramatically after 48 h (Wang et al. 2014b). In contrast to CSN, which is necessary for sexual development, the second deneddylase DEN1 is required for asexual development in *A. nidulans*. DEN1 is able to interact with CSN, which targets DEN1 for protein degradation. An increased amount of DEN1 partially compensates the lack of a functional CSN. Thus, a deneddylase disequilibrium impairs multicellular development, which suggests that control of deneddylase activity is important for multicellular development (Christmann et al. 2013; Schinke et al. 2016).

Binding of the exchange factor CAND1 (cullin-asociated-NEDD8-disocciated 1) locks the unneddylated CRL in an inactive state. The N-terminal domain of CAND1 blocks the cullin neddylation site, whereas the C-terminal domain inhibits cullin adaptor interaction (Mergner and Schwechheimer 2014).

In most fungi CAND1 is encoded by a single gene. However, in aspergilli the *cand1* homolog is divided into two separate genes, into *candA-N* encoding the smaller Cand1 N-terminus and *candA-C* for the larger C-terminal part. Either deletion results in an identical

Fig. 10 (continued) (CRL) by NEDD8 (N8), the deneddylase DEN1, and the COP9 signalosome (CSN) as well as by the cullin-associated NEDD8 dissociated protein CAND1. (1) Cullin-RING ligases (CRLs) bind the substrate protein via their substrate recognition unit consisting of the adaptor protein SKP1 and a specific F-box protein. The deneddylase DEN1 processes the NEDD8 precursor (N8), which then binds the CRL E3 ligase resulting in its activation. (2) The NEDD8-activated CRL catalyzes ubiquitination of the bound substrate. (3) The ubiquitinated substrate is recognized and degraded by the 26 proteasome (see A). The CSN removes NEDD8 from the CRL E3 ligase, which then is unable to bind E2-Ub but has a high affinity for CAND1. (4) Binding of CAND1 blocks the neddylation site and leads to disassembly of SKP1 and the F-box protein (5). Recruitment of a new substrate recognition unit (SKP1 and a specific F-box) results in CAND1 release. The newly assembled CRL is again activated by NEDD8 and binds new substrates [according to Lydeard et al. (2013) and Pierce et al. (2013)]

developmental and secondary metabolism phenotype in *A. nidulans*, which resembles *csn* mutants deficient in the CSN deneddylase (Helmstaedt et al. 2011), whereas deletion of *cand1* in *N. crassa* had little effect on conidial development and the circadian clock (Zhou et al. 2012).

In summary, proper targeting of regulatory proteins to the 26S proteasome is required for fruiting-body development in filamentous ascomycetes.

C. Transcriptional Regulation

Fruiting-body development in ascomycetes requires differentiation of many cell types that are specific to this process (Bistis et al. 2003; Han 2009; Lord and Read 2011; Dyer and O'Gorman 2012) and thus leads to drastic morphological changes compared to vegetative mycelium. These changes are reflected in vastly different transcriptomes of vegetative cells versus developmental stages (see Sect. IV.C.2), and these transcriptional changes are thought to organize the accompanying morphological changes. Transcription is regulated through several mechanisms including specific transcription factors as well as chromatin modifiers. A number of genes encoding such factors have already been identified as essential for fruiting-body development in different filamentous ascomycetes. The following sections give an overview of transcription factors, transcriptional changes, and the emerging role of chromatin modifiers in fruiting-body development.

1. Transcription Factors

The genomes of filamentous ascomycetes encode on average 450 transcription factor genes per genome (Todd et al. 2014). High-throughput screens of deletion mutants in *N. crassa* and *F. graminearum* showed that about 15–19% of transcription factor mutants had a defect in sexual development (Colot et al. 2006; Son et al. 2011b; Carrillo et al. 2017). Many **transcription factors** have already been characterized in detail with respect to their role in fruiting-body formation (Table 2). This section gives an overview of the varying processes that are controlled by transcription factors during sexual morphogenesis.

Among the first transcription factors that were identified as essential for fruiting-body formation in filamentous ascomycetes are several genes encoded by the mating-type (MAT) loci (Glass et al. 1990; Staben and Yanofsky 1990) (Table 2; see also Sect. I). Even though the gene content of the MAT loci varies, most species contain at least two *MAT* genes, *MAT1-1-1* and *MAT1-2-1*, either in one individual in case of homothallic species or in different individuals in case of heterothallic species. *MAT1-1-1* encodes an α domain transcription factor and *MAT1-2-1* a transcription factor with an HMG domain (Turgeon and Yoder 2000; Bennett and Turgeon 2016). Both genes were shown to be essential for fruiting-body formation in a number of species covering the phylogenetic range of filamentous ascomycetes (Table 2). The α domain was recently shown to bear structural similarity to the HMG domain, thus strengthening the hypothesis that ascomycete *MAT* genes are derived from an ancient MAT locus containing (a) HMG domain gene(s) (Idnurm et al. 2008; Martin et al. 2010). The involvement of HMG domain proteins in sexual development may be a conserved feature in fungi and metazoa, as the MAT1-2-1 (MatA) HMG box protein of *A. nidulans* can be functionally substituted by the human SRY (sex-determining region Y) protein (Czaja et al. 2014).

However, some variability exists with respect to *MAT* gene function. For example, it was shown in the homothallic *S. macrospora* that while *MAT1-2-1* is required for sexual development, *MAT1-1-1* is not (Pöggeler et al. 2006b; Klix et al. 2010). Surprisingly, it turned out that the *MAT1-1-2* gene, which does not encode any known DNA-binding domain, is essential for fruiting-body formation in this species. This result is in contrast to its close relative *N. crassa*, where *MAT1-1-1*, but not *MAT1-1-2*, is required for sexual development (Ferreira et al. 1998). Another example of a lineage-specific gene that does not encode a transcription factor but is present in a MAT locus and essential for sexual development is *MAT1-1-5* in *S. sclerotiorum* (Doughan and Rollins 2016). Thus, gene content and function at MAT loci varies to some extent in filamentous ascomycetes, but so far, at least two *MAT* genes per species,

Table 2 Transcription factor genes involved in fruiting-body development

Gene name(s)	Species	Class	References
Transcription factors encoded by mating-type genes			
MAT-1-1-1	*N. crassa*, *P. anserina*, *C. heterostrophus*, *A. nidulans*, *A. fumigatus*, *F. graminearum*, *D. zeae-maydis*, *S. sclerotiorum*	α domain	Glass et al. (1990), Debuchy and Coppin (1992), Turgeon et al. (1993), Saupe et al. (1996), Wirsel et al. (1998), Paoletti et al. (2007), Szewczyk and Krappmann (2010), Yun et al. (2013), Zheng et al. (2013), Doughan and Rollins (2016)
MAT-1-1-3	*F. graminearum*, *P. anserina*	HMG	Debuchy et al. (1993), Zheng et al. (2013)
MAT-1-2-1	*N. crassa*, *P. anserina*, *C. heterostrophus*, *S. macrospora*, *A. nidulans*, *A. fumigatus*, *F. graminearum*, *D. zeae-maydis*, *S. sclerotiorum*	HMG	Staben and Yanofsky (1990), Debuchy and Coppin (1992), Turgeon et al. (1993), Wirsel et al. (1998), Pöggeler et al. (2006b), Paoletti et al. (2007), Szewczyk and Krappmann (2010), Czaja et al. (2011), Yun et al. (2013), Zheng et al. (2013), Doughan and Rollins (2016)
Other transcription factors			
stuA, *asm-1*	*A. nidulans*, *N. crassa*, *F. graminearum*, *A. benhamiae*	APSES domain	Miller et al. (1992), Aramayo et al. (1996), Wu and Miller (1997), Lysoe et al. (2011), Kröber et al. (2017)
devR	*A. nidulans*	bHLH	Tüncher et al. (2004)
urdA	*A. nidulans*	bHLH	Oiartzabal-Arano et al. (2015)
atfA	*A. nidulans*	bZIP	Lara-Rojas et al. (2011)
cpcA	*A. nidulans*	bZIP	Hoffmann et al. (2000)
napA	*A. nidulans*	bZIP	Yin et al. (2013)
rsmA	*A. nidulans*	bZIP	Yin et al. (2013)
zipA	*A. nidulans*	bZIP	Yin et al. (2013)
zif1	*F. graminearum*, *M. oryzae*	bZIP	Wang et al. (2011)
asl-2, *ts*	*N. crassa*	bZIP	Colot et al. (2006), McCluskey et al. (2011)
Smjlb1	*S. macrospora*	bZIP	Voigt et al. (2013)
flbC	*A. nidulans*	C_2H_2	Kwon et al. (2010)
mtfA	*A. nidulans*	C_2H_2	Ramamoorthy et al. (2013)
nsdC	*A. nidulans*	C_2H_2	Kim et al. (2009)
sltA	*A. nidulans*	C_2H_2	Ramamoorthy et al. (2013)
pcs1	*F. graminearum*	C_2H_2	Jung et al. (2014)
VpCRZ1	*V. pyri*	C_2H_2	He et al. (2016)
fhpA	*A. nidulans*	FKH box	Lee et al. (2005)
FoxE2	*S. sclerotiorum*	FKH box	Wang et al. (2016a)
SsFKH1	*S. sclerotiorum*	FKH box	Fan et al. (2016)

(continued)

Table 2 (continued)

Gene name(s)	Species	Class	References
nsdD, *sub-1*, *pro44*, *bcltf1*	*A. nidulans*, *N. crassa*, *A. fumigatus*, *S. macrospora*, *B. cinerea*	GATA	Han et al. (2001), Colot et al. (2006), Szewczyk and Krappmann (2010), Nowrousian et al. (2012), Schumacher et al. (2014)
Cmwc-1	*C. militaris*	GATA	Yang et al. (2016)
asd4	*N. crassa*	GATA	Feng et al. (2000)
steA, *pp-1*, *ste12*, *cpst12*	*A. nidulans*, *N. crassa*, *S. macrospora*, *C. parasitica*, *A. benhamiae*	HD	Vallim et al. (2000), Li et al. (2005), Nolting and Pöggeler (2006b), Deng et al. (2007), Kröber et al. (2017)
pah2	*P. anserina*	HD	Coppin et al. (2012)
pah5	*P. anserina*	HD	Coppin et al. (2012)
fmf-1	*N. crassa*	HMG	Iyer et al. (2009)
PaHMG5, *FGSG_01366*	*P. anserina*, *F. graminearum*	HMG	Ait Benkhali et al. (2013), Kim et al. (2015)
PaHMG6	*P. anserina*	HMG	Ait Benkhali et al. (2013)
PaHMG8	*P. anserina*	HMG	Ait Benkhali et al. (2013)
PaHMG9	*P. anserina*	HMG	Ait Benkhali et al. (2013)
fsd-1	*N. crassa*	IgG-fold	Hutchinson and Glass (2010)
vib-1	*N. crassa*	IgG-fold	Hutchinson and Glass (2010)
Fvmads2, *Bcmads1*	*F. verticillioides*, *B. cinerea*	MADS box	Ortiz and Shim (2013), Zhang et al. (2016)
mcm1, *Fvmcm1*, *Fgmcm1*, *mcmA*	*S. macrospora*, *F. verticillioides*, *F. graminearum*, *A. nidulans*	MADS box	Nolting and Pöggeler (2006a), Ortiz and Shim (2013), Yang et al. (2015), Zhang et al. (2016)
flbD	*A. nidulans*	Myb domain	Arratia-Quijada et al. (2012)
myt1	*F. graminearum*	Myb domain	Lin et al. (2011)
myt2	*F. graminearum*	Myb domain	Lin et al. (2012)
myt3	*F. graminearum*	Myb domain	Kim et al. (2014)
veA, *vel1*	*A. nidulans*, *C. heterostrophus*, *T. reesei*	velvet domain	Kim et al. (2002b), Bayram et al. (2008b), Wu et al. (2012), Bazafkan et al. (2015))
velB, *vel2*	*A. nidulans*, *C. heterostrophus*	velvet domain	Bayram et al. (2008b), Wang et al. (2014a)
velC	*A. nidulans*	velvet domain	Park et al. (2014)
vosA, *vos1*	*A. nidulans*, *C. heterostrophus*	velvet domain	Ni and Yu (2007), Ahmed et al. (2013), Wang et al. (2014a)
rosA	*A. nidulans*	$Zn(II)_2Cys_6$	Vienken et al. (2005)
pro1, *adv-1*, *nosA*	*S. macrospora*, *N. crassa*, *A. nidulans*, *C. parasitica*	$Zn(II)_2Cys_6$	Masloff et al. (1999), Colot et al. (2006), Vienken and Fischer (2006), Sun et al. (2009)

Genes are sorted according to encoded transcription factor class and within class according to species name. This table does not contain transcription factor genes that were identified in large-scale screens of *N. crassa* (Colot et al. 2006; Chinnici et al. 2014; Carrillo et al. 2017) and *F. graminearum* (Son et al. 2011b; Kim et al. 2015) deletion strains
bHLH basic helix-loop-helix, *C_2H_2* C_2H_2 zinc finger, *FKH box* forkhead box, *HD* homeodomain, *HMG*high-mobility group

with at least one of them encoding one of the core transcription factors MAT1-1-1 or MAT1-2-1, were found to be essential for sexual development in all species that were investigated.

Apart from the mating-type genes, a number of other transcription factor genes were shown to be required for fruiting-body formation in different species (Table 2). Interestingly, $Zn(II)_2Cys_6$-encoding genes seem to be underrepresented among the characterized transcription factors compared to their prevalence in *Pezizomycotina* genomes, where they constitute the largest transcription factor class with more than one-quarter of the encoded transcription factors (Shelest 2008; Todd et al. 2014). Indeed, in an analysis of deletion mutants of 657 of the predicted 693 transcription factors of *F. graminearum*, $Zn(II)_2Cys_6$ transcription factors made up 45% of the deleted genes but only 17% of those genes with a phenotype in sexual development (Son et al. 2011b). A different result was obtained in a deletion analysis of 99 transcription factor genes of *N. crassa*, where $Zn(II)_2Cys_6$ transcription factors contributed 71% of the deleted genes and 66% of genes with a phenotype in sexual development (Colot et al. 2006). However, a recent study of 242 transcription factor deletion mutants of *N. crassa* analyzed 101 $Zn(II)_2Cys_6$ transcription factor genes, of which 7 showed a phenotype related to sexual development. These comprise only 15% of transcription factor genes with a sexual development-related phenotype, whereas the $Zn(II)_2Cys_6$ family makes up 42% of all predicted transcription factors in *N. crassa* (Carrillo et al. 2017). Thus, the available data suggest that $Zn(II)_2Cys_6$ proteins might be underrepresented among the transcription factors regulating development. The $Zn(II)_2Cys_6$ transcription factors are a fungal-specific class of proteins that expanded most strongly in the filamentous ascomycetes (Shelest 2008; Todd et al. 2014). It is possible that fruiting-body development evolved preferentially based on evolutionary older regulatory pathways or that the expansion of the $Zn(II)_2Cys_6$ class occurred after core developmental pathways evolved. However, more large-scale studies of species outside of the *Sordariomycetes* will be needed to address this question comprehensively.

Several transcription factors were shown to be involved in fruiting-body formation in only distantly related species, e.g., in *Sordariomycetes* and *Eurotiomycetes* (Table 2). Among these are homologs of the *A. nidulans* transcription factor StuA, which was first studied with respect to its role in asexual development but in addition is required for cleistothecia formation (Miller et al. 1992). A mutant of the *N. crassa* homolog ASM-1 has a similar phenotype, as the mutant does not form protoperithecia, and conidia are formed on stunted conidiophores (Aramayo et al. 1996). The *N. crassa asm-1* gene can complement the conidiation defects, but not the sexual development phenotype of the *A. nidulans* stuA mutant (Chung et al. 2015). However, it was shown previously that different levels of *stuA* transcript are required for correct progression of different developmental pathways in *A. nidulans* (Wu and Miller 1997); therefore failure of *asm-1* to complement the lack of fruiting-body formation in the stuA mutant might be related to non-wild-type-like expression. StuA homologs were also analyzed in two additional species from the *Sordariomycetes* and *Eurotiomycetes*, respectively, namely, *F. graminearum* and *Arthroderma benhamiae* (Lysoe et al. 2011; Kröber et al. 2017). In both species, the corresponding homologs are required for sexual development and additionally have phenotypes related to species-specific environments; the *F. graminearum* mutant is no longer pathogenic, whereas the dermatophyte *A. benhamiae* requires *stuA* for growth on keratin-containing substrates. Overall, the role of *stuA* in sexual development seems to be conserved across a wide range of filamentous ascomycetes.

Another transcription factor with a (partially) conserved role in sexual development is Ste12. This gene was first identified as an essential gene for sexual differentiation in the yeast *S. cerevisiae* (Johnson 1995). Subsequent studies in several filamentous ascomycetes showed a role for *ste12* homologs in all species that were analyzed (Table 2); however, the developmental phenotypes of ste12 mutants vary widely. The first *ste12* homolog that was analyzed in filamentous ascomycetes was *steA* from *A. nidulans*

(Vallim et al. 2000). The corresponding mutant makes Hülle cells, but no cleistothecia, ascogenous hyphae, or ascospores. A similar phenotype was observed in the *steA* mutant of another eurotiomycete, *A. benhamiae* (Kröber et al. 2017). A mutant in the *N. crassa* homolog *pp-1* is also unable to form fruiting bodies or even fruiting-body precursors, and in addition, ascospores from outcrossing the pp-1 mutant are ascospore lethal when carrying the mutant allele (Li et al. 2005). However, in *S. macrospora*, which is closely related to *N. crassa*, *ste12* deletion still allows fruiting-body formation, but ascospore development is impaired (Nolting and Pöggeler 2006b). Interestingly, despite different morphological outcomes, parts of the signaling cascades involving *ste12* appear to be conserved from yeast to filamentous ascomycetes. For example, it was shown in *S. macrospora* that STE12 interacts with the transcription factors Mcm1 and MAT1-1-1, similar to its counterpart in yeast (Nolting and Pöggeler 2006b). Mcm1 itself is another example of a transcription factor with a conserved role in sexual development in different ascomycetes (Nolting and Pöggeler 2006a; Ortiz and Shim 2013; Yang et al. 2015). In *A. nidulans*, SteA acts downstream of a MAPK cascade that controls the balance of sexual development, asexual development, and secondary metabolism in response to external signals and comprises elements that form a similar MAPK cascade regulating sexual development in yeast (Bayram et al. 2012). Thus, core regulatory pathways might be conserved, but it is likely that input and output pathways were rewired extensively during the evolution of fruiting-body development. A special case of such rewiring might be the interaction between hypovirus infection, virulence, and sexual development in *Cryphonectria parasitica* (Deng et al. 2007). In this sordariomycete, deletion of the *ste12* homolog *CpST12* has the same effects as hypovirus infection, namely, female sterility and reduced virulence. Furthermore, many genes that are differentially regulated in the CpST12 mutant are also differentially regulated upon hypovirus infection (see Sect. IV.C.2). Thus, hypovirus infection seems to interfere with pathways regulating sexual development in this species. Yet another example for this interference is the role of the *pro1* homolog in *C. parasitica*. *pro1* was first identified as a transcription factor essential for sexual development in *S. macrospora* (Masloff et al. 1999), whereas in *C. parasitica*, *pro1* is not only required for female fertility but also for stable hypovirus maintenance (Sun et al. 2009). Additional roles for *pro1* orthologs were also found in *N. crassa*, where deletion of the ortholog *adv-1* leads to a pleiotropic phenotype with defects in vegetative growth, conidiation, and protoperithecia formation (Colot et al. 2006). In *A. nidulans*, there are two *pro1* homologs, *nosA* and *rosA*, with *nosA* being more closely related to *pro1* than *rosA* (Vienken et al. 2005; Vienken and Fischer 2006). Both genes are involved in fruiting-body formation, but with opposite roles; while *nosA* is an activator required for sexual development, *rosA* is a repressor of fruiting-body formation.

A conserved role in fruiting-body development was also found for the GATA-type transcription factor NsdD. It was first identified in *A. nidulans*, where *nsdD* mutants make no cleistothecia or Hülle cells under standard conditions, whereas overexpression of *nsdD* leads to inappropriate formation of sexual structures, e.g., when the mutant is grown submerged in liquid medium (Han et al. 2001). In *A. nidulans*, *nsdD* is not only required for sexual development but is part of the regulatory circuits that control the balance between sexual and asexual morphogenesis. Deletion of *nsdD* leads to increased conidiation, and NsdD binds the promotor of the conidiation activator *brlA* as a repressor (Lee et al. 2014, 2016). The orthologs of *nsdD* in *Sordariomycetes* are also required for fruiting-body formation as was shown for *N. crassa sub-1* and *S. macrospora pro44* (Colot et al. 2006; Nowrousian et al. 2012). In addition to being blocked at the transition from protoperithecia to perithecia, the corresponding mutants show the additional phenotype of protoperithecia that are submerged in the agar medium. Thus, *sub-1*/*pro44* is not only involved in the development as such but also in the correct spatial placement of fruiting bodies within the mycelium. *nsdD*/*sub-1*/*pro44* orthologs are not only required for fruiting-body formation in the *Eurotiomycetes* and *Sordariomycetes* but

also in the *Leotiomycetes*. This was shown for *Botrytis cinerea* where the corresponding ortholog *bcltf1* is unable to make sclerotia, the structures from which fruiting bodies are initiated (Schumacher et al. 2014). A *nsdD/sub-1/pro44* ortholog is also present in the early-diverging lineage of *Pezizomycetes*, and the corresponding gene from the pezizomycete *P. confluens* can complement the *S. macrospora pro44* mutant, suggesting a conserved role for this gene for fruiting-body development in the ancestor of filamentous ascomycetes (Traeger et al. 2013).

However, a fruiting-body-related role is not conserved in all transcription factors that were investigated. One example to the contrary is the ortholog pair ASD4/AreB from *N. crassa* and *A. nidulans*, respectively (Feng et al. 2000; Wong et al. 2009). While *asd4* is involved in the formation of asci and ascospores in *N. crassa*, its ortholog *areB* in *A. nidulans* is involved in nitrogen metabolism and asexual differentiation, but not in sexual development. Similarly, the WC-1 homolog in *Cordyceps militaris* is essential for fruiting-body formation (Yang et al. 2016), whereas *wc-1* mutants of *N. crassa* are impaired in protoperithecia formation but still fertile (Degli-Innocenti and Russo 1984). One explanation in this case is that fruiting-body formation in *C. militaris* is light dependent, which is not the case in *N. crassa*, and generally rare in filamentous ascomycetes (see Sect. III.A.1). Therefore, it is possible that preexisting regulatory circuits for light regulation were recruited into regulating sexual development in those species where light influences this process.

Analysis of species that are able to form conidia in addition to undergoing sexual development has revealed that many transcription factors involved in sexual development are in fact regulating the balance between the two different morphogenetic pathways. This might make sense in the light of limited resources, e.g., nutrients, because both processes require a considerable investment of such resources until mature spores can be produced, and depending on the conditions, commitment to one or the other might be the most efficient way toward successful propagation. The regulation of this balance was most intensively studied in the homothallic *A. nidulans*, where in principle conidiation and fruiting-body formation can be carried out by a single individual. These studies have revealed a role for a number of transcription factors in regulating not only the balance between sexual and asexual development, but also secondary metabolism, another resource-intensive process that is tightly linked to environmental conditions. Apart from the GATA transcription factor NsdD mentioned above, the transcription factors FlbC, NapA, NsdC, RsmA, UrdA, and ZipA are part of this extensive regulatory network (Kim et al. 2009; Kwon et al. 2010; Yin et al. 2013; Oiartzabal-Arano et al. 2015). However, the best-studied player in this process might be the velvet protein VeA. The *veA1* mutant allele has been present for many years in a number of laboratory strains, used because it preferentially forms conidia (Käfer 1965). *veA* was identified as a gene required for fruiting-body formation in 2002 (Kim et al. 2002b) and has been studied intensively since (Yin and Keller 2011; Gerke and Braus 2014) (see Sect. III.A.1). The novel velvet domain present in VeA and several other proteins was only recently shown to be a DNA-binding domain (Ahmed et al. 2013). VeA was shown to participate in several protein complexes that shuttle between cytoplasm and nucleus depending on external signals. One such complex consists of VeA, a second velvet protein named VelB, and the putative methyltransferase LaeA. In darkness, VeA localizes to the nucleus and allows protein complex formation leading to sexual development and expression of a number of genes involved in secondary metabolism, whereas in light, VeA stays in the cytoplasm, thereby preventing formation of the nuclear complex (Bayram et al. 2008b). In addition, VeA can form an alternative complex with the methyltransferases VipC and VapB, which inhibits sexual development and instead promotes conidiation (Sarikaya-Bayram et al. 2014) (see Sect. IV.C.3). As part of the regulatory network controlling the balance between sexual and asexual development, the MAPK cascade that regulates SteA/Ste12 also regulates VeA through phosphorylation of VeA by the MAP kinase Fus3 (Bayram et al. 2012). By now, homologs of VeA and other velvet proteins have been identified as regulators of sexual development also in other ascomycetes (Table 2).

The transcription factors described so far mostly lead to a block at a certain developmental

stage when the corresponding genes are deleted. However, there are also transcription factors that are involved in modulating morphology or are required for morphogenesis of specific parts of the fruiting body. An example of the former is MYT2 in *F. graminearum*, deletion of which leads to larger perithecia, whereas overexpression leads to smaller perithecia (Lin et al. 2012). An interesting case is the transcription factor FlbD in *A. nidulans*. This protein was originally analyzed for its role in conidiophore differentiation but was also shown to be required for the formation of the fruiting-body peridium; mutants in *flbD* are able to produce ascospores without the surrounding peridium (Arratia-Quijada et al. 2012). Further analysis of factors like MYT2 or FlbD might help to elucidate the regulatory events involved in the morphogenesis of specific cell types during fruiting-body formation, a topic not much explored in fungi to date. What is also not well known is the spatial regulation of development, e.g., on which parts of the mycelium fruiting bodies are differentiated, and how the organization of different cell types within the fruiting body is regulated. It is reasonable to assume that transcription factors will play a role in these processes, similar to morphogenetic events in plants and animals. Future studies involving advanced microscopy techniques or single-cell transcriptomics might shed light on these questions.

2. Transcriptional Changes During Development

Fruiting-body development involves the differentiation of many cell types that are not present in the vegetative mycelium and consequently requires changes in the activity of a large number of genes compared to vegetative growth. This change is thought to occur to a large degree at the level of transcription, and consequently transcription factors are likely to play a role in this process. Therefore, **transcriptomics studies** have not only been used to compare transcriptomes of different developmental stages but also to analyze transcriptomes of developmental mutants, especially transcription factor mutants, which will be the focus of this section. For a review of transcriptomics of other developmental mutants, different growth conditions, and comparative transcriptomics approaches, see Nowrousian (2014).

Among the first transcription factor mutants that were analyzed using transcriptomics were *MAT* gene mutants. Studies using differential hybridization, microarrays, or RNA-seq have been performed for *MAT* gene mutants of *F. graminearum*, *G. fujikuroi*, *P. anserina*, *P. chrysogenum*, and *S. macrospora* (Lee et al. 2006; Pöggeler et al. 2006b; Keszthelyi et al. 2007; Klix et al. 2010; Bidard et al. 2011; Böhm et al. 2013; Kim et al. 2015). A recent study of *P. chrysogenum* used chromatin immunoprecipitation-sequencing (ChIP-seq) to identify direct target genes of the MAT1-1-1 protein (Becker et al. 2015). A recurrent finding in these studies was that there are many more genes regulated directly or indirectly by *MAT* genes than in the yeast *S. cerevisiae*, where the *MAT* genes directly regulate only about 30 genes (Galgoczy et al. 2004). Furthermore, most of the *MAT* target genes in yeast have roles in sexual development themselves, whereas function predictions of *MAT*-regulated genes in filamentous ascomycetes point to various roles outside of fruiting-body formation. Consequently, gene deletion studies of differentially regulated genes found that some but not all of the potential target genes are involved in sexual development (Kim et al. 2015). Thus, deletion and gene expression studies show that *MAT* genes in filamentous ascomycetes have acquired functions outside of sexual development in addition to their "classical" roles as sexual regulators. Furthermore, the regulatory networks surrounding the MAT genes might be only moderately conserved. For example, the HMG domain protein HMG5 in *P. anserina* was found to act upstream of *MAT1-1-1* (Ait Benkhali et al. 2013), whereas its ortholog *FGSG_01366* in *F. graminearum* is a potential target gene of MAT1-2-1 (Kim et al. 2015).

Transcriptomics analyses were also conducted for other transcription factor mutants. Ste12 mutants of *N. crassa* (*pp-1*) and *C. parasitica* (*CpST12*) were analyzed using

microarrays (Li et al. 2005; Deng et al. 2007). In *N. crassa*, it was shown that the expression patterns of the *pp-1* mutant were similar to those of a mutant of the *fus3*-homolog *mak-2*, indicating that similar to yeast, these two genes are involved in the same regulatory pathway. Many of the genes regulated differentially in the *C. parasitica* mutant are also regulated differentially upon hypovirus infection, confirming the influence of hypoviruses on the regulation of sexual development in this fungus.

The *stuA* mutant of *F. graminearum* was also used for transcriptome analysis. However, these studies were carried out under growth conditions that favor conidiation (Lysoe et al. 2011). In contrast, transcriptome studies were conducted for the pro1 mutant of *S. macrospora*, either using total mycelia under conditions allowing sexual development or using RNA from young fruiting bodies from the wild type and the pro1 mutant (Nowrousian et al. 2005, 2007; Teichert et al. 2012). In addition, ChIP-seq analyses were used to identify direct target genes of PRO1 in *S. macrospora* and its ortholog ADV-1 in *N. crassa* (Steffens et al. 2016; Dekhang et al. 2017). The transcriptomics analyses found large differences in expression patterns between the pro1 mutant and the wild type, including differential expression of a number of genes known to be involved in sexual development. Furthermore, the ChIP-seq analyses showed PRO1 binding to promoters of genes involved in pheromone signaling, ROS metabolism, and cell wall integrity. These pathways were shown previously to play a role in fruiting-body development (see Sects. III.B.2, IV.A.3, and IV.A.1), and it is possible that PRO1 acts as a master regulator controlling several important downstream pathways.

3. Chromatin Modifiers

Underlying the drastic morphological changes during fruiting-body development are corresponding genome-wide changes in gene expression. While specific transcription factors play a role in those expression changes, it seems likely that many of these are also facilitated or regulated at the level of chromatin. Therefore, **chromatin-modifying factors** are thought to play a role in fruiting-body formation. Chromatin modifications can include modification of the DNA itself, e.g., through methylation, positional changes of nucleosomes, incorporation of histone variants into nucleosomes, or a wide range of histone modifications (Zhang and Pugh 2011; Freitag 2014; Voss and Hager 2014). Several studies in recent years have uncovered a number of chromatin-modifying factors that are involved in fruiting-body formation and that cover a wide range of potential functions in chromatin modification.

Two such factors, HDF1 and FTL1, were studied in *F. graminearum* and are predicted to be homologs of members of the yeast Set3 complex that contains several histone deacetylase (HDAC) proteins and regulates HDAC activity (Ding et al. 2009; Li et al. 2011). In *S. cerevisiae*, the Set3 complex controls the progression through meiosis, with mutants showing accelerated meiosis and lower numbers of viable spores (Pijnappel et al. 2001). HDF1 from *F. graminearum* is an ortholog of the yeast HDAC Hos2, and the corresponding deletion mutant is no longer able to form perithecia under selfing conditions. Instead, the mutant forms conidia, indicating that HDF1 is required to maintain the correct balance between sexual and asexual development in *F. graminearum* (Li et al. 2011). FTL1 is homologous to the yeast Set3 complex protein Sif2, and similar to the HDF1 mutant, the FTL1 mutant is female sterile. Furthermore, HDAC activity in the mutant is reduced (Ding et al. 2009). These data suggest that chromatin regulation via histone modification plays an important role in priming the genome for sexual development.

Another chromatin modifier that is required for fruiting-body formation is the histone chaperone ASF1 in *S. macrospora* (Gesing et al. 2012). Histone chaperones comprise a heterogenic group of proteins that are characterized by their ability to handle non-nucleosomal histones in vivo and mediate the assembly of nucleosomes from isolated histones and DNA in vitro (Hammond et al. 2017). Histone chaperones are involved in essentially all processes involving chromatin, including DNA replication, repair, and transcription (Das et al. 2010). ASF1 is a conserved eukaryotic histone chaperone specific for

histones H3 and H4 and involved in a number of processes ranging from nucleosome assembly to regulation of transcription (Mousson et al. 2007). Interestingly, deletion of *asf1* is lethal in many organisms where it was investigated, and so far *S. macrospora* is the only multicellular organism where an *asf1* deletion mutant is viable. Apart from a slower vegetative growth rate, the mutant is unable to progress from immature to mature fruiting bodies (Gesing et al. 2012). A number of known developmental genes are transcriptionally deregulated in the *asf1* mutant, but the exact molecular mechanisms by which ASF1 causes changes in gene expression during fruiting-body formation are not clear yet.

Another player among chromatin modifiers involved in regulating sexual development is the protein complex comprising the methyltransferases VipC and VapB as well as the velvet protein VeA in *A. nidulans* (Sarikaya-Bayram et al. 2014). The methyltransferases were identified in a screen for VeA interaction partners. A mutant in *vipC* shows the opposite phenotype of the *veA* mutant, namely, the production of more fruiting bodies in the light. Depending on external signals, VipC and VapB either interact with a protein called VapA, which tethers the complex to the plasma membrane and prevents interaction with VeA, or VipC and VapB are released from VapA and translocate to the nucleus, where they can interact with VeA and inhibit sexual development and secondary metabolism (Sarikaya-Bayram et al. 2014). This is in contrast to the effect of VeA interacting with another putative methyltransferase, LaeA, in which case sexual development and secondary metabolism are promoted (Bok et al. 2005; Bayram et al. 2008b). However, in contrast to LaeA, for which the target molecules have not been identified yet, it is possible that VapB is a bona fide methyltransferase, as overexpression of *vapB* leads to a reduction in histone H3 lysine 9 trimethylation (H3K9me3) (Sarikaya-Bayram et al. 2014, 2015). Thus, it is possible that histone modifications play an important role in regulating the transition between sexual and asexual propagation. This is also supported by the finding that the white collar-1 homolog LreA interacts not only with the white collar-2 homolog LreB, which in turn interacts with the phytochrome FphA, which itself interacts with VeA (see Sect. III.A.1), but that LreA also interacts with the histone acetyltransferase GcnE and the deacetylase HdaA (Hedtke et al. 2015). An additional case in point comes from the analysis of another putative histone-modifying protein, RtfA, in *A. nidulans* (Ramamoorthy et al. 2012). *rtfA* was identified as a suppressor of ΔveA with respect to secondary metabolism. RtfA is homologous to the *S. cerevisiae* Rtf1 protein, which is a member of the Paf complex required for transcription-associated histone modifications (Warner et al. 2007). Deletion of *rtfA* in *A. nidulans* leads to loss of Hülle cells and cleistothecia but also to vegetative growth defects and the production of fewer conidia (Ramamoorthy et al. 2012). As *rtfA* was identified as a suppressor of ΔveA, it is possible that *veA* acts as a central hub for the control of chromatin landscape during different life phases of *A. nidulans* (Sarikaya-Bayram et al. 2015).

Overall, analysis of the role of chromatin modifications in regulating sexual development in filamentous ascomycetes is still in its infancy, but from results obtained so far, it seems likely that there are major regulatory events remaining to be discovered.

V. Conclusions

Filamentous ascomycetes generate multiple types of fruiting bodies and serve as model organisms to investigate eukaryotic multicellular development at the molecular level. Highly developed fungal genetic systems provide the experimental basis for forward and reverse genetic approaches to decipher components of cellular networks that direct and regulate the development of complex fruiting bodies. These experimental benefits of filamentous fungi are complemented by their relatively small genome size, and the ease of culturing makes them amenable to high-throughput screening. The data output of these technologies has led to new ideas about the mechanisms to build a eukaryotic multicellular structure. We just

begin to understand how genetic and environmental cues initiate and regulate fruiting-body formation at the molecular level.

While fruiting-body-dependent gene expression and its regulation is intensively studied, relatively little is known about signaling events that transmit outside-in signals and direct the coordinated formation of different cell types. Currently, we are aware of several multi-subunit protein complexes that are involved in fruiting-body development, and we begin to get a picture about how these complexes interact with each other and transduce signals that direct the initiation of the sexual cycle, and the subsequent formation of a dikaryon. However, the signaling events for maintenance of the dikaryon and the regulated entry into karyogamy remain obscure.

Acknowledgment The authors thank Gabriele Frenßen-Schenkel for preparing all figures and Hauke Kuhlmann and Marion Wolf for the technical help in preparing the text. We further acknowledge several colleagues, as mentioned in the text, who provided images for some of the figures.

References

Aguirre J, Rios-Momberg M, Hewitt D, Hansberg W (2005) Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol 13:111–118

Ahmed YL, Gerke J, Park HS, Bayram Ö, Neumann P, Ni M, Dickmanns A, Kim SC, Yu JH, Braus GH, Ficner R (2013) The velvet family of fungal regulators contains a DNA-binding domain structurally similar to NF-κB. PLoS Biol 11:e1001750

Ait Benkhali J, Coppin E, Brun S, Peraza-Reyes L, Martin T, Dixelius C, Lazar N, van Tilbeurgh H, Debuchy R (2013) A network of HMG-box transcription factors regulates sexual cycle in the fungus *Podospora anserina*. PLoS Genet 9:e1003642

Alvaro CG, Thorner J (2016) Heterotrimeric G protein-coupled receptor signaling in yeast mating pheromone response. J Biol Chem 291:7788–7795

Amselem J, Cuomo CA, van Kan JA, Viaud M, Benito EP, Couloux A, Coutinho PM, de Vries RP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L, Lapalu N, Plummer KM, Pradier JM, Quevillon E, Sharon A, Simon A, ten Have A, Tudzynski B, Tudzynski P, Wincker P, Andrew M, Anthouard V, Beever RE, Beffa R, Benoit I, Bouzid O, Brault B, Chen Z, Choquer M, Collemare J, Cotton P, Danchin EG, Da Silva C, Gautier A, Giraud C, Giraud T, Gonzalez C, Grossetete S, Guldener U, Henrissat B, Howlett BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Neuveglise C, Oeser B, Pearson M, Poulain J, Poussereau N, Quesneville H, Rascle C, Schumacher J, Segurens B, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yandava C, Yarden O, Zeng Q, Rollins JA, Lebrun MH, Dickman M (2011) Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS Genet 7:e1002230

Anding AL, Baehrecke EH (2017) Cleaning house: selective autophagy of organelles. Dev Cell 41:10–22

Araki Y, Ku W-C, Akioka M, May AI, Hayashi Y, Arisaka F, Ishihama Y, Ohsumi Y (2013) Atg38 is required for autophagy-specific phosphatidylinositol 3-kinase complex integrity. J Cell Biol 203:299–313

Aramayo R, Peleg Y, Addison R, Metzenberg R (1996) *Asm-1*+, a *Neurospora crassa* gene related to transcriptional regulators of fungal development. Genetics 144:991–1003

Arratia-Quijada J, Sánchez O, Scazzocchio C, Aguirre J (2012) FlbD, a Myb transcription factor of *Aspergillus nidulans*, is uniquely involved in both asexual and sexual differentiation. Eukaryot Cell 11:1132–1142

Balestrini R, Mainieri D, Soragni E, Garnero L, Rollino S, Viotti A, Ottonello S, Bonfante P (2000) Differential expression of chitin synthase III and IV mRNAs in ascomata of *Tuber borchii Vittad*. Fungal Genet Biol 31:219–232

Barnett HL, Lilly VG (1947) The effects of biotin upon the formation and development of perithecia, asci and ascospores by *Sordaria fimicola* Ces and de Not. Am J Bot 34:196–204

Bartoszewska M, Kiel JAKW (2010) The role of macroautophagy in development of filamentous fungi. Antioxid Redox Signal 14:2271–2287

Bayram O, Braus GH (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol Rev 36:1–24

Bayram O, Biesemann C, Krappmann S, Galland P, Braus GH (2008a) More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. Mol Biol Cell 19:3254–3262

Bayram Ö, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon N-J, Keller NP, Yu J-H, Braus GH (2008b) VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320:1504–1506

Bayram O, Braus GH, Fischer R, Rodriguez-Romero J (2010) Spotlight on *Aspergillus nidulans* photosensory systems. Fungal Genet Biol 47:900–908

Bayram Ö, Bayram ÖS, Ahmed YL, Maruyama JI, Valerius O, Rizzoli SO, Ficner R, Irniger S, Braus GH (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. PLoS Genet 8: e1002816

Bazafkan H, Dattenböck C, Böhmdorfer S, Tisch D, Stappler E, Schmoll M (2015) Mating type-dependent partner sensing as mediated by VEL1 in *Trichoderma reesei*. Mol Microbiol 96:1103–1118

Bazafkan H, Dattenbock C, Stappler E, Beier S, Schmoll M (2017) Interrelationships of VEL1 and ENV1 in light response and development in *Trichoderma reesei*. PLoS One 12:e0175946

Becker K, Beer C, Freitag M, Kück U (2015) Genome-wide identification of target genes of a mating-type α-domain transcription factor reveals functions beyond sexual development. Mol Microbiol 96:1002–1022

Beckmann EA, Köhler AM, Meister C, Christmann M, Draht OW, Rakebrandt N, Valerius O, Braus GH (2015) Integration of the catalytic subunit activates deneddylase activity in vivo as final step in fungal COP9 signalosome assembly. Mol Microbiol 97:110–124

Beier A, Teichert I, Krisp C, Wolters DA, Kück U (2016) Catalytic subunit 1 of protein phosphatase 2A is a subunit of the STRIPAK complex and governs fungal sexual development. MBio 7:e00870-00816

Bennett RJ, Turgeon BG (2016) Fungal sex: the ascomycota. Microbiol Spectr 4. https://doi.org/10.1128/microbiolspec.FUNK-0005-2016

Bernhards Y, Pöggeler S (2011) The phocein homologue SmMOB3 is essential for vegetative cell fusion and sexual development in the filamentous ascomycete *Sordaria macrospora*. Curr Genet 57:133–149

Bhattacharyya S, Yu H, Mim C, Matouschek A (2014) Regulated protein turnover: snapshots of the proteasome in action. Nat Rev Mol Cell Biol 15:122–133

Bidard F, Aït Benkhali J, Coppin E, Imbeaud S, Grognet P, Delacroix H, Debuchy R (2011) genome-wide gene expression profiling of fertilization competent mycelium in opposite mating types in the heterothallic fungus *Podospora anserina*. PLoS One 6:e21476

Bieszke JA, Braun EL, Bean LE, Kang SC, Natvig DO, Borkovich KA (1999) The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci U S A 96:8034–8039

Bistis GN (1981) Chemotrophic interactions between trichogynes and conidia of the opposite mating type in *Neurospora crassa*. Mycologia 73:959–975

Bistis GN (1983) Evidence for diffusible mating-type specific trichogyne attractants in *Neurospora crassa*. Exp Mycol 7:292–295

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Newsl 50:17–19

Bloemendal S, Lord KM, Rech C, Hoff B, Engh I, Read ND, Kück U (2010) A mutant defective in sexual development produces aseptate ascogonia. Eukaryot Cell 9:1856–1866

Bloemendal S, Bernhards Y, Bartho K, Dettmann A, Voigt O, Teichert I, Seiler S, Wolters DA, Pöggeler S, Kück U (2012) A homolog of the human STRIPAK complex controls sexual development in fungi. Mol Microbiol 84:310–323

Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R (2005) The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr Biol 15:1833–1838

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804

Böhm J, Hoff B, O'Gorman CM, Wolfers S, Klix V, Binger D, Zadra I, Kürnsteiner H, Pöggeler S, Dyer PS, Kück U (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. Proc Natl Acad Sci U S A 110:1476–1481

Böhm J, Dahlmann TA, Gümüser H, Kück U (2015) A MAT1-2 wild-type strain from *Penicillium chrysogenum*: functional mating-type locus characterization, genome sequencing and mating with an industrial penicillin-producing strain. Mol Microbiol 95:859–874

Bok JW, Balajee SA, Marr KA, Andes D, Nielsen KF, Frisvad JC, Keller NP (2005) LaeA, a regulator of morphogenetic fungal virulence factors. Eukaryot Cell 4:1574–1582

Bonnet C, Espagne E, Zickler D, Boisnard S, Bourdais A, Berteaux-Lecellier V (2006) The peroxisomal import proteins PEX2, PEX5 and PEX7 are differently involved in *Podospora anserina* sexual cycle. Mol Microbiol 62:157–169

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N, Plamann M, Goodrich-Tanrikulu M, Schulte U, Mannhaupt G, Nargang FE, Radford A, Selitrennikoff C, Galagan JE, Dunlap JC, Loros JJ, Catcheside D, Inoue H, Aramayo R, Polymenis M, Selker EU, Sachs MS, Marzluf GA, Paulsen I, Davis R, Ebbole DJ, Zelter A, Kalkman ER, O'Rourke R, Bowring F, Yeadon J, Ishii C, Suzuki K, Sakai W, Pratt R (2004) Lessons from the genome sequence of *Neurospora crassa:* tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Bornstein G, Grossman C (2015) COP9-Signalosome deneddylase activity is enhanced by simultaneous

neddylation: insights into the regulation of an enzymatic protein complex. Cell Div 10:5
Bouhouche K, Zickler D, Debuchy R, Arnaise S (2004) Altering a gene involved in nuclear distribution increases the repeat-induced point mutation process in the fungus *Podospora anserina*. Genetics 167:151–159
Braus GH, Irniger S, Bayram Ö (2010) Fungal development and the COP9 signalosome. Curr Opin Microbiol 13:672–676
Brodhun F, Feussner I (2011) Oxylipins in fungi. FEBS J 278:1047–1063
Brown LS, Dioumaev AK, Lanyi JK, Spudich EN, Spudich JL (2001) Photochemical reaction cycle and proton transfers in Neurospora rhodopsin. J Biol Chem 276:32495–32505
Brown NA, Dos Reis TF, Ries LN, Caldana C, Mah JH, Yu JH, Macdonald JM, Goldman GH (2015) G-protein coupled receptor-mediated nutrient sensing and developmental control in *Aspergillus nidulans*. Mol Microbiol 98:420–439
Brun S, Malagnac F, Bidard F, Lalucque H, Silar P (2009) Functions and regulation of the Nox family in the filamentous fungus *Podospora anserina*: a new role in cellulose degradation. Mol Microbiol 74:480–496
Buetow L, Huang DT (2016) Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nat Rev Mol Cell Biol 17:626–642
Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730
Busch S, Schwier EU, Nahlik K, Bayram Ö, Helmstaedt K, Draht OW, Krappmann S, Valerius O, Lipscomb WN, Braus GH (2007) An eight-subunit COP9 signalosome with an intact JAMM motif is required for fungal fruit body formation. Proc Natl Acad Sci U S A 104:8089–8094
Cai M, Zhou X, Zhou J, Niu C, Kang L, Sun X, Zhang Y (2010) Efficient strategy for enhancing aspergiolide A production by citrate feedings and its effects on sexual development and growth of marine-derived fungus *Aspergillus glaucus*. Bioresour Technol 101:6059–6068
Callaghan AA (1962) Observations on perithecium production and spore discharge in *Pleurage setosa*. Trans Br Mycol Soc 45:249–254
Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459
Cano-Domínguez N, Alvarez-Delfín K, Hansberg W, Aguirre J (2008) NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. Eukaryot Cell 7:1352–1361
Canovas D, Marcos JF, Marcos AT, Strauss J (2016) Nitric oxide in fungi: is there NO light at the end of the tunnel? Curr Genet 62:513–518
Carlile MJ, Friend J (1956) Carotenoids and reproduction in *Pyronema confluens*. Nature 178:369–370
Carrillo AJ, Schacht P, Cabrera IE, Blahut J, Prudhomme L, Dietrich S, Bekman T, Mei J, Carrera C, Chen V, Clark I, Fierro G, Ganzen L, Orellana J, Wise S, Yang K, Zhong H, Borkovich KA (2017) Functional profiling of transcription factor genes in *Neurospora crassa*. G3 (Bethesda) 7(9):2945–2956. https://doi.org/10.1534/g3.117.043331
Casas-Flores S, Herrera-Estrella A (2016) The bright and dark side of fungal life. In: Druzhinina LS, Kubicek CP (eds) The Mycota IV. Environmental and microbial relationships, 3rd edn. Springer, Berlin, pp 41–77
Casselton LA (2002) Mate recognition in fungi. Heredity 88:142–147
Chen CH, DeMay BS, Gladfelter AS, Dunlap JC, Loros JJ (2010) Physical interaction between VIVID and white collar complex regulates photoadaptation in *Neurospora*. Proc Natl Acad Sci U S A 107:16715–16720
Chen CL, Kuo HC, Tung SY, Hsu PW, Wang CL, Seibel C, Schmoll M, Chen RS, Wang TF (2012) Blue light acts as a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*). PLoS One 7:e44969
Cheng P, Yang Y, Gardner KH, Liu Y (2002) PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. Mol Cell Biol 22:517–524
Chinnici JL, Fu C, Caccamise LM, Arnold JW, Free SJ (2014) *Neurospora crassa* female development requires the PACC and other signal transduction pathways, transcription factors, chromatin remodeling, cell-to-cell fusion, and autophagy. PLoS One 9:e110603
Chona BL (1932) The effect of cultural conditions on the growth and sporulation of an organism belonging to the group species *Aspergillus glaucus*. Trans Br Mycol Soc 17:221–228
Christmann M, Schmaler T, Gordon C, Huang X, Bayram Ö, Schinke J, Stumpf S, Dubiel W, Braus GH (2013) Control of multicellular development by the physically interacting deneddylases DEN1/DenA and COP9 signalosome. PLoS Genet 9:e1003275
Chung D, Dellaire G (2015) The role of the COP9 signalosome and neddylation in DNA damage signaling and repair. Biomol Ther 5:2388–2416
Chung D, Upadhyay S, Bomer B, Wilkinson HH, Ebbole DJ, Shaw BD (2015) *Neurospora crassa* ASM-1 complements the conidiation defect in a *stuA*

mutant of *Aspergillus nidulans*. Mycologia 107: 298–306
Ciechanover A (1994) The ubiquitin-proteasome proteolytic pathway. Cell 79:13–21
Cohen-Kaplan V, Livneh I, Avni N, Cohen-Rosenzweig C, Ciechanover A (2016) The ubiquitin-proteasome system and autophagy: coordinated and independent activities. Int J Biochem Cell Biol 79:403–418
Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, Weiss RL, Borkovich KA, Dunlap JC (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. Proc Natl Acad Sci U S A 103:10352–10357
Coppin E, de Renty C, Debuchy R (2005) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4:407–420
Coppin E, Berteaux-Lecellier V, Bidard F, Brun S, Ruprich-Robert G, Espagne E, Aït-Benkhali J, Goarin A, Nesseir A, Planamente S, Debuchy R, Silar P (2012) Systematic deletion of homeobox genes in *Podospora anserina* uncovers their roles in shaping the fruiting body. PLoS One 7:e37488
Czaja W, Miller KY, Miller BL (2011) Complex mechanisms regulate developmental expression of the matA (HMG) mating type gene in homothallic *Aspergillus nidulans*. Genetics 189:795–808
Czaja W, Miller KY, Skinner MK, Miller BL (2014) Structural and functional conservation of fungal MatA and human SRY sex-determining proteins. Nat Commun 5:5434
D'Souza CA, Heitman J (2001) Conserved cAMP signaling cascades regulate fungal development and virulence. FEMS Microbiol Rev 25:349–364
Das C, Tyler JK, Churchill ME (2010) The histone shuffle: histone chaperones in an energetic dance. Trends Biochem Sci 35:476–489
Dasgupta A, Fuller KK, Dunlap JC, Loros JJ (2016) Seeing the world differently: variability in the photosensory mechanisms of two model fungi. Environ Microbiol 18:5–20
Davis RH (1995) Genetics of *Neurospora*. In: Kück U (ed) The Mycota II. Genetics and biotechnology, 1st edn. Springer, Berlin, pp 3–33
De Souza CP, Hashmi SB, Osmani AH, Andrews P, Ringelberg CS, Dunlap JC, Osmani SA (2013a) Functional analysis of the *Aspergillus nidulans* kinome. PLoS One 8:e58008
de Souza WR, Morais ER, Krohn NG, Savoldi M, Goldman MH, Rodrigues F, Caldana C, Semelka CT, Tikunov AP, Macdonald JM, Goldman GH (2013b) Identification of metabolic pathways influenced by the G-protein coupled receptors GprB and GprD in *Aspergillus nidulans*. PLoS One 8:e62088
Debuchy R, Coppin E (1992) The mating types of *Podospora anserina*: functional analysis and sequence of the fertilization domains. Mol Gen Genet 233:113–121
Debuchy R, Arnaise S, Lecellier G (1993) The mat- allele of *Podospora anserina* contains three regulatory genes required for the development of fertilized female organs. Mol Gen Genet 241:667–673
Debuchy R, Berteaux-Lecellier V, Silar P (2010) Mating systems and sexual morphogenesis in ascomycetes. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Washington, DC, pp 501–535
Degli-Innocenti F, Russo VEA (1984) Isolation of new white-collar mutants of *Neurospora crassa* and studies on their behavior in the blue light-induced formation of protoperithecia. J Bacteriol 159:757–761
Deka R, Ghosh A, Tamuli R, Borkovich KA (2016) Heterotrimeric G proteins. In: Hoffmeister D (ed) The Mycota III. Biochemistry and molecular biology, 3rd edn. Springer, Berlin, pp 119–144
Dekhang R, Wu C, Smith KM, Lamb TM, Peterson M, Bredeweg EL, Ibarra O, Emerson JM, Karunarathna N, Lyubetskaya A, Azizi E, Hurley JM, Dunlap JC, Galagan JE, Freitag M, Sachs MS, Bell-Pedersen D (2017) The *Neurospora* transcription factor ADV-1 transduces light signals and temporal information to control rhythmic expression of genes involved in cell fusion. G3 (Bethesda) 7:129–142
Dementhon K, Saupe SJ (2005) DNA-binding specificity of the IDI-4 basic leucine zipper factor of *Podospora anserina* defined by systematic evolution of ligands by exponential enrichment (SELEX). Eukaryot Cell 4:476–483
Dementhon K, Saupe SJ, Clavé C (2004) Characterization of IDI-4, a bZIP transcription factor inducing autophagy and cell death in the fungus *Podospora anserina*. Mol Microbiol 53:1625–1640
Deng F, Allen TD, Nuss DL (2007) Ste12 transcription factor homologue CpST12 is down-regulated by hypovirus infection and required for virulence and female fertility of the chestnut blight fungus *Cryphonectria parasitica*. Eukaryot Cell 6:235–244
Dettmann A, Heilig Y, Ludwig S, Schmitt K, Illgen J, Fleissner A, Valerius O, Seiler S (2013) HAM-2 and HAM-3 are central for the assembly of the Neurospora STRIPAK complex at the nuclear envelope and regulate nuclear accumulation of the MAP kinase MAK-1 in a MAK-2-dependent manner. Mol Microbiol 90:796–812
Dettmann A, Heilig Y, Valerius O, Ludwig S, Seiler S (2014) Fungal communication requires the MAK-2 pathway elements STE-20 and RAS-2, the NRC-1 adapter STE-50 and the MAP kinase scaffold HAM-5. PLoS Genet 10:e1004762
Ding S, Mehrabi R, Koten C, Kang Z, Wei Y, Seong K, Kistler HC, JR X (2009) Transducin beta-like gene FTL1 is essential for pathogenesis in *Fusarium graminearum*. Eukaryot Cell 8:867–876

Dirschnabel DE, Nowrousian M, Cano-Dominguez N, Aguirre J, Teichert I, Kück U (2014) New insights into the roles of NADPH oxidases in sexual development and ascospore germination in *Sordaria macrospora*. Genetics 196:729–744

Doherty FJ, Dawson S, Mayer RJ (2002) The ubiquitin-proteasome pathway of intracellular proteolysis. Essays Biochem 38:51–63

Doughan B, Rollins JA (2016) Characterization of MAT gene functions in the life cycle of *Sclerotinia sclerotiorum* reveals a lineage-specific MAT gene functioning in apothecium morphogenesis. Fungal Biol 120:1105–1117

Draht OW, Busch S, Hoffman K, Braus-Stromeyer S, Helmstaedt K, Goldman GH, Braus GH (2007) Amino acid supply of Aspergillus. CRC Press, New York

Droce A, Sorensen JL, Sondergaard TE, Rasmussen JJ, Lysoe E, Giese H (2017) PTR2 peptide transporters in *Fusarium graminearum* influence secondary metabolite production and sexual development. Fungal Biol 121:515–527

Dyer PS, Kück U (2017) Sex and the imperfect fungi. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.FUNK-0043-2017

Dyer PS, O'Gorman CM (2011) A fungal sexual revolution: Aspergillus and Penicillium show the way. Curr Opin Microbiol 14:649–654

Dyer PS, O'Gorman CM (2012) Sexual development and cryptic sexuality in fungi: insights from *Aspergillus* species. FEMS Microbiol Rev 36:165–192

Dyer PS, Ingram DS, Johnstone K (1993) Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population IV. Mycol Res 97:485–496

Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303

Ebersberger I, de Matos Simoes R, Kupczok A, Gube M, Kothe E, Voigt K, von Haeseler A (2012) A consistent phylogenetic backbone for the fungi. Mol Biol Evol 29:1319–1334

Engh I, Nowrousian M, Kück U (2007) Regulation of melanin biosynthesis via the dihydroxynaphthalene pathway is dependent on sexual development in the ascomycete *Sordaria macrospora*. FEMS Microbiol Lett 275:62–70

Esser K (1966) Die Phenoloxydasen des Ascomyceten *Podospora anserina*. III. Quantitative und Qualitative Enzymunterschiede nach Mutation an nicht gekoppelten Loci. Z Vererbungsl 97:327–344

Esser K (1982) Cryptogams. Cambridge University Press, Cambridge

Esser K, Straub J (1958) Genetische Untersuchungen an *Sordaria mcrospora* Auersw., Kompensation und Induktion bei genbedingten Entwicklungsdefekten. Z Vererbungsl 89:729–746

Fajardo-Somera RA, Jöhnk B, Bayram O, Valerius O, Braus GH, Riquelme M (2015) Dissecting the function of the different chitin synthases in vegetative growth and sexual development in *Neurospora crassa*. Fungal Genet Biol 75:30–45

Fan H, Yu G, Liu Y, Zhang X, Liu J, Zhang Y, Rollins JA, Sun F, Pan H (2016) An atypical forkhead-containing transcription factor SsFKH1 is involved in sclerotial formation and is essential for pathogenicity in *Sclerotinia sclerotiorum*. Mol Plant Pathol 18:963–975

Faretra F, Antonacci E (1987) Production of apothecia of *Botryotinia fuckeliana* (de Bary) Whetz. under controlled environmental conditions. Phytopathol Mediterranea 26:29–35

Feng B, Haas H, Marzluf GA (2000) ASD4, a new GATA factor of *Neurospora crassa*, displays sequence-specific DNA binding and functions in ascus and ascospore development. Biochemistry 39: 11065–11073

Feng Y, He D, Yao Z, Klionsky DJ (2014) The machinery of macroautophagy. Cell Res 24:24–41

Ferreira AV, An Z, Metzenberg RL, Glass NL (1998) Characterization of *mat A-2*, *mat A-3* and D*matA*mating-type mutants of *Neurospora crassa*. Genetics 148:1069–1079

Fischer R, Aguirre J, Herrera-Estrella A, Corrochano LM (2016) The complexity of fungal vision. Microbiol Spectr 4:1–22

Fleissner A, Leeder AC, Roca MG, Read ND, Glass NL (2009) Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion. Proc Natl Acad Sci U S A 106:19387–19392

Freitag M (2014) Fungal chromatin and its role in regulation of gene expression. In: Nowrousian M (ed) The Mycota XIII. Fungal genomics, 2nd edn. Springer, Berlin, pp 99–120

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. Science 297:815–819

Froehlich AC, Chen CH, Belden WJ, Madeti C, Roenneberg T, Merrow M, Loros JJ, Dunlap JC (2010) Genetic and molecular characterization of a cryptochrome from the filamentous fungus *Neurospora crassa*. Eukaryot Cell 9:738–750

Fu C, Iyer P, Herkal A, Abdullah J, Stout A, Free SJ (2011) Identification and characterization of genes required for cell-to-cell fusion in *Neurospora crassa*. Eukaryot Cell 10:1100–1109

Gagne JM, Downes BP, Shiu S-H, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci 99:11519–11524

Galgoczy DJ, Cassidy-Stone A, Llinas M, O'Rourke SM, Herskowitz I, DeRisi JL, Johnson AD (2004) Genomic dissection of the cell-type-specification circuit in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 101:18069–18074

Gao X, Schöttker B (2017) Reduction-oxidation pathways involved in cancer development: a systematic

review of literature reviews. Oncotarget. 10.18632/oncotarget.17128
Garcia-Martinez J, Brunk M, Avalos J, Terpitz U (2015) The CarO rhodopsin of the fungus *Fusarium fujikuroi* is a light-driven proton pump that retards spore germination. Sci Rep 5:7798
Ge L, Baskaran S, Schekman R, Hurley JH (2014) The protein-vesicle network of autophagy. Curr Opin Cell Biol 29:18–24
Geng F, Wenzel S, Tansey WP (2012) Ubiquitin and proteasomes in transcription. Annu Rev Biochem 81:177–201
Geng Z, Zhu W, Su H, Zhao Y, Zhang KQ, Yang J (2014) Recent advances in genes involved in secondary metabolite synthesis, hyphal development, energy metabolism and pathogenicity in *Fusarium graminearum* (teleomorph Gibberella zeae). Biotechnol Adv 32:390–402
Genschik P, Marrocco K, Bach L, Noir S, Criqui M-C (2014) Selective protein degradation: a rheostat to modulate cell-cycle phase transitions. J Exp Bot 65:2603–2615
Gerke J, Braus GH (2014) Manipulation of fungal development as source of novel secondary metabolites for biotechnology. Appl Microbiol Biotechnol 98:8443–8455
Gerke J, Bayram Ö, Feussner K, Landesfeind M, Shelest E, Feussner I, Braus GH (2012) Breaking the silence: protein stabilization uncovers silenced biosynthetic gene clusters in the fungus *Aspergillus nidulans*. Appl Environ Microbiol 78:8234–8244
Gesing S, Schindler D, Fränzel B, Wolters D, Nowrousian M (2012) The histone chaperone ASF1 is essential for sexual development in the filamentous fungus *Sordaria macrospora*. Mol Microbiol 84:748–765
Glass NL, Grotelueschen J, Metzenberg RL (1990) *Neurospora crassa* A mating-type region. Proc Natl Acad Sci U S A 87:4912–4916
Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428
Gomes-Rezende JA, Gomes-Alves AG, Menino JF, Coelho MA, Ludovico P, Gonçalves P, Sturme MHJ, Rodrigues F (2012) Functionality of the *Paracoccidioides* Mating α-Pheromone-Receptor System. PLoS One 7:e47033
Green KA, Becker Y, Fitzsimons HL, Scott B (2016) An *Epichloe festucae* homologue of MOB3, a component of the STRIPAK complex, is required for the establishment of a mutualistic symbiotic interaction with *Lolium perenne*. Mol Plant Pathol 17:1480–1492
Grimaldi B, Coiro P, Filetici P, Berge E, Dobosy JR, Freitag M, Selker EU, Ballario P (2006) The *Neurospora crassa* White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by NGF-1. Mol Biol Cell 17:4576–4583
Halliwell B, Gutteridge JMC (2015) Free radicals in biology and medicine, 5th edn. Oxford University Press, Oxford
Hammond CM, Strømme CB, Huang H, Patel DJ, Groth A (2017) Histone chaperone networks shaping chromatin function. Nat Rev Mol Cell Biol 18:141–158
Han KH (2009) Molecular genetics of *Emericella nidulans* sexual development. Mycobiology 37:171–182
Han KH, Han KY, JH Y, Chae KS, Jahng KY, Han DM (2001) The *nsdD* gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. Mol Microbiol 41:299–309
Han KH, Lee DB, Kim JH, Kim MS, Han KY, Kim WS, Park YS, Kim HB, Han DM (2003) Environmental factors affecting development of *Aspergillus nidulans*. J Microbiol 41:34–40
Harding RW, Melles S (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. Plant Physiol 72:996–1000
Harnett MM, Pineda MA, Latré de Laté P, Eason RJ, Besteiro S, Harnett W, Langsley G (2017) From Christian de Duve to Yoshinori Ohsumi: more to autophagy than just dining at home. Biomed J 40:9–22
He QY, Cheng P, Yang YH, Wang LX, Gardner KH, Liu Y (2002) White collar-1, a DNA binding transcription factor and a light sensor. Science 297:840–843
He Q, Cheng P, He Q, Liu Y (2005) The COP9 signalosome regulates the Neurospora circadian clock by controlling the stability of the SCFFWD-1 complex. Genes Dev 19:1518–1531
He F, Zhang X, Mafurah JJ, Zhang M, Qian G, Wang R, Safdar A, Yang X, Liu F, Dou D (2016) The transcription factor VpCRZ1 is required for fruiting body formation and pathogenicity in *Valsa pyri*. Microb Pathog 95:101–110
Hedtke M, Rauscher S, Röhrig J, Rodríguez-Romero J, Yu Z, Fischer R (2015) Light-dependent gene activation in *Aspergillus nidulans* is strictly dependent on phytochrome and involves the interplay of phytochrome and white collar-regulated histone H3 acetylation. Mol Microbiol 97:733–745
Helmstaedt K, Schwier EU, Christmann M, Nahlik K, Westermann M, Harting R, Grond S, Busch S, Braus GH (2011) Recruitment of the inhibitor Cand1 to the cullin substrate adaptor site mediates interaction to the neddylation site. Mol Biol Cell 22:153–164
Herskowitz I (1989) A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757
Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D,

Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the Fungi. Mycol Res 111:509–547

Hirsch HM (1954) Environmental factors influencing the differentiation of protoperithecia and their relation to tyrosinase and melanin formation in *Neurospora crassa*. Physiol Plant 7:72–97

Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, fleming's penicillium strain discloses the secret of its sex. Eukaryot Cell 7:465–470

Hoffmann B, Wanke C, Lapaglia SK, Braus GH (2000) c-Jun and RACK1 homologues regulate a control point for sexual development in *Aspergillus nidulans*. Mol Microbiol 37:28–41

Hohmann S (2009) Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*. FEBS Lett 583:4025–4029

Hurley J, Loros JJ, Dunlap JC (2015) Dissecting the mechanisms of the clock in *Neurospora*. Methods Enzymol 551:29–52

Hutchinson EA, Glass NL (2010) Meiotic regulators Ndt80 and Ime2 have different roles in *Saccharomyces* and *Neurospora*. Genetics 185:1271–1282

Hwang J, Pallas DC (2014) STRIPAK complexes: structure, biological function, and involvement in human diseases. Int J Biochem Cell Biol 47:118–148

Hynes MJ, Murray SL (2010) ATP-citrate lyase is required for production of cytosolic acetyl coenzyme A and development in *Aspergillus nidulans*. Eukaryot Cell 9:1039–1048

Hynes MJ, Murray SL, Khew GS, Davis MA (2008) Genetic analysis of the role of peroxisomes in the utilization of acetate and fatty acids in *Aspergillus nidulans*. Genetics 178:1355–1369

Idnurm A, Heitman J (2005) Photosensing fungi: phytochrome in the spotlight. Curr Biol 15:R829–R832

Idnurm A, Walton FJ, Floyd A, Heitman J (2008) Identification of the sex genes in an early diverged fungus. Nature 451:193–196

Idnurm A, Verma S, Corrochano LM (2010) A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol 47:881–892

Innocenti FD, Pohl U, Russo VEA (1983) Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem Photobiol 37:49–51

Irniger S (2011) The Ime2 protein kinase family in fungi: more duties than just meiosis. Mol Microbiol 80:1–13

Irniger S, Sarikaya-Bayram Ö, Bayram Ö (2016) Fungal MAP-kinase-mediated regulatory pathways. In: Hoffmeister D (ed) The Mycota III. Biochemistry and molecular biology, 3rd edn. Springer, Berlin, pp 97–117

Ivanova C, Baath JA, Seiboth B, Kubicek CP (2013) Systems analysis of lactose metabolism in *Trichoderma reesei* identifies a lactose permease that is essential for cellulase induction. PLoS One 8: e62631

Ivey FD, Hodge PN, Turner GE, Borkovich KA (1996) The G alpha i homologue *gna-1* controls multiple differentiation pathways in *Neurospora crassa*. Mol Biol Cell 7:1283–1297

Iyer SV, Ramakrishnan M, Kasbekar DP (2009) *Neurospora crassa fmf-1* encodes the homologue of the *Schizosaccharomyces pombe* Ste11p regulator of sexual development. J Genet 88:33–39

Jöhnk B, Bayram Ö, Abelmann A, Heinekamp T, Mattern DJ, Brakhage AA, Jacobsen ID, Valerius O, Braus GH (2016) SCF ubiquitin ligase F-box protein Fbx15 controls nuclear co-repressor localization, stress response and virulence of the human pathogen *Aspergillus fumigatus*. PLoS Pathog 12: e1005899

Johnson AD (1995) Molecular mechanisms of cell-type determination in budding yeast. Curr Opin Genet Dev 5:552–558

Jones SK Jr, Bennett RJ (2011) Fungal mating pheromones: choreographing the dating game. Fungal Genet Biol 48:668–676

Jones J, Wu K, Yang Y, Guerrero C, Nillegoda N, Pan Z-Q, Huang L (2008) A targeted proteomic analysis of the ubiquitin-like modifier Nedd8 and associated proteins. J Proteome Res 7:1274–1287

Jonkers W, Rep M (2009) Lessons from fungal F-box proteins. Eukaryot Cell 8:677–695

Jonkers W, Leeder AC, Ansong C, Wang Y, Yang F, Starr TL, Camp DG II, Smith RD, Glass NL (2014) HAM-5 functions as a MAP kinase scaffold during cell fusion in *Neurospora crassa*. PLoS Genet 10:e1004783

Jonkers W, Fischer MS, Do HP, Starr TL, Glass NL (2016) Chemotropism and cell fusion in *Neurospora crassa* relies on the formation of distinct protein complexes by HAM-5 and a novel protein HAM-14. Genetics 203:319–334

Josefsen L, Droce A, Sondergaard TE, Sørensen JL, Bormann J, Schäfer W, Giese H, Olsson S (2012) Autophagy provides nutrients for nonassimilating fungal structures and is necessary for plant colonization but not for infection in the necrotrophic plant pathogen *Fusarium graminearum*. Autophagy 8:326–337

Jung B, Park J, Son H, Lee YW, Seo YS, Lee J (2014) A putative transcription factor pcs1 positively regulates both conidiation and sexual reproduction in the cereal pathogen *Fusarium graminearum*. Plant Pathol J 30:236–244

Junttila MR, Li SP, Westermarck J (2007) Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival. FASEB J 22:954–965

Käfer E (1965) Origins of translocations in *Aspergillus nidulans*. Genetics 52:217–232

Kamei M, Yamashita K, Takahashi M, Fukumori F, Ichiishi A, Fujimura M (2016) Involvement of MAK-1 and MAK-2 MAP kinases in cell wall integrity in *Neurospora crassa*. Biosci Biotechnol Biochem 80:1843–1852

Kamerewerd J, Jansson M, Nowrousian M, Pöggeler S, Kück U (2008) Three alpha-subunits of heterotrimeric G proteins and an adenylyl cyclase have distinct roles in fruiting body development in the homothallic fungus *Sordaria macrospora*. Genetics 180:191–206

Kang JY, Chun J, Jun SC, Han DM, Chae KS, Jahng KY (2013) The MpkB MAP kinase plays a role in autolysis and conidiation of *Aspergillus nidulans*. Fungal Genet Biol 61:42–49

Kaur G, Sharma A, Guruprasad K, Pati PK (2014) Versatile roles of plant NADPH oxidases and emerging concepts. Biotechnol Adv 32:551–563

Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163

Kayano Y, Tanaka A, Akano F, Scott B, Takemoto D (2013) Differential roles of NADPH oxidases and associated regulators in polarized growth, conidiation and hyphal fusion in the symbiotic fungus *Epichloe festucae*. Fungal Genet Biol 56:87–97

Kazmierczak P, Kim DH, Turina M, Van Alfen NK (2005) A hydrophobin of the chestnut blight fungus, *Cryphonectria parasitica*, is required for stromal pustule eruption. Eukaryot Cell 4:931–936

Kemp HA, Sprague GF Jr (2003) Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast *Saccharomyces cerevisiae*. Mol Cell Biol 23:1750–1763

Kerscher O, Felberbaum R, Hochstrasser M (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. Annu Rev Cell Dev Biol 22:159–180

Keszthelyi A, Jeney A, Kerenyi Z, Mendes O, Waalwijk C, Hornok L (2007) Tagging target genes of the MAT-1-2-1 transcription factor in *Fusarium verticillioides* (*Gibberella fujikuroi* MP-A). Antonie Van Leeuwenhoek 91:373–391

Khaminets A, Behl C, Dikic I (2016) Ubiquitin-dependent and independent signals in selective autophagy. Trends Cell Biol 26:6–16

Khan IA, Lu J-P, Liu X-H, Rehman A, Lin F-C (2012) Multifunction of autophagy-related genes in filamentous fungi. Microbiol Res 167:339–345

Kim H, Borkovich KA (2006) Pheromones are essential for male fertility and sufficient to direct chemotropic polarized growth of trichogynes during mating in *Neurospora crassa*. Eukaryot Cell 5:544–554

Kim H, Metzenberg RL, Nelson MA (2002a) Multiple functions of mfa-1, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1:987–999

Kim HS, Kyu-Yong H, Kim KJ, Han DM, Jahng KY, Chae KS (2002b) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80

Kim H-K, Lee T, Yun S-H (2008) A putative pheromone signaling pathway is dispensable for self-fertility in the homothallic ascomycete *Gibberella zeae*. Fungal Genet Biol 45:1188–1196

Kim HR, Chae KS, Han KH, Han DM (2009) The *nsdC* gene encoding a putative C2H2-type transcription factor is a key activator of sexual development in *Aspergillus nidulans*. Genetics 182:771–783

Kim H, Wright SJ, Park G, Ouyang S, Krystofova S, Borkovich KA (2012) Roles for receptors, pheromones, G proteins, and mating type genes during sexual reproduction in *Neurospora crassa*. Genetics 190:1389–1404

Kim Y, Kim H, Son H, Choi GJ, Kim JC, Lee YW (2014) MYT3, a Myb-like transcription factor, affects fungal development and pathogenicity of *Fusarium graminearum*. PLoS One 9:e94359

Kim H-K, Jo S-M, Kim G-Y, Kim D-W, Kim Y-K, Yun S-H (2015) A large-scale functional analysis of putative target genes of mating-type loci provides insight into the regulation of sexual development of the cereal pathogen *Fusarium graminearum*. PLoS Genet 11:e1005486

Klionsky DJ, Cregg JM, Dunn WA Jr, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, Ohsumi Y (2003) A unified nomenclature for yeast autophagy-related genes. Dev Cell 5:539–545

Klix V, Nowrousian M, Ringelberg C, Loros JJ, Dunlap JC, Pöggeler S (2010) Functional characterization of MAT1-1-specific mating-type genes in the homothallic ascomycete *Sordaria macrospora* provides new insights into essential and nonessential sexual regulators. Eukaryot Cell 9:894–905

Krappmann S, Jung N, Medic B, Busch S, Prade RA, Braus GH (2006) The *Aspergillus nidulans* F-box protein GrrA links SCF activity to meiosis. Mol Microbiol 61:76–88

Kröber A, Etzrodt S, Bach M, Monod M, Kniemeyer O, Staib P, Brakhage AA (2017) The transcriptional regulators SteA and StuA contribute to keratin degradation and sexual reproduction of the dermatophyte *Arthroderma benhamiae*. Curr Genet 63:103–116

Krystofova S, Borkovich KA (2005) The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gβγ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot Cell 4:365–378

Kück U (2005) A *Sordaria macrospora* mutant lacking the *leu1* gene shows a developmental arrest during fruiting body formation. Mol Gen Genomics 274:307–315

Kück U, Pöggeler S (2009) Cryptic sex in fungi. Fungal Biol Rev 23:55–90

Kück U, Beier AM, Teichert I (2016) The composition and function of the striatin-interacting phosphatases and kinases (STRIPAK) complex in fungi. Fungal Genet Biol 90:31–38

Kües U, James TY, Heitman J (2011) Mating type in basidiomycetes: unipolar, bipolar and tetrapolar. In: Pöggeler S, Wöstemeyer J (eds) The Mycota XIV. Evolution of fungi and fungal-like organisms, 1st edn. Springer, Heidelberg, pp 97–160

Kumar S, Yoshida Y, Noda M (1993) Cloning of a cDNA which encodes a novel ubiquitin-like protein. Biochem Biophys Res Commun 195:393–399

Kwon NJ, Garzia A, Espeso EA, Ugalde U, JH Y (2010) FlbC is a putative nuclear C_2H_2 transcription factor regulating development in *Aspergillus nidulans*. Mol Microbiol 77:1203–1219

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158

Lara-Ortiz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50:1241–1255

Lara-Rojas F, Sánchez O, Kawasaki L, Aguirre J (2011) *Aspergillus nidulans* transcription factor AtfA interacts with the MAPK SakA to regulate general stress responses, development and spore functions. Mol Microbiol 80:436–454

Lee EK, Diehl JA (2014) SCFs in the new millennium. Oncogene 33:2011–2018

Lee BY, Han SY, Choi HG, Kim JH, Han KH, Han DM (2005) Screening of growth- or development-related genes by using genomic library with inducible promoter in *Aspergillus nidulans*. J Microbiol 43:523–528

Lee SH, Lee S, Choi D, Lee YW, Yun SH (2006) Identification of the down-regulated genes in a *mat1-2*-deleted strain of *Gibberella zeae*, using cDNA subtraction and microarray analysis. Fungal Genet Biol 43:295–310

Lee J, Leslie JF, Bowden RL (2008) Expression and function of sex pheromones and receptors in the homothallic ascomycete Gibberella zeae. Eukaryot Cell 7:1211–1221

Lee SH, Han YK, Yun SH, Lee YW (2009a) Roles of the glyoxylate and methylcitrate cycles in sexual development and virulence in the cereal pathogen *Gibberella zeae*. Eukaryot Cell 8:1155–1164

Lee SH, Lee J, Lee S, Park EH, Kim KW, Kim MD, Yun SH, Lee YW (2009b) GzSNF1 is required for normal sexual and asexual development in the ascomycete *Gibberella zeae*. Eukaryot Cell 8:116–127

Lee S, Son H, Lee J, Min K, Choi GJ, Kim JC, Lee YW (2011) Functional analyses of two acetyl coenzyme A synthetases in the ascomycete *Gibberella zeae*. Eukaryot Cell 10:1043–1052

Lee MK, Kwon NJ, Choi JM, Lee IS, Jung S, Yu JH (2014) NsdD is a key repressor of asexual development in *Aspergillus nidulans*. Genetics 197:159–173

Lee MK, Kwon NJ, Lee IS, Jung S, Kim SC, Yu JH (2016) Negative regulation and developmental competence in Aspergillus. Sci Rep 6:28874

Lehneck R, Elleuche S, Pöggeler S (2014) The filamentous ascomycete *Sordaria macrospora* can survive in ambient air without carbonic anhydrases. Mol Microbiol 92:931–944

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ (2005) A mitogen-activated protein kinase pathway essential for mating and contributing to vegetative growth in *Neurospora crassa*. Genetics 170:1091–1104

Li L, Wright SJ, Krystofova S, Park G, Borkovich KA (2007) Heterotrimeric G protein signaling in filamentous fungi. Annu Rev Microbiol 61:423–452

Li Y, Wang C, Liu W, Wang G, Kang Z, Kistler HC, Xu JR (2011) The HDF1 histone deacetylase gene is important for conidiation, sexual reproduction, and pathogenesis in *Fusarium graminearum*. Mol Plant-Microbe Interact 24:487–496

Li W-w, Li J, Bao J-k (2012) Microautophagy: lesser-known self-eating. Cell Mol Life Sci 69:1125–1136

Lichius A, Lord KM (2014) Chemoattractive mechanisms in filamentous fungi. Open Mycol J 8:28–57

Lichius A, Lord KM, Jeffree CE, Oborny R, Boonyarungsrit P, Read ND (2012) Importance of MAP kinases during protoperithecial morphogenesis in *Neurospora crassa*. PLoS One 7:e42565

Lin Y, Son H, Lee J, Min K, Choi GJ, Kim JC, Lee YW (2011) A putative transcription factor MYT1 is required for female fertility in the ascomycete *Gibberella zeae*. PLoS One 6:e25586

Lin Y, Son H, Min K, Lee J, Choi GJ, Kim JC, Lee YW (2012) A putative transcription factor MYT2 regulates perithecium size in the ascomycete *Gibberella zeae*. PLoS One 7:e37859

Linke R, Thallinger GG, Haarmann T, Eidner J, Schreiter M, Lorenz P, Seiboth B, Kubicek CP (2015) Restoration of female fertility in *Trichoderma reesei* QM6a provides the basis for inbreeding in this industrial cellulase producing fungus. Biotechnol Biofuels 8:155

Lisa-Santamaría P, Jiménez A, Revuelta JL (2012) The protein factor-arrest 11 (Far11) is essential for the

toxicity of human caspase-10 in yeast and participates in the regulation of autophagy and the DNA damage signaling. J Biol Chem 287:29636–29647

Liu T-B, Liu X-H, Lu J-P, Zhang L, Min H, Lin F-C (2010) The cysteine protease MoAtg4 interacts with MoAtg8 and is required for differentiation and pathogenesis in *Magnaporthe oryzae*. Autophagy 6:74–85

Liu H, Wang Q, He Y, Chen L, Hao C, Jiang C, Li Y, Dai Y, Kang Z, Xu JR (2016) Genome-wide A-to-I RNA editing in fungi independent of ADAR enzymes. Genome Res 26:499–509

Lord KM, Read ND (2011) Perithecium morphogenesis in *Sordaria macrospora*. Fungal Genet Biol 49:388–399

Lydeard JR, Schulman BA, Harper JW (2013) Building and remodelling Cullin–RING E3 ubiquitin ligases. EMBO Rep 14:1050–1061

Lysoe E, Pasquali M, Breakspear A, Kistler HC (2011) The transcription factor FgStuAp influences spore development, pathogenicity, and secondary metabolism in *Fusarium graminearum*. Mol Plant-Microbe Interact 24:54–67

Maerz S, Ziv C, Vogt N, Helmstaedt K, Cohen N, Gorovits R, Yarden O, Seiler S (2008) The nuclear Dbf2-related kinase COT1 and the mitogen-activated protein kinases MAK1 and MAK2 genetically interact to regulate filamentous growth, hyphal fusion and sexual development in *Neurospora crassa*. Genetics 179:1313–1325

Malagnac F, Lalucque H, Lepère G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41:982–997

Marschall R, Tudzynski P (2016) Reactive oxygen species in development and infection processes. Semin Cell Dev Biol 57:138–146

Martin T, Lu SW, van Tilbeurgh H, Ripoll DR, Dixelius C, Turgeon BG, Debuchy R (2010) Tracing the origin of the fungal α1 domain places its ancestor in the HMG-box superfamily: implication for fungal mating-type evolution. PLoS One 5:e15199

Martin SH, Wingfield BD, Wingfield MJ, Steenkamp ET (2011) Causes and consequences of variability in peptide mating pheromones of ascomycete fungi. Mol Biol Evol 28:1987–2003

Masloff S, Pöggeler S, Kück U (1999) The *pro1*+ gene from *Sordaria macrospora* encodes a C_6 zinc finger transcription factor required for fruiting body development. Genetics 152:191–199

May GS, Xue T, Kontoyiannis DP, Gustin MC (2005) Mitogen activated protein kinases of *Aspergillus fumigatus*. Med Mycol 43(Suppl 1):S83–S86

Mayrhofer S, Pöggeler S (2005) Functional characterization of an alpha-factor-like *Sordaria macrospora* peptide pheromone and analysis of its interaction with its cognate receptor in *Saccharomyces cerevisiae*. Eukaryot Cell 4:661–672

Mayrhofer S, Weber JM, Pöggeler S (2006) Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. Genetics 172:1521–1533

McCluskey K, Wiest AE, Grigoriev IV, Lipzen A, Martin J, Schackwitz W, Baker SE (2011) Rediscovery by whole genome sequencing: classical mutations and genome polymorphisms in *Neurospora crassa*. G3 (Bethesda) 1:303–316

Meister C, Kolog Gulko M, Köhler AM, Braus GH (2016) The devil is in the details: comparison between COP9 signalosome (CSN) and the LID of the 26S proteasome. Curr Genet 62:129–136

Mergner J, Schwechheimer C (2014) The NEDD8 modification pathway in plants. Front Plant Sci 5. https://doi.org/10.3389/fpls.2014.00103

Miller KY, Wu J, Miller BL (1992) StuA is required for cell pattern formation in *Aspergillus*. Genes Dev 6:1770–1782

Mochida K, Oikawa Y, Kimura Y, Kirisako H, Hirano H, Ohsumi Y, Nakatogawa H (2015) Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. Nature 522:359–362

Molowitz R, Bahn M, Hock B (1976) The control of fruiting body formation in the ascomycete *Sordaria macrospora* Auersw. by arginine and biotin: a two-factor analysis. Planta 128:143–148

Montenegro-Montero A, Canessa P, Larrondo LF (2015) Around the fungal clock: recent advances in the molecular study of circadian clocks in *Neurospora* and other fungi. Adv Genet 92:107–184

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Moore-Landecker E (1992) Physiology and biochemistry of ascocarp induction and development. Mycol Res 96:705–716

Mousson F, Ochsenbein F, Mann C (2007) The histone chaperone Asf1 at the crossroads of chromatin and DNA checkpoint pathways. Chromosoma 116:79–93

Murray SL, Hynes MJ (2010) Metabolic and developmental effects resulting from deletion of the *citA* gene encoding citrate synthase in *Aspergillus nidulans*. Eukaryot Cell 9:656–666

Nahlik K, Dumkow M, Bayram Ö, Helmstaedt K, Busch S, Valerius O, Gerke J, Hoppert M, Schwier E, Opitz L, Westermann M, Grond S, Feussner K, Goebel C, Kaever A, Meinicke P, Feussner I, Braus GH (2010) The COP9 signalosome mediates transcriptional and metabolic response to hormones, oxidative stress protection and cell wall rearrangement during fungal development. Mol Microbiol 78:964–979

Naider F, Becker JM (2004) The α-factor mating pheromone of *Saccharomyces cerevisiae*: a model for studying the interaction of peptide hormones and G protein-coupled receptors. Peptides 25:1441–1463

Nazarko TY, Ozeki K, Till A, Ramakrishnan G, Lotfi P, Yan M, Subramani S (2014) Peroxisomal Atg37 binds Atg30 or palmitoyl-CoA to regulate phagophore formation during pexophagy. J Cell Biol 204:541–557

Nelson MA, Metzenberg RL (1992) Sexual development genes of *Neurospora crassa*. Genetics 132:149–162

Ni M, Yu JH (2007) A novel regulator couples sporogenesis and trehalose biogenesis in *Aspergillus nidulans*. PLoS One 2:e970

Nolting N, Pöggeler S (2006a) A MADS box protein interacts with a mating-type protein and is required for fruiting body development in the homothallic ascomycete *Sordaria macrospora*. Eukaryot Cell 5:1043–1056

Nolting N, Pöggeler S (2006b) A STE12 homologue of the homothallic ascomycete *Sordaria macrospora* interacts with the MADS box protein MCM1 and is required for ascosporogenesis. Mol Microbiol 62:853–868

Nolting N, Bernhards Y, Pöggeler S (2009) SmATG7 is required for viability in the homothallic ascomycete *Sordaria macrospora*. Fungal Genet Biol 46:531–542

Nordzieke S, Zobel T, Fränzel B, Wolters DA, Kück U, Teichert I (2015) A fungal SLMAP homolog plays a fundamental role in development and localizes to the nuclear envelope, ER, and mitochondria. Eukaryot Cell:345–358

Nowrousian M (2014) Genomics and transcriptomics to analyze fruiting body development. In: Nowrousian M (ed) The Mycota XIII. Fungal genomics, 2nd edn. Springer, Berlin, pp 149–172

Nowrousian M, Masloff S, Pöggeler S, Kück U (1999) Cell differentiation during sexual development of the fungus *Sordaria macrospora* requires ATP citrate lyase activity. Mol Cell Biol 19:450–460

Nowrousian M, Ringelberg C, Dunlap JC, Loros JJ, Kück U (2005) Cross-species microarray hybridization to identify developmentally regulated genes in the filamentous fungus *Sordaria macrospora*. Mol Gen Genomics 273:137–149

Nowrousian M, Frank S, Koers S, Strauch P, Weitner T, Ringelberg C, Dunlap JC, Loros JJ, Kück U (2007) The novel ER membrane protein PRO41 is essential for sexual development in the filamentous fungus *Sordaria macrospora*. Mol Microbiol 64:923–937

Nowrousian M, Teichert I, Masloff S, Kück U (2012) Whole-genome sequencing of *Sordaria macrospora* mutants identifies developmental genes. G3 (Bethesda) 2:261–270

Oda K, Hasunuma K (1997) Genetic analysis of signal transduction through light-induced protein phosphorylation in *Neurospora crassa* perithecia. Mol Gen Genet 256:593–601

Ohsumi Y (2014) Historical landmarks of autophagy research. Cell Res 24:9–23

Oiartzabal-Arano E, Garzia A, Gorostidi A, Ugalde U, Espeso EA, Etxebeste O (2015) Beyond asexual development: modifications in the gene expression profile caused by the absence of the *Aspergillus nidulans* transcription factor FlbB. Genetics 199:1127–1142

Okamoto K (2014) Organellophagy: eliminating cellular building blocks via selective autophagy. J Cell Biol 205:435–445

Olmedo M, Ruger-Herreros C, Luque EM, Corrochano LM (2010) A complex photoreceptor system mediates the regulation by light of the conidiation genes con-10 and con-6 in *Neurospora crassa*. Fungal Genet Biol 47:352–363

Ortiz CS, Shim WB (2013) The role of MADS-box transcription factors in secondary metabolism and sexual development in the maize pathogen *Fusarium verticillioides*. Microbiology 159:2259–2268

Palmer GE, Askew DS, Williamson PR (2008) The diverse roles of autophagy in medically important fungi. Autophagy 4:982–988

Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, Lutzoni F, Debeaupuis J-P, Latgé J-P, Denning DW, Dyer PS (2005) Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr Biol 15:1242–1248

Paoletti M, Seymour FA, Alcocer MJC, Kaur N, Calvo AM, Archer DB, Dyer PS (2007) Mating type and the genetic basis of self-fertility in the model fungus *Aspergillus nidulans*. Curr Biol 17:1384–1389

Park G, Pan S, Borkovich KA (2008) Mitogen-activated protein kinase cascade required for regulation of development and secondary metabolism in *Neurospora crassa*. Eukaryot Cell 7:2113–2122

Park G, Servin JA, Turner GE, Altamirano L, Colot HV, Collopy P, Litvinkova L, Li L, Jones CA, Diala FG, Dunlap JC, Borkovich KA (2011) Global analysis of serine-threonine protein kinase genes in *Neurospora crassa*. Eukaryot Cell 10:1553–1564

Park HS, Nam TY, Han KH, Kim SC, Yu JH (2014) VelC positively controls sexual development in *Aspergillus nidulans*. PLoS One 9:e89883

Peraza-Reyes L, Berteaux-Lecellier V (2013) Peroxisomes and sexual development in fungi. Front Physiol 4:244. https://doi.org/10.3389/fphys.2013.00244

Peraza-Reyes L, Malagnac F (2016) Sexual development in fungi. In: Wendland J (ed) The Mycota I. Growth, differentiation and sexuality, 3rd edn. Springer, Berlin, pp 407–455

Peraza-Reyes L, Zickler D, Berteaux-Lecellier V (2008) The peroxisome RING-finger complex is required for meiocyte formation in the fungus *Podospora anserina*. Traffic 9:1998–2009

Pierce NW, Lee JE, Liu X, Sweredoski Michael J, Graham Robert LJ, Larimore Elizabeth A, Rome M, Zheng N, Clurman Bruce E, Hess S, Shan S-o, Deshaies Raymond J (2013) Cand1 promotes

assembly of new SCF complexes through dynamic exchange of F box proteins. Cell 153:206–215
Pijnappel WW, Schaft D, Roguev A, Shevchenko A, Tekotte H, Wilm M, Rigaut G, Séraphin B, Aasland R, Stewart AF (2001) The *S. cerevisiae* SET3 complex includes two histone deacetylases, Hos2 and Hst1, and is a meiotic-specific repressor of the sporulation gene program. Genes Dev 15: 2991–3004
Pinan-Lucarré B, Paoletti M, Dementhon K, Coulary-Salin B, Clavé C (2003) Autophagy is induced during cell death by incompatibility and is essential for differentiation in the filamentous fungus *Podospora anserina*. Mol Microbiol 47:321–333
Pinan-Lucarré B, Balguerie A, Clavé C (2005) Accelerated cell death in *Podospora* autophagy mutants. Eukaryot Cell 4:1765–1774
Pöggeler S (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37:403–411
Pöggeler S (2011) Pheromone receptors in filamentous ascomycetes. In: Pöggeler S, Wöstemeyer J (eds) The Mycota XIV. Evolution of fungi and fungal-like organism. Springer, Berlin, pp 73–96
Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280:9–17
Pöggeler S, Kück U (2004) A WD40 repeat protein regulates fungal cell differentiation and can be replaced functionally by the mammalian homologue striatin. Eukaryot Cell 3:232–240
Pöggeler S, Risch S, Kück U, Osiewacz HD (1997) Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics 147:567–580
Pöggeler S, Nowrousian M, Kück U (2006a) Fruiting body development in ascomycetes. In: Kües U, Fischer R (eds) The Mycota I. Growth, differentiation and sexuality, 2nd edn. Springer, Berlin, pp 325–355
Pöggeler S, Nowrousian M, Ringelberg C, Loros JJ, Dunlap JC, Kück U (2006b) Microarray and real time PCR analyses reveal mating type-dependent gene expression in a homothallic fungus. Mol Gen Genomics 275:492–503
Pollack JK, Harris SD, Marten MR (2009) Autophagy in filamentous fungi. Fungal Genet Biol 46:1–8
Pracheil T, Liu Z (2013) Tiered assembly of the yeast Far3-7-8-9-10-11 complex at the endoplasmic reticulum. J Biol Chem 288:16986–16997
Prade RA, Cruz AK, Terenzi HF (1984) Regulation of tyrosinase during the vegetative and sexual life-cycles of *Neurospora crassa*. Arch Microbiol 140:236–242
Purschwitz J, Müller S, Kastner C, Schöser M, Haas H, Espeso EA, Atoui A, Calvo AM, Fischer R (2008) Functional and physical interaction of blue- and red-light sensors in *Aspergillus nidulans*. Curr Biol 18:255–259
Rabut G, Peter M (2008) Function and regulation of protein neddylation. EMBO Rep 9:969–976
Ragnelli AM, Pacioni G, Aimola P, Lanza B, Miranda M (1992) Truffle melanogenesis: correlation with reproductive differentiation and ascocarp ripening. Pigment Cell Res 5:205–212
Rahikainen M, Pascual J, Alegre S, Durian G, Kangasjarvi S (2016) PP2A phosphatase as a regulator of ROS signaling in plants. Antioxidants (Basel) 5:pii: E8. doi:https://doi.org/10.3390/antiox5010008
Ramamoorthy V, Shantappa S, Dhingra S, Calvo AM (2012) veA-dependent RNA-pol II transcription elongation factor-like protein, RtfA, is associated with secondary metabolism and morphological development in *Aspergillus nidulans*. Mol Microbiol 85:795–814
Ramamoorthy V, Dhingra S, Kincaid A, Shantappa S, Feng X, Calvo AM (2013) The putative C2H2 transcription factor MtfA is a novel regulator of secondary metabolism and morphogenesis in *Aspergillus nidulans*. PLoS One 8:e74122
Raudaskoski M, Kothe E (2010) Basidiomycete mating type genes and pheromone signaling. Eukaryot Cell 9:847–859
Read ND, Goryachev AB, Lichius A (2012) The mechanistic basis of self-fusion between conidial anastomosis tubes during fungal colony initiation. Fungal Biol Rev 26:1–11
Reggiori F, Klionsky DJ (2013) Autophagic processes in yeast: mechanism, machinery and regulation. Genetics 194:341–361
Richthammer C, Enseleit M, Sanchez-Leon E, März S, Heilig Y, Riquelme M, Seiler S (2012) RHO1 and RHO2 share partially overlapping functions in the regulation of cell wall integrity and hyphal polarity in *Neurospora crassa*. Mol Microbiol 85:716–733
Roca MG, Weichert M, Siegmund U, Tudzynski P, Fleissner A (2012) Germling fusion via conidial anastomosis tubes in the grey mould *Botrytis cinerea* requires NADPH oxidase activity. Fungal Biol 116:379–387
Rockwell NC, Lagarias JC (2010) A brief history of phytochromes. ChemPhysChem 11:1172–1180
Ropars J, Lopez-Villavicencio M, Dupont J, Snirc A, Gillot G, Coton M, Jany JL, Coton E, Giraud T (2014) Induction of sexual reproduction and genetic diversity in the cheese fungus *Penicillium roqueforti*. Evol Appl 7:433–441
Rosen S, Yu JH, Adams TH (1999) The *Aspergillus nidulans* sfaD gene encodes a G protein beta subunit that is required for normal growth and repression of sporulation. EMBO J 18:5592–5600
Sacristán-Reviriego A, Martin H, Molina M (2015) Identification of putative negative regulators of yeast signaling through a screening for protein phosphatases acting on cell wall integrity and mating MAPK pathways. Fungal Genet Biol 77:1–11

Sarikaya-Bayram O, Bayram O, Feussner K, Kim JH, Kim HS, Kaever A, Feussner I, Chae KS, Han DM, Han KH, Braus GH (2014) Membrane-bound methyltransferase complex VapA-VipC-VapB guides epigenetic control of fungal development. Dev Cell 29:406–420

Sarikaya-Bayram Ö, Palmer JM, Keller N, Braus GH, Bayram Ö (2015) One Juliet and four Romeos: VeA and its methyltransferases. Front Microbiol 6:1

Saupe S, Stenberg L, Shiu KT, Griffiths AJ, Glass NL (1996) The molecular nature of mutations in the *mt A-1* gene of the *Neurospora crassa* A idiomorph and their relation to mating-type function. Mol Gen Genet 250:115–122

Scheckhuber CQ, Osiewacz HD (2008) *Podospora anserina*: a model organism to study mechanisms of healthy ageing. Mol Gen Genomics 280:365–374

Schindler D, Nowrousian M (2014) The polyketide synthase gene *pks4* is essential for sexual development and regulates fruiting body morphology in *Sordaria macrospora*. Fungal Genet Biol 68:48–59

Schinke J, Kolog Gulko M, Christmann M, Valerius O, Stumpf SK, Stirz M, Braus GH (2016) The DenA/DEN1 interacting phosphatase DipA controls septa positioning and phosphorylation-dependent stability of cytoplasmatic DenA/DEN1 during fungal development. PLoS Genet 12:e1005949

Schmoll M, Wang T-F (2016) Sexual development in *Trichoderma*. In: Wendland J (ed) The Mycota I. Growth, differentiation and sexuality, 3rd edn. Springer, Berlin, pp 457–474

Schmoll M, Esquivel-Naranjo EU, Herrera-Estrella A (2010a) *Trichoderma* in the light of day – physiology and development. Fungal Genet Biol 47:909–916

Schmoll M, Seibel C, Tisch D, Dorrer M, Kubicek CP (2010b) A novel class of peptide pheromone precursors in ascomycetous fungi. Mol Microbiol 77:1483–1501

Schmoll M, Dattenbock C, Carreras-Villasenor N, Mendoza-Mendoza A, Tisch D, Aleman MI, Baker SE, Brown C, Cervantes-Badillo MG, Cetz-Chel J, Cristobal-Mondragon GR, Delaye L, Esquivel-Naranjo EU, Frischmann A, Gallardo-Negrete Jde J, Garcia-Esquivel M, Gomez-Rodriguez EY, Greenwood DR, Hernandez-Onate M, Kruszewska JS, Lawry R, Mora-Montes HM, Munoz-Centeno T, Nieto-Jacobo MF, Nogueira Lopez G, Olmedo-Monfil V, Osorio-Concepcion M, Pilsyk S, Pomraning KR, Rodriguez-Iglesias A, Rosales-Saavedra MT, Sanchez-Arreguin JA, Seidl-Seiboth V, Stewart A, Uresti-Rivera EE, Wang CL, Wang TF, Zeilinger S, Casas-Flores S, Herrera-Estrella A (2016) The genomes of three uneven siblings: footprints of the lifestyles of three *Trichoderma* species. Microbiol Mol Biol Rev 80:205–327

Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lucking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol 58:224–239

Schumacher J (2017) How light affects the life of Botrytis. Fungal Genet Biol 106:26–41

Schumacher J, Simon A, Cohrs KC, Viaud M, Tudzynski P (2014) The transcription factor BcLTF1 regulates virulence and light responses in the necrotrophic plant pathogen *Botrytis cinerea*. PLoS Genet 10:e1004040

Schuster A, Tisch D, Seidl-Seiboth V, Kubicek CP, Schmoll M (2012) Roles of protein kinase A and adenylate cyclase in light-modulated cellulase regulation in *Trichoderma reesei*. Appl Environ Microbiol 78:2168–2178

Scott B, Eaton CJ (2008) Role of reactive oxygen species in fungal cellular differentiations. Curr Opin Microbiol 11:488–493

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012a) ENVOY is a major determinant in regulation of sexual development in *Hypocrea jecorina* (*Trichoderma reesei*). Eukaryot Cell 11:885–895

Seibel C, Tisch D, Kubicek CP, Schmoll M (2012b) The role of pheromone receptors for communication and mating in *Hypocrea jecorina* (*Trichoderma reesei*). Fungal Genet Biol 49:814–824

Seidl V, Seibel C, Kubicek CP, Schmoll M (2009) Sexual development in the industrial workhorse *Trichoderma reesei*. Proc Natl Acad Sci U S A 106:13909–13914

Seo JA, Han KH, JH Y (2004) The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol Microbiol 53:1611–1623

Seo JA, Han KH, JH Y (2005) Multiple roles of a heterotrimeric G-protein gamma-subunit in governing growth and development of *Aspergillus nidulans*. Genetics 171:81–89

Shelest E (2008) Transcription factors in fungi. FEMS Microbiol Lett 286:145–151

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Shim WB, Sagaram US, Choi YE, So J, Wilkinson HH, Lee YW (2006) FSR1 is essential for virulence and female fertility in *Fusarium verticillioides* and *F. graminearum*. Mol Plant-Microbe Interact 19:725–733

Sikhakolli UR, Lopez-Giraldez F, Li N, Common R, Townsend JP, Trail F (2012) Transcriptome analyses during fruiting body formation in *Fusarium graminearum* and *Fusarium verticillioides* reflect species life history and ecology. Fungal Genet Biol 49:663–673

Singh NS, Shao N, McLean JR, Sevugan M, Ren L, Chew TG, Bimbo A, Sharma R, Tang X, Gould KL, Balasubramanian MK (2011) SIN-inhibitory phosphatase complex promotes Cdc11p dephosphorylation and propagates SIN asymmetry in fission yeast. Curr Biol 21:1968–1978

Smith KM, Sancar G, Dekhang R, Sullivan CM, Li SJ, Tag AG, Sancar C, Bredeweg EL, Priest HD, McCormick RF, Thomas TL, Carrington JC, Stajich JE, Bell-Pedersen D, Brunner M, Freitag M (2010) Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for *Neurospora* white collar complex. Eukaryot Cell 9:1549–1556

Son H, Lee J, Park AR, Lee YW (2011a) ATP citrate lyase is required for normal sexual and asexual development in *Gibberella zeae*. Fungal Genet Biol 48:408–417

Son H, Seo YS, Min K, Park AR, Lee J, Jin JM, Lin Y, Cao P, Hong SY, Kim EK, Lee SH, Cho A, Lee S, Kim MG, Kim Y, Kim JE, Kim JC, Choi G, Yun SH, Lim JY, Kim M, Lee YH, Choi YD, Lee YW (2011b) A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. PLoS Pathog 7:e1002310

Son H, Min K, Lee J, Choi GJ, Kim JC, Lee YW (2012) Mitochondrial carnitine-dependent acetyl coenzyme A transport is required for normal sexual and asexual development of the ascomycete *Gibberella zeae*. Eukaryot Cell 11:1143–1153

Son H, Park AR, Lim JY, Shin C, Lee YW (2017) Genome-wide exonic small interference RNA-mediated gene silencing regulates sexual reproduction in the homothallic fungus *Fusarium graminearum*. PLoS Genet 13:e1006595

Sontag EM, Vonk WIM, Frydman J (2014) Sorting out the trash: the spatial nature of eukaryotic protein quality control. Curr Opin Cell Biol 26:139–146

Sorensen JL, Akk E, Thrane U, Giese H, Sondergaard TE (2013) Production of fusarielins by *Fusarium*. Int J Food Microbiol 160:206–211

Spellig T, Bölker M, Lottspeich F, Frank RW, Kahmann R (1994) Pheromones trigger filamentous growth in *Ustilago maydis*. EMBO J 13:1620–1627

Staben C, Yanofsky C (1990) *Neurospora crassa* ***a*** mating-type region. Proc Natl Acad Sci U S A 87:4917–4921

Stanley RE, Ragusa MJ, Hurley JH (2014) The beginning of the end: how scaffolds nucleate autophagosome biogenesis. Trends Cell Biol 24:73–81

Steffens EK, Becker K, Krevet S, Teichert I, Kück U (2016) Transcription factor PRO1 targets genes encoding conserved components of fungal developmental signaling pathways. Mol Microbiol 102:792–809

Sun Q, Choi GH, Nuss DL (2009) Hypovirus-responsive transcription factor gene *pro1* of the chestnut blight fungus *Cryphonectria parasitica* is required for female fertility, asexual spore development, and stable maintenance of hypovirus infection. Eukaryot Cell 8:262–270

Suzuki K, Kubota Y, Sekito T, Ohsumi Y (2007) Hierarchy of Atg proteins in pre-autophagosomal structure organization. Genes Cells 12:209–218

Szewczyk E, Krappmann S (2010) Conserved regulators of mating are essential for *Aspergillus fumigatus* cleistothecium formation. Eukaryot Cell 9:774–783

Tanaka K, Hirayama K, Yonezawa H, Sato G, Toriyabe A, Kudo H, Hashimoto A, Matsumura M, Harada Y, Kurihara Y, Shirouzu T, Hosoya T (2015) Revision of the *Massarineae* (*Pleosporales, Dothideomycetes*). Stud Mycol 82:75–136

Teichert I, Nowrousian M (2011) Evolution of genes for secondary metabolism in fungi. In: Pöggeler S, Wöstemeyer J (eds) The Mycota XIV. Evolution of fungi and fungal-like organisms, 1st edn. Springer, Berlin, pp 231–255

Teichert I, Wolff G, Kück U, Nowrousian M (2012) Combining laser microdissection and RNA-seq to chart the transcriptional landscape of fungal development. BMC Genomics 13:511

Teichert I, Nowrousian M, Pöggeler S, Kück U (2014a) The filamentous fungus *Sordaria macrospora* as a genetic model to study fruiting body development. Adv Genet 87:199–244

Teichert I, Steffens EK, Schnass N, Fränzel B, Krisp C, Wolters DA, Kück U (2014b) PRO40 is a scaffold protein of the cell wall integrity pathway, linking the MAP kinase module to the upstream activator protein kinase C. PLoS Genet 10:e1004582

Tisch D, Schmoll M (2013) Targets of light signalling in *Trichoderma reesei*. BMC Genomics 14:657

Tisch D, Kubicek CP, Schmoll M (2011) New insights into the mechanism of light modulated signaling by heterotrimeric G-proteins: ENVOY acts on gna1 and gna3 and adjusts cAMP levels in *Trichoderma reesei* (*Hypocrea jecorina*). Fungal Genet Biol 48:631–640

Tisch D, Schuster A, Schmoll M (2014) Crossroads between light response and nutrient signalling: ENV1 and PhLP1 act as mutual regulatory pair in *Trichoderma reesei*. BMC Genomics 15:425

Todd RB, Zhou M, Ohm RA, Leeggangers HA, Visser L, de Vries RP (2014) Prevalence of transcription factors in ascomycete and basidiomycete fungi. BMC Genomics 15:214

Traeger S, Nowrousian M (2015) Analysis of circadian rhythms in the basal filamentous ascomycete *Pyronema confluens*. G3 (Bethesda) 5:2061–2071

Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar A, Marcet-Houben M, Pöggeler S, Stajich JE, Nowrousian M (2013) The genome and devel-

opment-dependent transcriptomes of *Pyronema confluens*: a window into fungal evolution. PLoS Genet 9:e1003820

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2005) Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*. Microbiology 151:1809–1821

Tüncher A, Reinke H, Martic G, Caruso ML, Brakhage AA (2004) A basic-region helix-loop-helix protein-encoding gene (*devR*) involved in the development of *Aspergillus nidulans*. Mol Microbiol 52:227–241

Turgeon G, Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet Biol 31:1–5

Turgeon BG, Bohlmann H, Ciuffetti LM, Christiansen SK, Yang G, Schäfer W, Yoder OC (1993) Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol Gen Genet 238:270–284

Turina M, Prodi A, Alfen NKV (2003) Role of the *Mf1-1* pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40:242–251

Turrà D, El Ghalid M, Rossi F, Di Pietro A (2015) Fungal pathogen uses sex pheromone receptor for chemotropic sensing of host plant signals. Nature 527:521–524

Turrà D, Nordzieke D, Vitale S, El Ghalid M, Di Pietro A (2016) Hyphal chemotropism in fungal pathogenicity. Semin Cell Dev Biol 57:69–75

Vallim MA, Miller KY, Miller BL (2000) Aspergillus SteA (sterile12-like) is a homeodomain-C2/H2-Zn +2 finger transcription factor required for sexual reproduction. Mol Microbiol 36:290–301

Van Dijck P, Brown NA, Goldman GH, Rutherford J, Xue C, Van Zeebroeck G (2017) Nutrient sensing at the plasma membrane of fungal cells. Microbiol Spectr 5. https://doi.org/10.1128/microbiolspec.FUNK-0031-2016

Videira A, Duarte M (2002) From NADH to ubiquinone in *Neurospora* mitochondria. Biochim Biophys Acta 1555:187–191

Vienken K, Fischer R (2006) The $Zn(II)_2Cys_6$ putative transcription factor NosA controls fruiting body formation in *Aspergillus nidulans*. Mol Microbiol 61:544–554

Vienken K, Scherer M, Fischer R (2005) The Zn(II) 2Cys6 putative *Aspergillus nidulans* transcription factor repressor of sexual development inhibits sexual development under low-carbon conditions and in submersed culture. Genetics 169:619–630

Vitale S, Partida-Hanon A, Serrano S, Martínez-del-Pozo Á, Di Pietro A, Turrà D, Bruix M (2017) Structure-activity relationship of α mating pheromone from the fungal pathogen *Fusarium oxysporum*. J Biol Chem 292:3591–3602

Vittal V, Stewart MD, Brzovic PS, Klevit RE (2015) Regulating the regulators: recent revelations in the control of E3 ubiquitin ligases. J Biol Chem 290:21244–21251

Voigt O, Pöggeler S (2013a) Autophagy genes *Smatg8* and *Smatg4* are required for fruiting-body development, vegetative growth and ascospore germination in the filamentous ascomycete *Sordaria macrospora*. Autophagy 9:33–49

Voigt O, Pöggeler S (2013b) Self-eating to grow and kill: autophagy in filamentous ascomycetes. Appl Microbiol Biotechnol 97:9277–9290

Voigt O, Herzog B, Jakobshagen A, Pöggeler S (2013) bZIP transcription factor SmJLB1 regulates autophagy-related genes *Smatg8* and *Smatg4* and is required for fruiting-body development and vegetative growth in *Sordaria macrospora*. Fungal Genet Biol 61:50–60

von Zeska Kress MR, Harting R, Bayram Ö, Christmann M, Irmer H, Valerius O, Schinke J, Goldman GH, Braus GH (2012) The COP9 signalosome counteracts the accumulation of cullin SCF ubiquitin E3 RING ligases during fungal development. Mol Microbiol 83:1162–1177

Voss TC, Hager GL (2014) Dynamic regulation of transcriptional states by chromatin and transcription factors. Nat Rev Genet 15:69–81

Wang C-L, Shim W-B, Shaw BD (2010a) *Aspergillus nidulans* striatin (StrA) mediates sexual development and localizes to the endoplasmic reticulum. Fungal Genet Biol 47:789–799

Wang J, Hu Q, Chen H, Zhou Z, Li W, Wang Y, Li S, He Q (2010b) Role of individual subunits of the *Neurospora crassa* CSN complex in regulation of deneddylation and stability of cullin proteins. PLoS Genet 6:e1001232

Wang Y, Liu W, Hou Z, Wang C, Zhou X, Jonkers W, Ding S, Kistler HC, JR X (2011) A novel transcriptional factor important for pathogenesis and ascosporogenesis in *Fusarium graminearum*. Mol Plant-Microbe Interact 24:118–128

Wang W, Wu D, Pan H, Turgeon BG (2014a) Vel2 and Vos1 hold essential roles in ascospore and asexual spore development of the heterothallic maize pathogen *Cochliobolus heterostrophus*. Fungal Genet Biol 70:113–124

Wang Z, Lopez-Giraldez F, Lehr N, Farré M, Common R, Trail F, Townsend JP (2014b) Global gene expression and focused knockout analysis reveals genes associated with fungal fruiting body development in *Neurospora crassa*. Eukaryot Cell 13:154–169

Wang L, Liu Y, Liu J, Zhang Y, Zhang X, Pan H (2016a) The *Sclerotinia sclerotiorum* FoxE2 gene is required for apothecial development. Phytopathology 106:484–490

Wang Z, Li N, Li JG, Dunlap JC, Trail F, Townsend JP (2016b) The fast-evolving *phy-2* gene modulates sexual development in response to light in the model fungus *Neurospora crassa*. MBio 7

Warner MH, Roinick KL, Arndt KM (2007) Rtf1 is a multifunctional component of the Paf1 complex that regulates gene expression by directing cotranscriptional histone modification. Mol Cell Biol 27:6103–6115

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324:1076–1080

Werner A, Herzog B, Frey S, Pöggeler S (2016) Autophagy-associated protein SmATG12 is required for fruiting-body formation in the filamentous ascomycete *Sordaria macrospora*. PLoS One 11: e0157960

Willer M, Hoffmann L, Styrkarsdottir U, Egel R, Davey J, Nielsen O (1995) Two-step activation of meiosis by the *mat1* locus in *Schizosaccharomyces pombe*. Mol Cell Biol 15:4964–4970

Wirsel S, Horwitz B, Yamaguchi K, Yoder OC, Turgeon BG (1998) Single mating type-specific genes and their 3' UTRs control mating and fertility in *Cochliobolus heterostrophus*. Mol Gen Genet 259:272–281

Wong KH, Hynes MJ, Todd RB, Davis MA (2009) Deletion and overexpression of the *Aspergillus nidulans* GATA factor AreB reveals unexpected pleiotropy. Microbiology 155:3868–3880

Wu J, Miller BL (1997) *Aspergillus* asexual reproduction and sexual reproduction are differentially affected by transcriptional and translational mechanisms regulating stunted gene expression. Mol Cell Biol 17:6191–6201

Wu D, Oide S, Zhang N, Choi MY, Turgeon BG (2012) ChLae1 and ChVel1 regulate T-toxin production, virulence, oxidative stress response, and development of the maize pathogen *Cochliobolus heterostrophus*. PLoS Pathog 8:e1002542

Yamasaki A, Noda NN (2017) Structural biology of the Cvt pathway. J Mol Biol 429:531–542

Yang C, Liu H, Li G, Liu M, Yun Y, Wang C, Ma Z, JR X (2015) The MADS-box transcription factor FgMcm1 regulates cell identity and fungal development in *Fusarium graminearum*. Environ Microbiol 17:2762–2776

Yang T, Guo M, Yang H, Guo S, Dong C (2016) The blue-light receptor CmWC-1 mediates fruit body development and secondary metabolism in *Cordyceps militaris*. Appl Environ Microbiol 100:743–755

Yao Z, Delorme-Axford E, Backues SK, Klionsky DJ (2015) Atg41/Icy2 regulates autophagosome formation. Autophagy 11:2288–2299

Yin W, Keller NP (2011) Transcriptional regulatory elements in fungal secondary metabolism. J Microbiol 49:329–339

Yin WB, Reinke AW, Szilágyi M, Emri T, Chiang YM, Keating AE, Pócsi I, Wang CC, Keller NP (2013) bZIP transcription factors affecting secondary metabolism, sexual development and stress responses in *Aspergillus nidulans*. Microbiology 159:77–88

Yin Z, Pascual C, Klionsky DJ (2016) Autophagy: machinery and regulation. Microb Cell 3:588–596

Yu CCC (1954) The culture and spore germination of *Ascobolus* with emphasis on *A. magnificus*. Am J Bot 41:21–30

Yu Z, Armant O, Fischer R (2016) Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. Nat Microbiol 1:16019

Yun SH, Yoder OC, Turgeon BG (2013) Structure and function of the mating-type locus in the homothallic ascomycete, *Didymella zeae-maydis*. J Microbiol 51:814–820

Zhang Z, Pugh BF (2011) High-resolution genome-wide mapping of the primary structure of chromatin. Cell 144:175–186

Zhang L, Baasiri RA, van Alfen NK (1998) Viral repression of the fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

Zhang Z, Li H, Qin G, He C, Li B, Tian S (2016) The MADS-Box transcription factor Bcmads1 is required for growth, sclerotia production and pathogenicity of *Botrytis cinerea*. Sci Rep 6:33901

Zheng Q, Hou R, Zhang J, Ma J, Wu Z, Wang GL, Wang C, JR X (2013) The MAT locus genes play different roles in sexual reproduction and pathogenesis in *Fusarium graminearum*. PLoS One 8: e66980

Zhou Z, Wang Y, Cai G, He Q (2012) Neurospora COP9 signalosome integrity plays major roles for hyphal growth, conidial development, and circadian function. PLoS Genet 8:e1002712

Fungal Inteins: Distribution, Evolution, and Applications

Skander Elleuche[1], Stefanie Pöggeler[2,3]

CONTENTS

I. Introduction

Since the late 1970s, it has become clear that the coding sequence of many eukaryotic genes is disrupted by genetic elements called intervening sequences, which must be removed prior to host gene function. Intervening sequences can be classified into introns and inteins. Introns are excised from the primary RNA transcript by a process termed splicing. In contrast to introns, inteins are internal in-frame insertions transcribed and translated together with their host protein and excised at the protein level (Fig. 1). By analogy to introns and exons, the terms *intein* for internal protein sequence and *extein* for external protein sequence of the precursor have been proposed. Upstream exteins are termed the N-extein and downstream exteins the C-extein. The posttranslational process that excises the internal region from a precursor protein with subsequent ligation of the N- and C-extein is termed protein splicing (Callahan et al. 2011; Perler et al. 1994; Saleh and Perler 2006). Thus, protein splicing is a processing event in which the expression of a single gene results in production of two stable proteins, the mature protein and the intein (Fig. 1).

Protein splicing elements were first described in fungi. In 1990, two groups reported an in-frame insertion in the *Saccharomyces cerevisiae VMA1* gene encoding a vacuolar membrane H^+-ATPase (Hirata et al. 1990; Kane et al. 1990). The nucleotide sequence of the *Saccharomyces cerevisiae VMA1* gene predicts a polypeptide of 1071 amino acids with a calculated molecular mass of 118 kDa, which was much larger than the 67 kDa value estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The N- and C-terminal regions of the deduced sequence were shown to be very similar to those of the catalytic subunits of vacuolar membrane H^+-ATPases of other organisms, while the internal region containing 454 amino acid residues displayed no detectable sequence similarities to any known ATPase subunits and instead exhibits similarity to a yeast endonuclease encoded by the *HO* gene. Subsequently, it was demonstrated that

[1] Miltenyi Biotec GmbH, Bergisch Gladbach, Germany
[2] Abteilung Genetik eukaryotischer Mikroorganismen, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Göttingen, Germany; e-mail: spoegge@gwdg.de
[3] Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University Göttingen, Göttingen, Germany

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

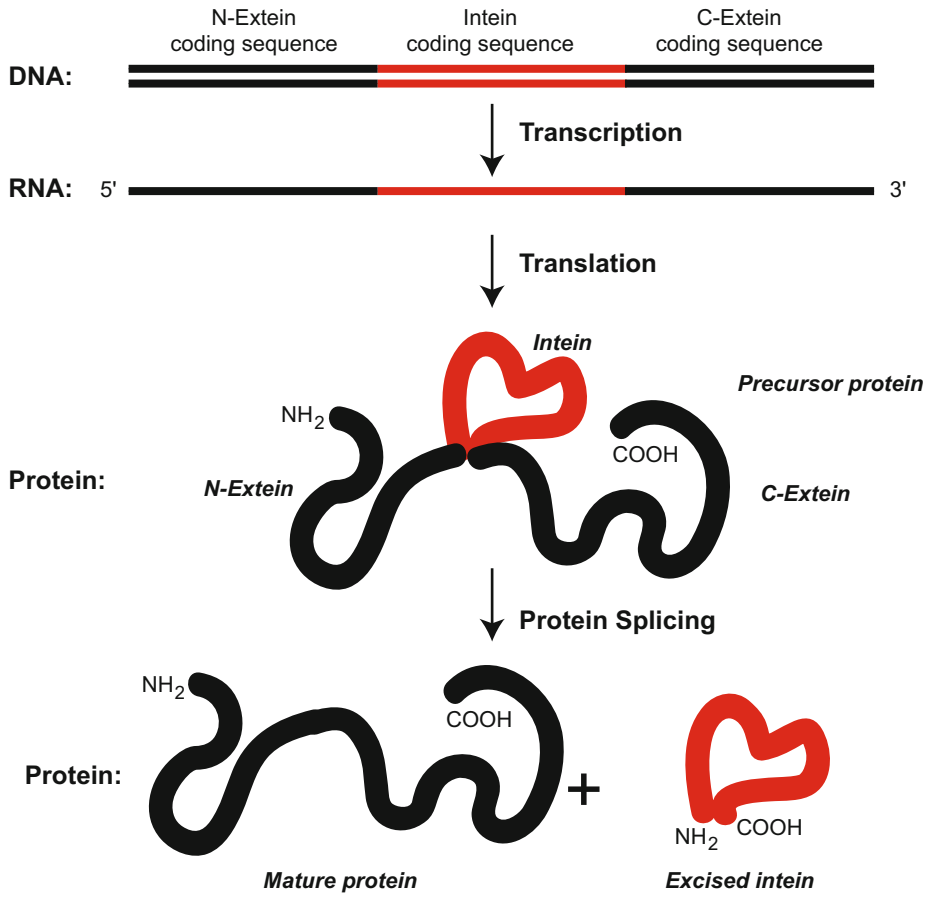


Fig. 1 Protein splicing of inteins. The intein-coding sequence is transcribed into mRNA and translated to a nonfunctional protein precursor, which then undergoes a self-catalyzed rearrangement in which the intein is excised and the exteins are joined to yield the mature protein

the insertion was still present in the mRNA and translated together with the VMA1 protein, and that the insertion spliced itself out of the protein. (Kane et al. 1990)

By now, more than 1000 inteins have been recognized in the genomes of bacteria, archaea, and eukaryotes as well as in viruses. Inteins have predominantly been identified in prokaryotes, but are also found in 71 eukaryotic genomes (Novikova et al. 2016). Eukaryotic inteins are encoded within the nuclear genomes of fungi, as well as within the nuclear genomes and plastomes of some unicellular green algae (Novikova et al. 2014). For information, see the intein registry InBase at http://www.inteins.com/ (Perler 2002) which was generated by individual entries of intein researchers, but was not updated since 2010. Since then the growing number of fully sequenced microbial genomes encourages computer-aided identification in diverse species (Novikova et al. 2016).

According to accepted nomenclature, intein names include a genus and species designation, abbreviated with three letters, and a host gene designation. For example, the *S. cerevisiae* VMA1 intein is called *Sce* VMA1. When a protein contains more than one intein, it is numbered with Roman numerals (Perler et al. 1994).

This review focuses on fungal inteins and will include the characteristics, as well as the distribution of fungal inteins. It summarizes

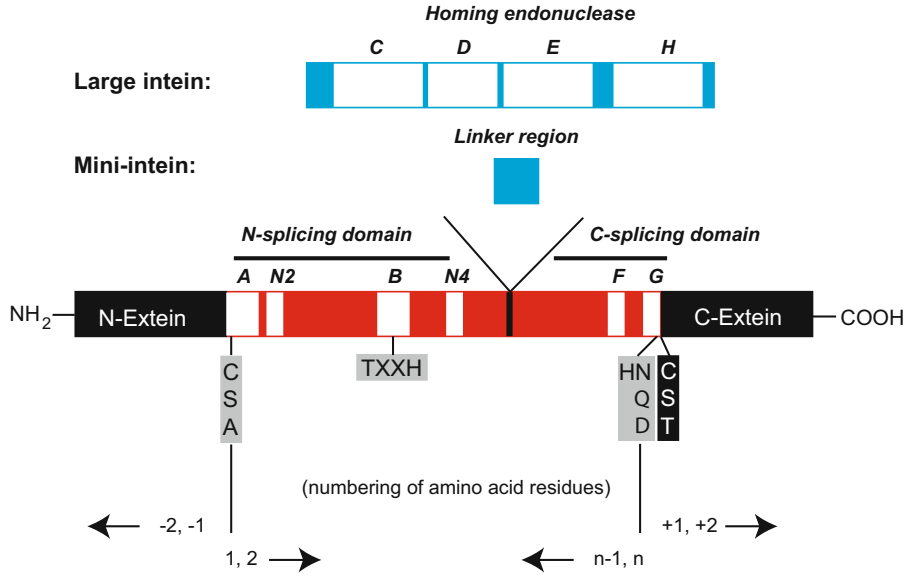


Fig. 2 Conserved elements in a large intein and mini-intein. The white areas A, N2, B, N4, C, D, E, F, and G are conserved intein motifs identified by Pietrokovski (1994, 1998) and Perler et al. (1997). The exteins are illustrated in black and the intein sequence in gray. The site of insertion of the homing endonuclease and the linker region in large and mini-inteins, respectively, is indicated by the dark vertical line. Conserved amino acid residues of the intein and the C-extein are indicated below

the recent progress in understanding the protein splicing mechanism, how inteins evolve, and how they can be used for technical applications.

A. General Characteristics of Inteins

Inteins are classified into two groups: large and minimal (mini-) inteins, depending on whether or not they contain a homing endonuclease domain (Liu 2000) (Fig. 2). Homing endonucleases are encoded by open reading frames that are embedded within introns and inteins (Barzel et al. 2011; Chevalier and Stoddard 2001; Liu 2000; Stoddard 2005). They are site-specific, double-strand DNA endonucleases that promote the lateral transfer of their own coding region and flanking sequences between genomes by a recombination-dependent process known as "homing" (Fig. 3). Genomic DNA encoding an intein-free allele of the potentially intein-carrying gene is cut by the homing endonuclease, and during DNA repair, the intein is subsequently copied into the empty allele (Belfort and Bonocora 2014; Gimble 2000; Gimble and Thorner 1993). The nomenclature of homing endonucleases is patterned after that of restriction enzymes. A three-letter, genus- and species-specific designation is followed by a Roman numeral to distinguish multiple enzymes from a single organism. Intron-encoded endonucleases are characterized by the prefix I (for intron), whereas intein-derived endonucleases are characterized by the prefix PI (for protein insert; i.e., the endonuclease of *Sce* VMA1 is termed PI *Sce*I) (Belfort and Roberts 1997).

Six families of homing endonucleases containing highly conserved amino acid sequences have been identified; including the LAGLIDADG, the GIY-YIG, the His-Cys box, the H-N-H, PD-(D/E)xK and the VsR endonucleases (Belfort and Bonocora 2014; Piégu et al. 2015). Fungal intein homing endonucleases belong to the LAGLIDADG family and are characterized by two copies of the conserved LAGLIDADG motif. (Belfort and Roberts 1997)

Mini-inteins are not bifunctional and contain only a splicing domain without endonuclease function. Because several splicing-efficient mini-inteins have been engineered from large inteins by deleting the central endonuclease

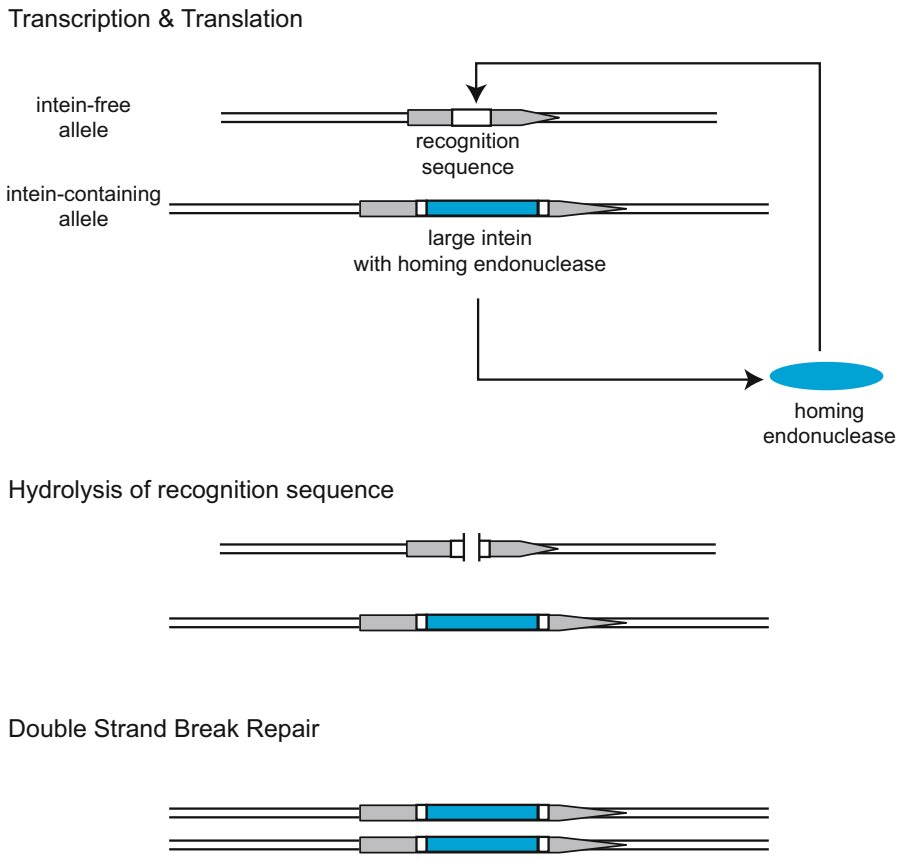


Fig. 3 Intein homing. The large intein encodes an endonuclease that in heterozygous cells recognizes a recognition sequence in the intein-less allele on the homologous chromosome. The recognition site is hydrolyzed by the intein-encoded endonuclease, while the chromosome carrying the intein is protected from being hydrolyzed. After cleavage, the recominational repair system of the cell is stimulated, and the intein-containing chromosome is used as a template for repair. The result of intein homing is a homozygote with two copies of the intein-containing gene

domain, it is clear that the endonuclease domain is not involved in protein splicing (Chong and Xu 1997; Derbyshire et al. 1997; Shingledecker et al. 1998). The splicing domain of large inteins seems to be split by the endonuclease domain into N-terminal and C-terminal subdomains. Both intein subdomains contain conserved blocks of amino acids: blocks A, N2, B, and N4 for the N-terminal subdomains and blocks G and F for the C-terminal subdomains (Perler et al. 1997; Pietrokovski 1994, 1998) (Fig. 2). These domains can also be identified in mini-inteins. However, motifs C, D, E, and H of the endonuclease domain are missing; instead they have a small linker region (Fig. 2). In addition, all known inteins have a Ser, Cys, or Ala at the N-terminus and end in His-Asn, His-Gln, or His-Asp. There is no conserved residue preceding the intein at the C-terminus of the N-extein, but all inteins have an invariant Ser, Thr, or Cys at the N-terminus of the C-extein (Mills et al. 2014; Perler 2002). According to the most common amino acid numbering system in intein precursors, minus and plus signs are used to designate N-extein and C-extein residues, respectively. Numbering begins at the N-terminus of the intein and C-extein and

proceeds to the C-terminus. Numbering of the N-extein begins at its C-terminus and proceeds to its N-terminus (Fig. 2) (Saleh and Perler 2006).

Three-dimensional structures of inteins reveal that the N- and C-terminal splicing domains form a common horseshoe-like 12-β-strand scaffold termed the Hedgehog (Hint) module (Ding et al. 2003; Hall et al. 1997; Klabunde et al. 1998; Koonin 1995; Perler 1998; Shah and Muir 2014; Sun et al. 2005). In addition to Hedgehog proteins, fungal inteins also share sequence and structure properties with bacterial intein-like sequences (BILs) (Amitai et al. 2003; Shah and Muir 2014).

Hedgehog proteins are essential signaling molecules for animal embryonic development and possess an autoprocessing activity that results in an intramolecular cleavage of the full-length Hedgehog protein, and covalent attachment of a cholesterol moiety to the newly generated amino-terminal fragment. Cholesterol anchors the signaling domain to the cell surface (Beachy et al. 1997; Ingham 2001). Until now, Hedgehog proteins have been described only in metazoa.

BIL-containing proteins are identified in diverse bacterial species but in contrast to inteins, BILs often do not have, Ser, Thr or Cys as the obligatory nucleophilic residue and they are commonly found in variable regions of secreted proteins. (Dori-Bachash et al. 2009; Swithers et al. 2009)

Combining all these characteristics, four main criteria help to differentiate true inteins from other in-frame insertions: (i) an intein-containing protein has a sequence lacking in homologs of other organisms; (ii) the intervening sequence is over 100 amino acids and (iii) contains the conserved intein motifs A, B, F, and G; (iv) the observed protein product is the same size as the predicted open reading frame without the intein (Perler et al. 1997).

B. Protein Splicing Mechanism

The posttranslational removal of the intein is an autocatalytic process that depends only on the information of the intein plus the first downstream extein residue (Fig. 1). Protein splicing is extremely rapid, and to date, precursors have not been identified in native systems. The first direct evidence for protein splicing was demonstrated for an intein derived from the thermostable DNA polymerase of *Pyrococcus* species GB-D. The intein from *Pyrococcus* was cloned into a foreign gene, and the purified precursor was shown to be capable of in vitro protein splicing in the absence of other proteins or cofactors (Xu et al. 1993).

Protein splicing of standard class 1 inteins typically involves four steps: (i) an N-O or N-S acyl shift at the N-extein/intein junction; (ii) transesterification, which transfers the N-extein to the side chain of the first residue (+1) of the C-extein; (iii) cyclization of a conserved asparagine residue at the C-terminus of the intein and cleavage of the peptide bond, resulting in the release of the intein; and (iv) rearrangement to a peptide bond of the ester/thioester bond linking the N- and C-exteins. In addition to this typical intein splicing mechanism, off-pathway N- or C-terminal cleavage of inteins occurs when conserved residues are mutated or inteins are inserted in heterologous host proteins. The details of the chemical process involved in protein splicing have been comprehensively described and reviewed (Gogarten et al. 2002; Liu 2000; Mills et al. 2014; Noren et al. 2000; Saleh and Perler 2006; Starokadomskyy 2007).

A small fraction of inteins exist as two fragments are encoded by two separately transcribed and translated genes (Perler 2002). These so-called split inteins self-associate and catalyze protein splicing activity in *trans*. The first native split intein capable of protein trans-splicing was identified in the catalytic subunit alpha of DNA polymerase III DnaE of the cyanobacterium *Synechocystis* sp. strain PCC6803 (Wu et al. 1998).

The N- and C-terminal halves are encoded by two separate genes, *dnaE-n* and *dnaE-c*, respectively. These two genes are located 745,226 bp apart in the genome and are on opposite DNA strands. (Wu et al. 1998)

Native split inteins have been identified in diverse cyanobacteria such as *Nostoc punctiforme* and the archaeon *Nanoarchaeum equitans* (Aranko et al. 2014; Choi et al. 2006; Iwai

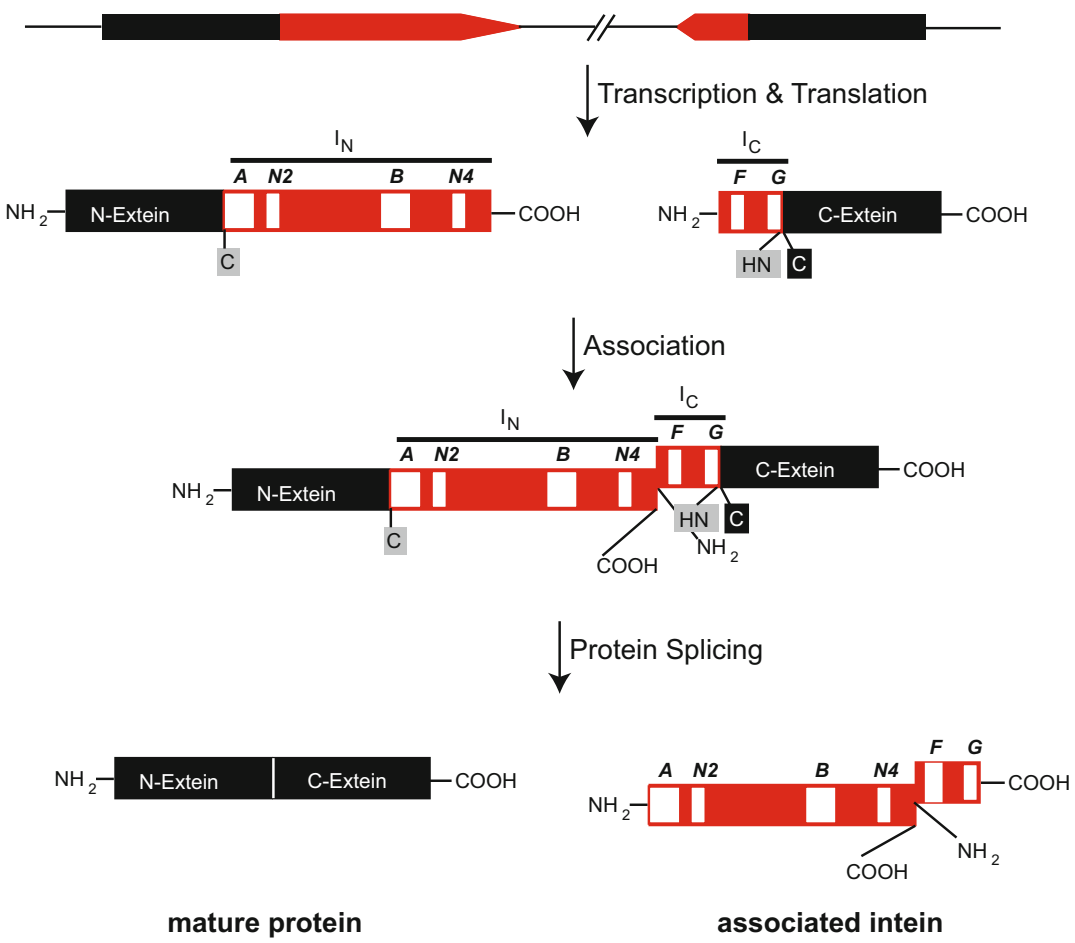


Fig. 4 Protein splicing in *trans*. The precursor is encoded by two genes: one gene encodes the N-extein and the N-terminal intein (I_N); the other gene encodes the C-terminal part of the intein (I_C) and the C-extein. Both genes are separately transcribed and translated; the intein fragments associate and form a functional intein. N- and C-exteins become ligated to the mature protein, and the intein fragments usually remain associated in solution

et al. 2006), but so far they have not been found in fungi. *Trans*-splicing inteins have, however, been artificially engineered from *cis*-splicing bacterial and fungal inteins (Elleuche and Pöggeler 2007; Li 2015; Mills et al. 1998; Mootz and Muir 2002; Southworth et al. 1998; Volkmann and Iwai 2010). In the majority of cases, naturally or artificially split inteins are separated between motifs B and F resulting in N-terminal intein fragments (I_N) of 25–40 amino acids and C-terminal intein fragments (I_C) of ~100 amino acids (Fig. 4) (Aranko et al. 2014). In addition, it was demonstrated that inteins can be artificially split at other sites. The *Ssp* DnaB mini-intein can be split at different loop regions between β-strands and still maintain the ability to splice in *trans*. Even a three-piece split intein can function in protein *trans*-splicing (Sun et al. 2004).

Naturally split inteins, as well as engineered split inteins, can be used for various applications in protein chemistry, e.g., protein purification, regulation of protein activity, and analysis of protein–protein interactions (Elleuche and Pöggeler 2010, 2016; Li 2015; Topilina and Mills 2014) (see Sect. IV).

II. Inteins in Fungi

In fungi, only *cis*-splicing mini- and large inteins have been detected, and all are encoded by nuclear genes. They have been identified in the four main groups: in chytrids, zygomycetes, ascomycetes, and basidiomycetes. Most of them are embedded into homologs of the *S. cerevisiae VMA1* gene or within the spliceosomal *prp8* gene, but they can also be found in genes encoding glutamate synthases, chitin synthases, threonyl-tRNA synthetases, or subunits of DNA-directed RNA polymerases (Poulter et al. 2007).

A. VMA1 Inteins Inserted in the Vacuolar ATPase, Subunit A

The *VMA1* gene of many saccharomycete yeasts consists of a single open reading frame that comprises two independent regions of genetic information for VMA1, the catalytic 67-kDa subunit of the vacuolar H^+-ATPase and for an intein varying in length from 389 to 517 residues (Table 1). Vacuolar H^+-ATPases are involved in the intravesicular acidification of vacuoles, endosomes, and the trans-Golgi network (Nelson and Harvey 1999). The intein is inserted in the highly conserved nucleoside triphosphate hydrolase P-loop domain catalyzing the hydrolysis of the β–γ phosphate bond of a bound nucleoside triphosphate for energy gain (Leipe et al. 2004). All inteins found in *VMA1* genes of saccharomycete yeasts are large inteins and contain two separate domains: a splicing domain and an endonuclease domain (Table 1). The presence of the VMA1 intein is not consistent among saccharomycete yeast species with closely related species differing in the presence of the intein (Fernandes et al. 2016; Koufopanou et al. 2002). Under laboratory conditions, *VMA1* mutants of *S. cerevisiae* are viable but lack the vacuolar H^+-ATPase and are defective in vacuolar acidification. Moreover, they are calcium-sensitive and exhibit low spore viability as well as slow growth (Hirata et al. 1990). In *Candida albicans*, a functional link between the V-ATPase and membrane lipid composition affecting filamentation and pathogenicity has recently been reported (Fernandes et al. 2016). Interestingly, *C. albicans* does not have any intein, and therefore the loss of the intein might have contributed to its pathogenicity. In contrast, many *Candida* species isolated from environment have retained inteins, leading to the hypothesis that intein splicing can act as posttranslation expression regulator under specific environmental conditions, as was recently demonstrated for intein splicing in archaea and bacteria (Callahan et al. 2011; Topilina et al. 2015a, b).

Alleles of the *VMA1* gene lacking the intein element are present in most species of *Pezizomycotina* and basidiomycetes. However, a BLAST analysis at the National Center for Biotechnology Information (NCBI) revealed the presence of a VMA1 intein-encoding sequence in genomes from 12 members of the *Pezizomycotina* and in the basidiomycete *Mixia osmundae* (Pöggeler, unpublished) (Table 1). Until now, all VMA1 inteins that have been identified are inserted at an identical insertion site within the P-loop of the catalytic domain.

The best-characterized VMA1 intein is *Sce* VMA1 from *S. cerevisiae*. As shown by crystal structure, the protein splicing domain of *Sce* VMA1 is structurally related to bacterial mini-inteins and to the *Drosophila* Hedgehog protein autoprocessing domain (Duan et al. 1997; Hall et al. 1997; Koonin 1995; Moure et al. 2002; Pietrokovski 1998). Site-directed mutagenesis experiments and deletion of the endonuclease domain have shown that the endonuclease activity is not required for protein splicing (Chong and Xu 1997; Gimble and Stephens 1995). The two-domain structure (self-splicing and endonuclease domain) of PI-*Sce*I was confirmed by X-ray crystallography. However, despite the apparent structural autonomy of the protein splicing and endonuclease domains, the two domains appear to collaborate by interacting with the homing site DNA (Duan et al. 1997; Moure et al. 2002; Werner et al. 2002). Furthermore, it was shown that the two intein domains are functionally independent and have

Table 1 Characteristics of VMA1 inteins inserted in the vacuolar ATPase, subunit A

Species	Intein size (), endonuclease	Activity	References
Ascomycota, Saccharomycotina			
Saccharomyces cerevisiae	454, +	Experimental	Hirata et al. (1990), Kane et al. (1990)
Saccharomyces castellii CBS4309	517, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Saccharomyces castellii IFO1992	517, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Saccharomyces cariocanus	454, +	Theoretical	Perler (2002)
Saccharomyces dairenensis	501, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Saccharomyces exiguous	499, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Saccharomyces pastorianus	454, +	Theoretical	Okuda et al. (2003)
Saccharomyces unisporus	414, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Kazachstania exiguus	502, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Candida apicola	389, +	Theoretical	Fernandes et al. (2016)
Candida bracarensis	420, +	Theoretical	Fernandes et al. (2016)
Candida castellii	367, +	Theoretical	Fernandes et al. (2016)
Candida glabrata	415, +	Theoretical	Okuda et al. (2003)
Candida homilentoma	491, +	Theoretical	Fernandes et al. (2016)
Candida intermedia	455, +	Theoretical	Fernandes et al. (2016)
Candida maltosa	451	Theoretical	Fernandes et al. (2016)
Candida metapsilosis	454, +	Theoretical	Prandini et al. (2013)
Candida nivariensis	424, +	Theoretical	Fernandes et al. (2016)
Candida orthopsilosis A and B	530, +	Theoretical	Prandini et al. (2013)
Candida sorboxylosa	374, +	Theoretical	Fernandes et al. (2016)
Candida sojae	501, +	Theoretical	Fernandes et al. (2016)
Candida tropicalis	471, +	Experimental	Gu et al. (1993), Steuer et al. (2004)
Debaryomyces hansenii	394, +	Theoretical	Dujon et al. (2004)
Pichia stipitis	449, +	Theoretical	Bakhrat et al. (2006), Jeffries et al. (2007)
Kluyveromyces lactis CBS683	410, +	Theoretical	Koufopanou et al. (2002), Okuda et al. (2003)
Kluyveromyces lactis IFO1267	410, +	Theoretical	Okuda et al. (2003)
Kluyveromyces lactis NRRLY1140	410, +	Theoretical	Dujon et al. (2004)
Vanderwaltozyma polyspora	433, +	Theoretical	Koufopanou et al. (2002)
Lodderomyces elongisporus	421, +	Theoretical	Perler (2002)
Torulaspora globosa	456, +	Theoretical	Koufopanou et al. (2002)
Torulaspora pretoriensis	455, +	Theoretical	Koufopanou et al. (2002)
Zygosaccharomyces bailii	456, +	Theoretical	Koufopanou et al. (2002)
Zygosaccharomyces bisporus	450, +	Theoretical	Koufopanou et al. (2002)
Zygosaccharomyces rouxii	450, +	Theoretical	Koufopanou et al. (2002)
Schizosaccharomyces japonicus	476, +	Theoretical	Perler (2002)
Ascomycota, Pezizomycotina			
Fonsecaea erecta	555, +	Theoretical	Pöggeler, unpublished
Fonsecaea monophora	477, +	Theoretical	Pöggeler, unpublished
Fonsecaea nubica	477, +	Theoretical	Pöggeler, unpublished
Fonsecaea pedrosoi CBS 271.37	477, +	Theoretical	Pöggeler, unpublished
Pseudocercospora musae	554, +	Theoretical	Pöggeler, unpublished
Rhinocladiella mackenziei CBS 650.93	494, +	Theoretical	Pöggeler, unpublished
Sporothrix brasiliensis 5110	499, +	Theoretical	Pöggeler, unpublished
Sporothrix insectorum RCEF 264	487, +	Theoretical	Pöggeler, unpublished
Stachybotrys chartarum IBT 40288	504, +	Theoretical	Pöggeler, unpublished
Sporothrix schenckii ATCC 58251	499, +	Theoretical	Pöggeler, unpublished
Sporothrix schenckii 1099-18	499, +	Theoretical	Pöggeler, unpublished
Basidiomycota, Pucciniomycotina			
Mixia osmundae IAM 14324	460, +	Theoretical	Pöggeler, unpublished

separate evolutionary origins (Dalgaard et al. 1997; Derbyshire et al. 1997; Okuda et al. 2003) (see Sect. III).

B. PRP8 Inteins in Fungi

After the discovery of the VMA1 intein, a second, non-allelic intein in fungi was identified within the PRP8 protein of the basidiomycete *Cryptococcus neoformans*. Many fungal inteins have also been found at an allelic insertion site in various fungi; some of these have been characterized experimentally in detail.

1. Distribution of PRP8 Inteins in Fungi

A nuclear-encoded intein has been identified inside the *prp8* gene in two strains (Cn 3511 and JEC 21) of the human pathogen *C. neoformans* (Butler et al. 2001). The PRP8 protein is a major component of the spliceosome and is highly conserved among eukaryotes (Grainger and Beggs 2005). Therefore, the PRP8 protein is essential for cell viability (Luo et al. 1999), and PRP8 inteins must be active, because inactivity would lead to host lethality. The *Cne* PRP8 intein (Table 2) is a mini-intein composed of only 172 amino acids, and lacks the endonuclease domain, present in the experimentally characterized VMA1 intein of *S. cerevisiae* (Butler et al. 2001) (see Sect. II.A).

Liu and Yang (2004) detected highly conserved PRP8 inteins ranging in size from 170 to 172 amino acids in the genomes of different *C. neoformans* serotypes. While PRP8 inteins of serotype A are 172-aa in length, PRP8 inteins of serotype B/C are 170-aa, and inteins of serotype D are 171-aa in length (Butler and Poulter 2005). *C. neoformans* serotype AD carries two distinct PRP8 inteins, indicating that strains of this serotype may be diploid (Poulter et al. 2007).

Isolates of *C. neoformans* have been classified into five different serotypes and three different varieties. They are named *C. neoformans* var. *grubii* (serotype A), var. *gattii* (serotypes B, C), var. *neoformans* (serotype D) and a hybrid of serotypes A and D (serotype AD). (Nelson and Lodes 2006)

Besides the PRP8 mini-inteins identified in *C. neoformans* and *C. gattii*, a large intein was found within the PRP8 protein of *Cryptococcus laurentii* (Butler and Poulter 2005). To date, no inteins have been identified in any other species of the genus *Cryptococcus* (Butler et al. 2006), but interestingly a large intein of 502-aa is present in the *prp8* gene of two members of the *Ustilagomycotina*: the wheat pathogen *Tilletia indica* and the ryegrass pathogen *Tilletia walkeri* (Table 2). Allelic PRP8 inteins that occupy the same insertion site as the *Cne* PRP8 have been identified in various species of the phylum *Ascomycota*. Approximately half of the PRP8 inteins are large inteins and contain an endonuclease domain (Table 2). The *Aspergillus fumigatus* PRP8 intein is an exceptionally long PRP8 intein and is comprised of 819 (*Afu*-FRR0163 PRP8) and 820 (*Afu*-Af293 PRP8) amino acids (Table 2). In addition to a 454-aa endonuclease domain, a 222-aa sequence is integrated after block B of the *Afu*-FRR0163 PRP8 intein. At the same position, a so-called tongs subdomain of 69-aa is integrated in the *Sce* VMA1 intein. On this basis, the 222-aa domain of *Afu* PRP8 was defined as a putative tongs domain, although there is no apparent sequence homology between the *Afu* PRP8 and the *Sce* VMA1 domains (Liu and Yang 2004). A tongs domain was also identified in the large PRP8 intein from *Botrytis cinerea* and other members of the genera *Botrytis* and *Botryotinia* (Table 2) The domain of *Bci* PRP8 is at exactly the same position as the putative tongs domain of *A. fumigatus* and shares a high degree of similarity (Bokor et al. 2012; Poulter et al. 2007). A similar domain at this insertion site is present in the large PRP8 inteins of *Aspergillus tubingensis*, *Fusarium fujikuroi*, *Penicillium subrubescens*, *Pseudogymnoascus* sp., *Pyrenophora tritici-repentis*, and *Rhinocladiella mackenziei* (Table 2). Overall the tongs domain seems not to be an essential feature of the intein function, because the domain is located in a region of the intein where

Table 2 Characteristics of PRP8 inteins inserted in a subunit of the spliceosome

Species	Intein size, endonuclease	Activity	References
Chytridiomycota			
Batrachochytrium dendrobatidis JEL423 (PRP8-a)	465, +	Theoretical	Perler (2002)
Batrachochytrium dendrobatidis JEL423 (PRP8-b)	465, +	Theoretical	Perler (2002)
Spizellomyces punctatus	473, +	Theoretical	Perler (2002)
Blastocladiomycota			
Allomyces macrogynus ATCC 38327	233, −	Theoretical	Pöggeler, unpublished
Mucoromycota, *Mortierellomycotina*			
Mortierella elongata AG-77	366, +	Theoretical	Pöggeler, unpublished
Mucoromycota, *Mucoromycotina*			
Phycomyces blakesleeanus NRRL 155	198, −	Theoretical	Perler (2002)
Rhizopus microsporus	459, +	Theoretical	Pöggeler, unpublished
Ascomycota, *Pezizomycotina*			
Aschersonia aleyrodis RCEF 2490	164, −	Theoretical	Pöggeler, unpublished
Aspergillus brevipes FRR2439	165, −	Theoretical	Butler et al. (2006)
Aspergillus cristatus	153, −	Theoretical	Pöggeler, unpublished
Aspergillus fumigatus FRR0163	820, + (T)	Experimental	Liu and Yang (2004)
Aspergillus fumigatus var. *ellipticus Af293*	819, + (T)	Theoretical	Poulter et al. (2007)
Aspergillus giganteus NRRL6136	167, −	Theoretical	Butler et al. (2006)
Aspergillus nidulans FGSC A4	605, +	Experimental	Liu and Yang (2004)
Aspergillus ochraceoroseus	163, −	Theoretical	Pöggeler, unpublished
Aspergillus rambellii	163, −	Theoretical	Pöggeler, unpublished
Aspergillus ruber CBS 135680	153, −	Theoretical	Pöggeler, unpublished
Aspergillus tubingensis CBS 134.48	885, + (T)	Theoretical	Pöggeler, unpublished
Aspergillus viridinutans FRR0577	169, −	Theoretical	Butler et al. (2006)
Arthroderma benhamiae CBS 112371	501, +	Theoretical	Novikova et al. (2016)
Arthroderma otae CBS 113480	551, +	Theoretical	Novikova et al. (2016)
Beauveria bassiana ARSEF 2860	163, −	Theoretical	Novikova et al. (2016)
Bipolaris zeicola 26-R-13	544, +	Theoretical	Novikova et al. (2016)
Blastomyces dermatitidis	526, +	Experimental	Theodoro et al. (2011)
Blumeria graminis f. sp. *tritici 96224*	183, −	Theoretical	Pöggeler, unpublished
Botryotinia calthae	518, +	Theoretical	Bokor et al. (2012)
Botryotinia convoluta	839, + (T)	Theoretical	Bokor et al. (2012)
Botryotinia draytonii	828, + (T)	Theoretical	Bokor et al. (2012)
Botryotinia ficariarum	546, +	Theoretical	Bokor et al. (2012)
Botryotinia fuckeliana B05.10	811, + (T)	Theoretical	Perler (2002)
Botryotinia globosa	834, + (T)	Theoretical	Bokor et al. (2012)
Botryotinia polyblastis	831, + (T)	Theoretical	Bokor et al. (2012)
Botryotinia ranunculi	546, +	Theoretical	Bokor et al. (2012)
Botryotinia sphaerosperma	834, + (T)	Theoretical	Bokor et al. (2012)
Botryotinia squamosa	546, +	Theoretical	Bokor et al. (2012)
Botrytis cinerea B05.10	838, + (T)	Theoretical	Bokor et al. (2010), Butler et al. (2006)
Botrytis croci	839, + (T)	Theoretical	Bokor et al. (2012)
Botrytis elliptica	546, +	Theoretical	Bokor et al. (2012)
Botrytis fabae	656, +	Theoretical	Bokor et al. (2012)
Botrytis hyacinthi	839, + (T)	Theoretical	Bokor et al. (2012)
Botrytis paeoniae	537, +	Theoretical	Bokor et al. (2012)
Botrytis porri	512, +	Theoretical	Bokor et al. (2012)
Capronia epimyces CBS 606.96	150, −	Theoretical	Novikova et al. (2016)
Cladophialophora bantiana CBS 173.52	427, +	Theoretical	Pöggeler, unpublished
Cladophialophora immunda	201, −	Theoretical	Pöggeler, unpublished
Cladophialophora psammophila CBS 110553	426, +	Theoretical	Novikova et al. (2016)
Cordyceps confragosa	155, −	Theoretical	Pöggeler, unpublished
Diaporthe helianthi	539, + partial (T)	Theoretical	Pöggeler, unpublished
Drechmeria coniospora	158, −	Theoretical	Pöggeler, unpublished

(continued)

Table 2 (continued)

Species	Intein size, endonuclease	Activity	References
Emmonsia crescens UAMH 3008	586, +	Theoretical	Pöggeler, unpublished
Emmonsia parva	526, +	Experimental	Theodoro et al. (2011)
Emmonsia pasteuriana UAMH 9510	549, +	Theoretical	Pöggeler, unpublished
Eutypa lata UCREL1	170, −	Theoretical	Pöggeler, unpublished
Endocarpon pusillum Z07020	200, −	Theoretical	Novikova et al. (2016)
Erysiphe necator	186, −	Theoretical	Pöggeler, unpublished
Eupenicillium baarnense CBS 134.41	168, −	Experimental	Elleuche et al. (2009)
Eupenicillium crustaceum CBS 244.32	158, −	Experimental	Elleuche et al. (2009)
Exophiala aquamarina CBS 119918	181, −	Theoretical	Pöggeler, unpublished
Exophiala oligosperma	189, −	Theoretical	Pöggeler, unpublished
Fonsecaea nubica	195, −	Theoretical	Pöggeler, unpublished
Fusarium fujikuroi IMI 58289	955, + (T)	Theoretical	Novikova et al. (2016)
Grosmannia clavigera kw1407	148, −	Theoretical	Pöggeler, unpublished
Histoplasma capsulatum/Hca PRP8	534, +	Experimental	Butler et al. (2006), Liu and Yang (2004)
Isaria fumosorosea ARSEF 2679	155, −	Theoretical	Pöggeler, unpublished
Metarhizium acridum CQMa 102	166, −	Theoretical	Novikova et al. (2016)
Metarhizium album ARSEF 1941	166, −	Theoretical	Pöggeler, unpublished
Metarrhizium anisopolia	166, −	Theoretical	Novikova et al. (2016)
Metarhizium brunneum ARSEF 3297	166, −	Theoretical	Pöggeler, unpublished
Metarhizium guizhouense ARSEF 977	166, −	Theoretical	Pöggeler, unpublished
Metarhizium majus ARSEF 297	166, −	Theoretical	Pöggeler, unpublished
Metarhizium rileyi RCEF 4871	166, −	Theoretical	Pöggeler, unpublished
Metarhizium robertsii ARSEF 23	166, −	Theoretical	Pöggeler, unpublished
Nannizzia gypsea CBS 118893	491, +	Theoretical	Novikova et al. (2016)
Neonectria ditissima	159, −	Theoretical	Pöggeler, unpublished
Neosartorya aurata NRRL 4378	164, −	Theoretical	Butler et al. (2006)
Neosartorya fenelliae NRRL 5534	155, −	Theoretical	Butler et al. (2006)
Neosartorya fischeri FRR0181	517, +	Theoretical	Butler et al. (2006)
Neosartorya glabra FRR 2163	155, −	Theoretical	Butler et al. (2006)
Neosartorya glabra FRR 1833	169, −	Theoretical	Perler (2002)
Neosartorya pseudofischeri FRR 0186	169, −	Theoretical	Butler et al. (2006)
Neosartorya quadricincta NRRL 4175	169, −	Theoretical	Butler et al. (2006)
Neosartorya spinosa FRR4595	169, −	Theoretical	Butler et al. (2006)
Paracoccidioides brasiliensis Pb18	573, +	Experimental	Butler et al. (2006), Butler and Poulter (2005), Theodoro et al. (2011)
Paracoccidioides lutzii	570, +	Experimental	Theodoro et al. (2011)
Penicillium camemberti	161, −	Theoretical	Pöggeler, unpublished
Penicillium chrysogenum	157, −	Experimental	Elleuche et al. (2006)
Penicillium digitatum PHI26	161, −	Theoretical	Pöggeler, unpublished
Penicillium digitatum Pd1	161, −	Theoretical	Pöggeler, unpublished
Penicillium expansum	162, −	Experimental	Elleuche et al. (2006)
Penicillium freii	161, −	Theoretical	Pöggeler, unpublished
Penicillium griseofulvum	158, −	Theoretical	Pöggeler, unpublished
Penicillium italicum	161, −	Theoretical	Pöggeler, unpublished
Penicillium nordicum	161, −	Theoretical	Pöggeler, unpublished
Penicillium oxalicum	160, −	Theoretical	Pöggeler, unpublished
Penicillium roqueforti FM164	158, −	Theoretical	Pöggeler, unpublished
Penicillium subrubescens	926, + (T)	Theoretical	Pöggeler, unpublished
Penicillium vulpinum	161, −	Experimental	Elleuche et al. (2006)
Phaeomoniella chlamydospora	563, +	Theoretical	Pöggeler, unpublished
Pseudogymnoascus sp. *03VT05*	818, + (T)	Theoretical	Pöggeler, unpublished
Pyrenophora tritici-repentis Pt-1C-BF	842, + (T)	Theoretical	Perler (2002)
Rhinocladiella mackenziei CBS 650.93	842, + (T)	Theoretical	Pöggeler, unpublished

(continued)

Table 2 (continued)

Species	Intein size, endonuclease	Activity	References
Sphaerulina musiva SO2202	486, +	Theoretical	Novikova et al. (2016)
Sporothrix brasiliensis 5110	522, +	Theoretical	Novikova et al. (2016)
Sporothrix schenckii ATCC 58251	522, +	Theoretical	Pöggeler, unpublished
Stachybotrys chartarum IBT 7711	163, −	Theoretical	Pöggeler, unpublished
Stachybotrys chlorohalonata IBT 40285	163, −	Theoretical	Pöggeler, unpublished
Trichophyton equinum CBS 127.97	502, +	Theoretical	Pöggeler, unpublished
Trichophyton interdigitale H6	501, +	Theoretical	Novikova et al. (2016)
Trichophyton rubrum CBS 118892	501, +	Theoretical	Novikova et al. (2016)
Trichophyton soudanense CBS 452.61	502, +	Theoretical	Novikova et al. (2016)
Trichophyton tonsurans CBS 112818	501, +	Theoretical	Pöggeler, unpublished
Trichophyton violaceum	502, +	Theoretical	Pöggeler, unpublished
Trichophyton verrucosum HKI 0517	501, +	Theoretical	Novikova et al. (2016)
Uncinocarpus reesii	180, −	Theoretical	Butler et al. (2006)
Valsa mali	613, +	Theoretical	Pöggeler, unpublished
Verticillium dahliae VdLs.17/*Vda* VdLs.17 PRP8	505, +	Theoretical	Novikova et al. (2016)
Verticillium longisporum/Vlo PRP8	492, +	Theoretical	Pöggeler, unpublished
Xylona heveae TC161	181, −	Theoretical	Pöggeler, unpublished
Basidiomycota, *Agaricomycotina*			
Cryptococcus gattii (serotype C/B)	170, −	Theoretical	Butler and Poulter (2005), Liu and Yang (2004)
Cryptococcus laurentii CBS139	522, +	Theoretical	Butler and Poulter (2005)
Cryptococcus neoformans grubii (serotype A)	171, −	Theoretical	Liu and Yang (2004)
Cryptococcus neoformans neoformans (*serotype D*)	172, −	Experimental	Butler et al. (2001), Liu and Yang (2004)
Basidiomycota, *Ustilaginomycotina*			
Tilletia indica	502, +	Theoretical	Pöggeler, unpublished
Tilletia walkeri	502, +	Theoretical	Pöggeler, unpublished

(T) large tongs domain present

mutations frequently occur (Poulter et al. 2007). Deletions of selected amino acids within this region in a homologous mini-intein from *Penicillium chrysogenum* did also not result in the complete loss of intein function (Elleuche et al. 2008).

Phylogenetic analysis based on the internal transcribed spacer sequences (ITS) of *Penicillium* and *Eupenicillium* species revealed that species containing a PRP8 intein are distinct from species lacking the intein (Elleuche et al. 2009). Moreover, we and others observed that introns are often inserted close to the empty insertion sites in intein-free alleles (Butler et al. 2006; Elleuche et al. 2009). In the genus *Eupenicillium*, all introns are identified in intein-free *prp8* alleles located between 13-bp and 15-bp downstream of the PRP8 intein insertion site (Elleuche et al. 2009). To date, however, introns at the same insertion site as the PRP8 intein have not been found in any fungus.

Analysis of the intein–extein boundaries of *prp8* alleles in species of the genus *Botrytis* and other taxa revealed that without any exception, species with PRP8 inteins contain an AGY serine codon at position +1 of the C-extein, whereas empty alleles predominately have a TCN +1 serine codon, and some exhibit a + 1 AGY codon (Bokor et al. 2012). This led Bokor et al. (2012) to speculate that empty *prp8* alleles with +1 TCN codons have never carried an intein, while empty alleles with a +1 AGY codon had precisely lost the intein.

A BLAST survey at NCBI (February 2017) of genomic fungal sequences identified 122 PRP8 inteins, which were detected in different fungal species of the phyla *Chytridiomycota*, *Mucoromycota*, *Ascomycota*, and *Basidiomycota*.

The 955-aa PRP8 intein of *Fusarium fujikuroi* is the largest PRP8 intein described to date. This intein as other large inteins from *Pyrenophora tritici-repentis*, *Aspergillus tubingensis*, and *Penicillium subrubescens* appears to possess a large insertion after block C within the endonuclease domain. The smallest PRP8 intein of 148-aa was identified in *Grosmannia clavigera* (Table 2). Only 62 out of the 123 fungal PRP8 inteins (approximately 50%) contain an endonuclease domain, in contrast to the VMA1 inteins of yeasts, where all known inteins contain an endonuclease domain (Table 1). Of the known inteins of all three domains of life, only about 15% lack the endonuclease domain (Perler 2002).

Nearly all PRP8 inteins have clear homology, based on a high degree of sequence identity and the position of their insertion within the *prp8* gene. The vast majority of fungal PRP8 inteins occupy the same insertion site as the prototype allele of *Cne* PRP8. The PRP8 inteins of only two chytrid fungi are not homologous and were identified at two different non-allelic positions: the PRP8 intein of *Spizellomyces punctatus Spu* PRP8 and *Bde*-JEL423 PRP8-1, one of the two PRP8 inteins of *Batrachochytrium dendrobatidis* (Perler 2002).

Although thought to be exclusive to fungi, a PRP8 intein was identified in an Atlantic population of the photosynthetic protist *Bathycoccus* and in parasitic and predatory marine opithokonts *Capsaspora owczarzaki* and *Salpingoeca rosetta*, respectively. PRP8 inteins of the protists have new insertion sites not identified in fungi so far. (Monier et al. 2013)

Nonetheless, PRP8 inteins are not universal. Various fungal species have been analyzed to date that do not contain an intein in the *prp8* gene (Bokor et al. 2012; Elleuche et al. 2006; Poulter et al. 2007).

2. Activity of Fungal PRP8 Inteins

One of the most interesting questions concerning inteins is the splicing activity. Since most inteins are embedded in essential proteins that are often involved in nucleic acid metabolism, they must be removed from the precursor protein for the mature protein to perform its function (Novikova et al. 2016).

Most experiments on the functionality of fungal inteins were done by expression in heterologous systems, mainly using *Escherichia coli* as a host. To track the splicing reaction, spliced products were usually visualized by SDS-PAGE and Western blot after overexpression of plasmid-borne fusion constructs. When inteins are embedded in foreign proteins, they can splice themselves out, so heterologous extein sequences such as maltose-binding protein, GST- or His-tags, and thioredoxin often serve as tags. All of these can be easily detected by Western blot (Elleuche et al. 2006, 2009; Liu and Yang 2004; Theodoro et al. 2011). The yield of the spliced products varies with the extein sequence used, although activity is often higher when the splicing reaction occurs with native extein flanks (Perler 2005; von der Heyde et al. 2015; Xu et al. 1993).

For the PRP8 inteins, the most well-characterized is the splicing activity of PRP8 mini-inteins from *C. neoformans* and from species of the genus *Penicillium* (Elleuche et al. 2006; Liu and Yang 2004; Pearl et al. 2007a, b). To characterize the splicing activity of the *Cne* PRP8, as well as large PRP8 inteins from *Aspergillus nidulans*, *A. fumigatus*, and *Histoplasma capsulatum* and fungal pathogens from *Ajellomycetaceae* family, each intein was cloned into a model gene encoding 5-aa of its native extein sequences on each site of the intein and an N-terminal maltose-binding protein and a C-terminal thioredoxin tag (Liu and Yang 2004; Theodoro et al. 2011). After overexpression, SDS-PAGE and Western blot analysis with an anti-thioredoxin antibody, splicing products were identified. All inteins investigated by Liu and Yang (2004) were demonstrated to be highly active when heterologously expressed in *E. coli*. The *Hca* PRP8 intein exhibited a 75% decrease in splicing activity when shifted from 25 to 37 °C. This temperature shift is an important physiological stimulus for the morphotypic transition from mold to yeast of *H. capsulatum* (Woods 2002). Temperature-independent splicing activity was also reported for three *Penicillium* PRP8 inteins (Elleuche et al. 2006).

Another system to characterize PRP8 intein activity was applied by Pearl et al. (2007a), based on an assay previously developed for the *Mycobacterium tuberculosis* RecA large intein (Buskirk et al. 2004; Davis et al. 1992; Skretas and Wood 2005). This method interrupted the α-complementation peptide of *E. coli* β-galactosidase, allowing assessment of intein function in vivo in *E. coli*, in combination with immunoblot analysis of the tagged intein sequences. *E. coli* loses its ability to grow on lactose, if the inserted intein is inactive. The authors examined in detail the influence of adjacent extein residues by replacing amino acids −1, −2 and +1, +2 with various other residues. The results confirmed that splicing efficiency depends on the composition of extein sequences (Pearl et al. 2007a). A similar result was obtained for the *Pex* PRP8 intein, which was able to undergo protein splicing within the green fluorescent protein (GFP). Only the +1-residue (a Serine) was conserved in the GFP, and as expected, the yield of spliced product was reduced compared to results obtained with native flanking extein regions (Elleuche et al. 2006).

To gain further insights into the splicing mechanism of fungal inteins, alanine scanning mutagenesis was applied to investigate the influence of single amino acid residues in *Cne* PRP8 on protein splicing (Pearl et al. 2007b). Similar to bacterial inteins and the large *Sce* VMA1 intein (Shingledecker et al. 2000; Southworth et al. 1999), mutation of the first, penultimate, and last residue as well as mutation in a highly conserved TxxH motif in block B strongly inhibited or completely blocked the splicing reaction. Furthermore, five new residues were found to be crucial for splicing activity in this study. All of these residues are located in the well-conserved block F and have not been previously determined to be involved in the protein splicing of bacterial inteins (Pearl et al. 2007b).

As mentioned above, large inteins derived from bacteria or the VMA1 intein of *S. cerevisiae* remain active after the endonuclease domain has been deleted, indicating that inteins are highly robust genetic elements. To define the catalytic and structural elements involved in protein splicing of naturally occurring mini-inteins, an analysis of the structural requirements of protein splicing has been conducted for the 157-aa *Pch* PRP8 intein, which is among the smallest known nuclear-encoded active splicing protein elements (Table 2). Amino acid sequences of *Pch* PRP8 can be deleted at two different sites without affecting splicing activity. One site corresponds to the insertion site of the endonuclease domain in large allelic PRP8 inteins. The other site was detected at a new position corresponding to the insertion site of a putative tongs domain of a large fungal PRP8 intein (see above). The smallest functional intein found after deleting eight and six amino acids at two different sites comprises only 143 residues and is the smallest functional eukaryotic intein engineered so far (Elleuche et al. 2008). Moreover, it was demonstrated that the *Pch* PRP8 intein is capable of protein splicing in *trans* (Elleuche and Pöggeler 2007). After artificially splitting the *Pch* PRP8 intein into an N- and a C-terminal domain at three different sites, protein *trans*-splicing has been shown to occur at two sites. One of the functional split sites corresponds to the insertion site of the endonuclease domain in allelic large PRP8 inteins, and the other was detected near the insertion site of the putative tongs domain, located N-terminal to the endonuclease insertion site (Elleuche and Pöggeler 2007).

C. Other Fungal Inteins

To date, 12 non-allelic sites of intein gene insertion in nine different nuclear genes have been described in fungi (Perler 2002). In the yeasts, *Debaryomyces hansenii*, *Candida* (*Pichia*) *guilliermondii*, *Candida carpophila*, and in the filamentous ascomycete *Podospora anserina*, inteins have been discovered within the *glt1* gene encoding glutamate synthase. These belong to the group of large inteins and are all embedded at exactly the same site of the GLT1 protein. A further GLT1 intein was identified in the GLT1 protein in the plant pathogen *Phaeosphaeria nodorum* (Poulter et al. 2007). A BLAST search at NCBI identified a large intein of 656-aa at an allelic position in the GLT1 protein of *Fusarium fujikuroi* (Pöggeler, unpublished, Table 3)

Table 3 Characteristics of fungal inteins within other proteins

Species	Host protein	Intein size, endonuclease	Activity	References
Chitin synthetase II				
Podospora anserina	CHS2	649, +	Theoretical	Butler et al. (2005)
Gaeumannomyces graminis var. tritici R3-111a-1	CHS2	659, +	Theoretical	Pöggeler, unpublished
Diaporthe helianthi	CHS2-b	607, +	Theoretical	Pöggeler, unpublished
Fusarium fujikuroi IMI 58289	CHS2-b	635, +	Theoretical	Pöggeler, unpublished
Glutamate synthase				
Candida carpophila	GLT1	557, +	Theoretical	Fernandes et al. (2016)
Debaryomyces hansenii CBS767	GLT1	607, +	Theoretical	Butler et al. (2005)
Meyerozyma guilliermondii ATCC 6260	GLT1	555, +	Theoretical	Novikova et al. (2016)
Phaeosphaeria nodorum SN15	GLT1	635, +	Theoretical	Poulter et al. (2007)
Pichia (Candida) guilliermondii	GLT1	553, +	Theoretical	Butler et al. (2005)
Podospora anserina	GLT1	682, +	Theoretical	Butler et al. (2005)
Fusarium fujikuroi IMI 58289	GLT1	656, +	Theoretical	Novikova et al. (2016)
Threonyl-tRNA synthetase				
Candida parapsilosis CLIB214	ThrRS	182, −	Theoretical	Poulter et al. (2007)
Candida maltosa	ThRS	444, +	Theoretical	Fernandes et al. (2016)
Candida metapsilosis	ThrRS	172, −	Theoretical	Prandini et al. (2013)
Candida orthopsilosis A	ThrRS	180, −	Theoretical	Prandini et al. (2013)
Candida orthopsilosis B	ThrRS	439, +	Theoretical	Prandini et al. (2013)
Candida sojae	ThrRS	338, +	Theoretical	Fernandes et al. (2016))
Candida tropicalis ATCC750	ThrRS	345, +	Theoretical	Perler (2002))
RNA polymerase I RPA2				
Baudoinia panamericana UAMH 10762	RPA2	484, +	Theoretical	Pöggeler, unpublished
Phaeosphaeria nodorum SN15/ Pno RPA2	RPA2	456, +	Theoretical	Goodwin et al. (2006)
Pyrenochaeta sp. *DS3sAY3a*	RPA2	484, +	Theoretical	Pöggeler, unpublished
Tilletia indica	RPA2	425, +	Theoretical	Pöggeler, unpublished
Tilletia walkeri	RPA2	425, +	Theoretical	Pöggeler, unpublished
RNA polymerase II RPB2				
Batrachochytrium dendrobatidis JEL197	RPB2-c	488, +	Theoretical	Goodwin et al. (2006)
Coelomomyces stegomyiae	RPB2-b	362, +	Theoretical	Goodwin et al. (2006)
Pseudocercospora musae	RPB2-d	491, +	Theoretical	Pöggeler, unpublished
Spiromyces aspiralis NRRL 22631	RPB2-b	354, +	Theoretical	Goodwin et al. (2006)
Tilletia caries	RPB2-d	407, +	Theoretical	Pöggeler, unpublished
Tilletia controversa	RPB2-d	407, +	Theoretical	Pöggeler, unpublished
Tilletia indica	RPB2-d	404, +	Theoretical	Pöggeler, unpublished
Tilletia walkeri	RPB2-d	401, +	Theoretical	Pöggeler, unpublished
Trichosporon asahii var. asahii CBS 2479	RPB2-a	519, +	Theoretical	Pöggeler, unpublished
RNA polymerase III RPC2				
*Batrachochytrium dendrobatidis JEL423/Bde-*JEL423 RPC2	RPC2-a	488, +	Theoretical	Perler (2002)
Melanopsichium pennsylvanicum 4	RPC2-b	606, +		
eIF-5B				
Batrachochytrium dendrobatidis JEL423	eIF-5B	419, +	Theoretical	Perler (2002)

In addition to the GLT1 intein, *P. anserina* contains a non-allelic large intein in the chitin synthase gene *chs2* (Butler et al. 2005). Both GLT1 and CHS2 inteins contain domains that indicate the presence of a homing endonuclease of the LAGLIDADG type. Interestingly, the *glt1* gene of *A. nidulans* contains a currently unannotated intron that occupies the close to the insertion site of the *Dha* GLT1 (Butler et al. 2005). Phylogenetic analysis revealed that *Pan* CHS2 is closely related to the GLT1 inteins. Therefore, Poulter et al. (2007) proposed that *Pan* CHS2 may have been derived from an ectopic movement of a GLT1 intein into a non-allelic CHS2 site. Interestingly, a BLAST search at NCBI identified a CHS2 intein in *Gaeumannomyces graminis* var. *tritici* at the same insertion site. The intein is 659-aa in size and exhibits an identity of 52% to *Pan* CHS2. Similar to the *P. anserina* CHS2 in the *G. graminis* CHS2, a conserved valine residue at the putative +1-extein position is replaced by a cysteine residue (Butler et al. 2005) (Pöggeler, unpublished). In addition, the BLAST search identified that in *Fusarium fujikuroi* and *Diaporthe helianthi*, the CHS2 protein carries a large intein at a non-allelic position. The inteins of *F. fujikuroi* and *D. helianthi* are 52% and 51%, respectively, identical to *Pan* CHS2 (Table 3, Pöggeler, unpublished).

A large intein and a mini-intein have also been detected in the threonyl-tRNA synthetase gene *thrRS* of the yeast *Candida tropicalis* and *Candida parapsilosis*, respectively. The large *Ctr* ThrRS intein is 345-aa in length with some endonuclease-resembling domains, but the region seems to have accumulated multiple of mutations that might result in an inactive endonuclease (Poulter et al. 2007). The allelic 182-aa mini-intein of *C. parapsilosis* (*Cpa* ThrRS) and *Candida metapsilosis* has lost the endonuclease domain (Poulter et al. 2007; Prandini et al. 2013). In different isolates of *Candida orthopsilosis*, either a mini-intein or a large intein, while in *Candida sojae* and *Candida maltosa*, a large intein, occurred at the same insertion site (Fernandes et al. 2016; Prandini et al. 2013). In contrast to the large ThrRS intein of *C. tropicalis*, the endonuclease domain of the *Cor* ThrRS and *Cma* ThrRS intein contains all essential residues and was suggested to be active (Fernandes et al. 2016; Prandini et al. 2013).

Except for mini-inteins of PRP8 proteins, *Cpa* ThrRS is the sole example of a eukaryotic mini-intein.

In prokaryotes, inteins are often embedded in proteins involved in replication, in transcription, or in related processes such as the metabolism of nucleotides (Liu 2000; Novikova et al. 2016).

In general, eukaryotes encode three RNA polymerases involved in the synthesis of messenger, ribosomal, and transfer RNAs (Cramer et al. 2008). A detailed investigation of eukaryotic RNA polymerase subunits resulted in the identification of inteins within this functional category of genes. The inteins are found in the second largest subunit of either RNA polymerase I or RNA polymerase II or RNA polymerase III. The insertion sites of inteins in RNA polymerases are among the most highly conserved regions of these subunits (Goodwin et al. 2006).

A 456-aa intein is embedded within a subunit of the RNA polymerase I (RPA2) of the plant pathogen *P. nodorum*. *Pno* RPA2 contains degenerated motifs of an endonuclease domain that no longer seems to be active (Goodwin et al. 2006). Large inteins at an allelic position of the RPA2 protein were identified in the genome sequence of the ascomycetes *Baudoinia panamericana, Pyrenochaeta* sp. and the basidiomycetes *Tilletia indica* and *Tilletia walkeri*. (Pöggeler unpublished, Table. 3)

Nine large inteins were also identified within the gene encoding the second largest subunit of RNA polymerase II (RPB2). Two of them are inserted at the same site in the zygomycete *Spiromyces aspiralis* (*Sas* RPB2-b) and the chytrid *Coelomomyces stegomyiae* (*Cst* RPB2-b). One intein, *Bde* RPB2-c, was identified in another chytridiomycete, *Batrachochytrium dendrobatidis,* at a non-allelic position. Five new inteins were identified by BLAST searches at NCBI at another position in the RPB2 protein in the ascomycete *Pseudocercospora musae* and the basidiomycetes *Tilletia caries, Tilletia controversa*, *T. indica* and *T. walkeri* (Pöggeler unpublished, Table 3). Another non-allelic RPB2 intein namely at the a-site was described in the RNA polymerase II of the green algae *Chlamydomonas reinhardtii* (Goodwin et al. 2006). Interestingly, an intein is inserted at the same position in the basidiomycetous yeast *Trichosporon asahii*. (Pöggeler unpublished, Table 3).

According to InBase, an endonuclease-containing intein was identified in the second largest subunit of RNA polymerase III in the chytrid *B. dendrobatidis* (Perler 2002). The sequences of the *Bde* RPB2 and *Bde* RPC2, as well as the regions of the insertion sites, are very similar at the amino acid level. However, the remaining parts of the exteins are not conserved, which suggests an intein transfer that recently occurred (Poulter, Butler and Goodwin unpublished results at InBase). A BLAST search at NCBI revealed that the basidiomycetous smut fungus *Melanopsichium pennsylvanicum* harbors a large intein in the RPC2 protein at a non-allelic position. (Pöggeler unpublished, Table 3)

According to InBase, a 419-aa intein is inserted in the translation initiation factor 5B. (Perler 2002) (Table 3).

Inteins have been described in four fungal phyla: *Chytridiomycota*, *Zygomycota*, *Ascomycota*, and *Basidiomycota*. Only the PRP8 intein and the RPB2 intein have been discovered in all fungal phyla. In fungi, inteins are embedded within nine different genes, and with few exceptions, they are inserted at allelic sites (Tables 1–3). In eukaryotes, only a few species are known that contain more than one intein in their genome. As can be seen from Tables 1 and 3, four inteins (in PRP8 at two different insertion sites) can be found in the genome of the chytrid *B. dendrobatidis*, and three inteins have been identified in the ascomycete *F. fujikuroi* and in the basidiomycetes *T. indica* and *T. walkeri*. Prokaryotes often contain a variety of inteins in their genomes, for example, 19 inteins were identified in the genome of the methanogenic archaeon *Methanococcus jannaschii* (Pietrokovski 1998).

III. Mobility, Evolution, and Domestication of Inteins

A variety of inteins can invade DNA sequences by virtue of the endonuclease encoded within them (Chevalier and Stoddard 2001). The lateral transfer of an intervening sequence (either an intron or intein) into a homologous intronless/intein-less allele is termed “homing” and must be distinguished from the transposition process to non-allelic sites (Dujon 1989). Phylogenetic analyses have revealed that the evolutionary biology of mobile inteins is highly dynamic. Further, VMA1 inteins have been shown to be the ancestors of the HO endonuclease required for mating-type switching in yeast.

A. Mobility of Fungal Inteins

Intein homing relies on endonucleases that create specific double-strand breaks at cognate alleles that do not contain these mobile elements. Mating of an intein-less and intein-containing strain leads to intein transmission to about 75%–100% of the meiotic progeny (Bokor et al. 2010). This non-Mendelian inheritance pattern ensures the persistence of homing endonuclease genes in populations and leads to their invasion of new species by horizontal transmission. Intein-containing genes are not deleterious to their host organisms because they have evolved in association with an intein that is removed by protein splicing (Chevalier and Stoddard 2001; Gogarten et al. 2002). Fungal intein-encoded endonucleases belong to the LAGLIDADG family and are characterized by two copies of this conserved motif. As described in Sect. II.A, the VMA1 intein endonuclease PI-*Sce*I of *S. cerevisiae* is among the most well-characterized homing endonucleases. PI-*Sce*I initiates the mobility of the intein by cleaving at intein-less alleles of the *VMA1* gene (Gimble and Thorner 1992). Subsequent to purification of PI-*Sce*I, genetic and biochemical studies demonstrated that the endonuclease makes numerous base-specific and phosphate backbone contacts with its 31-bp asymmetrical recognition site (Gimble and Thorner 1993; Gimble and Wang 1996).

It binds to a 36-bp DNA substrate on intein-free *VMA1* alleles and cleaves in a Mg^{2+}- or Mn^{2+}-dependent reaction to yield 4-bp extensions with 3′-overhangs (Gimble and Wang 1996; Moure et al. 2002; Noël et al. 2004; Wende et al. 1996). The intein-containing allele is immune to hydrolysis by PI-*Sce*I, as the VMA1 intein disrupts the 31-bp recognition sequence.

Homing of the *Sce* VMA1 intein occurs only during meiosis and not during mitosis, even if intein-containing and intein-free *VMA1* alleles coexist in the same cell and the *VMA1* gene is expressed in mitosis (Gimble and Thorner 1992). A functional genomic approach revealed that at least two karyopherins, Srp1p and Kap142p, are required for the nuclear localization of PI-*Sce*I. The nuclear localization of PI-*Sce*I was shown to be induced by inactivation of TOR kinases. Moreover, inactivation of TOR signaling or acquisition of an extranuclear localization signal in the PI-*Sce*I coding region leads to artificial nuclear localization of the endonuclease and thereby induces homing even during mitosis. Thus, the intein-encoded endonuclease utilizes the host systems of nutrient signal transduction and nucleocytoplasmic transport to ensure the propagation of its coding region (Nagai et al. 2003) Furthermore, it was demonstrated that the repair of PI-*Sce*I-induced double-strand breaks occurs at the same period as that of SPO11-initiated meiotic recombination and that it is dependent on the host homologous recombination system as well as on premeiotic DNA replication (Fukuda et al. 2003, 2004). Several VMA1-derived endonucleases were shown to fail to cleave their own intein-less DNA substrate (Posey et al. 2004). Only two enzymes have been demonstrated to be active, PI-*Zba*I from *Zygosaccharomyces bailii* and PI-*Sca*I from *Saccharomyces cariocanus*. Sequence alignment of active-site residues revealed that inactive endonucleases lack one or both of the conserved acidic residues corresponding to D-218 and D-326 in PI-*Sce*I (Posey et al. 2004). The presence or absence of a large intein in the *prp8* gene of different isolates of *B. cinerea* allowed assaying the homing endonuclease activity of the *B. cinerea* PRP8 large intein during sexual reproduction. During meiosis, the homing endonuclease of the *B. cinerea* PRP8 intein is capable of triggering gene conversion together with its adjacent sequences into an empty *prp8* allele. The gene conversion always included 25 bp of the flanking sequence but exceeded in some cases up to 450 bp. The efficiency of intein insertion into the intein-less allele was found to be 100% (Bokor et al. 2010).

The activity of the PRP8 endonuclease domain from *Paracoccidioides brasiliensis* was shown to be inactive in an *E. coli* in vitro assay. However, in the *P. brasiliensis* PRP8 endonuclease, one essential aspartate residue in motif E is substituted by a glycine or serine residue (Theodoro et al. 2011).

Most of the homing endonuclease domains of the PRP8 inteins, except *Pbr* PRP8, *Cla* PRP8, *Plu* PRP8, and *Epa* PRP8, were demonstrated to have both of the conserved aspartate residues (Poulter et al. 2007; Theodoro et al. 2008, 2011). From the PRP8 inteins which have been recently identified, the PRP8 inteins of *Nannizzia gypsea*, *Rhinocladiella mackenziei*, and *Sphaerulina musiva* have no conserved aspartate residue in motif E.

Furthermore, an analysis of the frequency of synonymous versus non-synonymous (d_S/d_N) changes indicated that PRP8-encoded endonucleases have been selectively constrained (Bokor et al. 2012; Butler et al. 2006). Only in the *Ajellomycetaceae* family the d_S/d_N ratio was shown to be much higher in the splicing domain than in the endonuclease domain (Theodoro et al. 2011).

A high value of the quotient of the frequency of synonymous (d_S) versus the frequency of non-synonymous changes (d_N) implies that the endonuclease is functional. (Butler et al. 2006)

Together, these data suggest that many PRP8-derived endonucleases might be active. Fungal GLT1-derived endonucleases, except for *Cgu* GLT1, also possess the two conserved aspartate residues, while the first aspartate residue has been substituted in the *Pan* CHS2 endonuclease domain. The other fungal inteins (Table 3) even do not exhibit clear evidence of the conserved motifs C, D, E, and H (Poulter et al. 2007). Thus, it seems that only *Dha* GTL1, *Pan* GTL1, and *Pno* GTL1 might contain an active endonuclease domain (see Sect. II.C).

B. Evolution of Fungal Inteins

Goddard and Burt (1999) proposed a cycle model consisting of recurrent cycles of intron

invasion, maintenance, degeneration, and loss for mobile introns with an endonuclease activity. A modified model was applied to large inteins (Burt and Koufopanou 2004; Gogarten et al. 2002; Koufopanou et al. 2002). According to this model, a mobile intein initially invades a genome by horizontal transmission. Once situated in the new genome, it is vertically transmitted to successive generations and spread out in the population by homing. When the intein is fixed in the population, there will be little or no selection against point or deletion mutations, and the activity of the endonuclease will be destroyed. After the loss of activity, the endonuclease domain of the intein may eventually be deleted leading to mini-inteins. The splicing domain is predicted to be under a different selection regime. Since the functionality of the splicing domain is critical for the function of the host protein which in most cases is an essential protein, a strong purifying selection to preserve function will occur. Only a precise deletion of the intein splicing domain will ensure host protein function and results in a genome that is devoid of the entire mobile intein. Before degeneration of the endonuclease mutation of the endonuclease, the domain may create an enzyme that evolves a new specificity and can be transferred by horizontal transfer to a new host genome with the recognition site for the altered enzyme. At this point, the cycle begins again.

Regarding VMA1 inteins of saccharomycete yeasts, several lines of evidences support the model of the homing cycle. First, Koufopanou et al. (2002) and Okuda et al. (2003) reported that horizontal transmission of VMA1 inteins from different strains of *S. cerevisiae*, saccharomycete yeasts, and other yeast species has been a regular occurrence in their evolutionary history. Second, the evolutionary state of VMA1-derived endonucleases from 12 yeast species was addressed by assaying their endonuclease activities (Posey et al. 2004).

As stated above, only two enzymes have been shown to be active. PI-*Zba*I cleaves the *Z. bailii* recognition sequence significantly faster than the *S. cerevisiae* site, which differs at six nucleotide positions. A mutational analysis has indicated that PI-*Zba*I cleaves the *S. cerevisiae* substrate poorly due to the absence of a DNA-protein contact that is established by PI-*Sce*I. These findings demonstrated that intein homing endonucleases evolve altered specificities as they adapt to recognize alternative target sites. (Posey et al. 2004)

Finally, in the tetraploid wild-wine *S. cerevisiae* strain DH1-1A, it was shown that an intein-containing *VMA1* allele and an inteinless *VMA1* allele are encoded in one genome. The molecular reason for the coexistence of both alleles in one genome was shown to be due to a loss of activity in the PI-*Sce*I analogue encoded by the DH1-1A *VMA1* intein and mutations in the 31-bp recognition site of the intein-free allele of DH1-1A (Gimble 2001).

However, analyses of the PRP8 inteins suggest that the homing cycle model is not generally applicable (Gogarten and Hilario 2006). Compared to the endonucleases of VMA1 inteins, the homing endonucleases of PRP8 inteins show high d_S/d_N values (Butler et al. 2006).

In addition, the level of synonymous change in the PRP8 endonuclease has reached saturation, while the *VMA1* endonuclease is not saturated by synonymous substitutions. These results imply that VMA1 inteins are of more recent origin than the PRP8 inteins. Furthermore, phylogeny of the euascomycete PRP8 inteins provides no evidence for horizontal transfer. Only basidiomycetes of the genus *Cryptococcus* may have gained the PRP8 intein by horizontal transmission (Butler et al. 2006; Butler and Poulter 2005). Based on these results, Butler et al. (2006) proposed a modified model for the evolution of inteins present in euascomycetes. They suggested that inteins might be maintained by balancing selection. They may not become fixed in a population due to a decreased fitness of the host organism, and thus, the homing cycle might only operate in subpopulations. This scenario would allow continuous selection for the endonuclease function during extended periods of vertical transmission. In *B. cinerea* the intein appears to sustain without reaching fixation (Bokor et al. 2012). This is in agreement with mathematical models which have demonstrated computationally that intein-free and intein-containing alleles with

and without a functional endonuclease domain can coexist in populations through long evolutionary periods (Barzel et al. 2011; Yahara et al. 2009). In this system, the three allele types persist through oscillations typical for predator–prey systems (Yahara et al. 2009). Recently, Naor et al. (2016) used the natural cell fusion ability of the archaeon *Haloferax volcanii* to determine the dispersal efficiency of the DNA polymerase B intein in a natural population. Competition between intein-less and intein-carrying strains revealed substantial fitness cost of over 7% for the intein and that the intein was not able to efficiently integrate into the intein-less population. This result suggest that survival of large active inteins does not include the homing cycle proceeding to completion, but that fitness differences connected with the presence or absence of an intein appear to play a role for the coexistence of large inteins with active endonucleases and empty target sites (Naor et al. 2016).

C. Domestication of Fungal Inteins

Although Naor et al. (2016) demonstrated a fitness cost for the intein, per se, it cannot be ruled out that inteins contribute to the host fitness rather than being detrimental. It has been reported that PI-*Sce*I binds but does not cut the promoter of the *S. cerevisiae GSH11* gene encoding a high-affinity glutathione transporter. *GSH11* is not expressed in a PI-*Sce*I-deleted strain, and the inability to express *GSH11* has been shown to be overcome by the introduction of the coding region of PI-*Sce*I or the entire *VMA1* gene (Miyake et al. 2003).

Other potential positive roles for the presence of inteins as positive sensors have been reported recently (Callahan et al. 2011; Novikova et al. 2016; Topilina et al. 2015a, b). The deep-sea archaeon *Pyrococcus abyssi* carries an intein in the molybdate-biosynthesis MoaA enzyme with an N-terminal cysteine at position 1 and a cysteine at position 3 in the N-extein which is part of a conserved CXXXCXXC motif necessary for Fe–S formation and the oxygen-labile oxidoreductase activity of MoaA. It was demonstrated that the MoaA protein production is posttranslationally blocked by a Cys-3-to-Cys1 disulfide bond under hyperoxia, thereby preventing oxidative damage to the mature enzyme (Callahan et al. 2011).

Topolina et al. (2015a) discovered an oxidative and nitrosative stress-sensitive intein in the iron–sulfur cluster assembly protein SufB of *Mycobacterium tuberculosis*. This effect results from oxidative and nitrosative modification of the cysteine residue at position +1 of the C-extein, which coordinates the [Fe–S] cluster of the active SufB protein and is essential for splicing. Under oxidative and nitrosative, the Cys +1 of the SufB precursor forms either a disulfide bond, resulting in precursor accumulation, or is modified, leading to N-terminal cleavage. The posttranslation modification prevents the functional protein from being damaged under adverse conditions. Similarly, splicing of the intein located in the ATPase domain of the RadA recombinase of the hyperthermophilic archaeon *Pyrococcus horikoshii* is regulated by temperature stress. At low temperatures, an intein–extein interaction locks the intein in an inactive state. At high temperatures of 65° corresponding to the ambient temperature of *P. horikoshii*, the intein becomes unlocked for full activity (Topilina et al. 2015b).

A recent bioinformatics survey of available genome data revealed a biased distribution of inteins in ATPase domains of proteins involved in replication and recombination, processes that must be slowed down during stress conditions. It has been therefore suggested that intein splicing may act as panic buttons that reversibly inhibit detrimental ATP consumption (Novikova et al. 2016). As outlined by these examples from bacteria and archaea, fungal inteins may similarly play important regulatory roles for their hosts.

Another example for the domestication of a fungal intein is the HO endonuclease, required for mating-type switching in various yeast species (Haber 1998). Phylogenetic analyses revealed that HO and the VMA1 intein of *S. cerevisiae* are close relatives (Dalgaard et al. 1997). The *HO* gene is thought to have arisen by an ectopic insertion of the coding region of the *VMA1* intein (Butler et al. 2004). The HO endonuclease is encoded by a freestanding gene

showing 50% similarity to the full-length VMA1 intein including the splicing domain, but is unique among LAGLIDADG endonucleases in having a 120-residue C-terminal putative zinc finger domain. Mutational analysis indicated that in addition to the splicing domain and the zinc finger domain, conserved residues between HO and catalytic, active-site residues in PI-*Sce*I, and other related homing endonucleases are essential for HO activity (Bakhrat et al. 2004). Phylogenetic analysis from several yeast species revealed a single origin of the *HO* gene from the *VMA1* intein. In contrast to *VMA1* inteins, *HO* shows no evidence for degeneration or horizontal transmission (Bakhrat et al. 2006; Koufopanou and Burt 2004).

IV. Application of Inteins

Inteins were recently employed in biotechnology and medicine. Due to their robustness and autocatalytic activity, they can easily be coupled to foreign extein sequences making them highly adaptable for versatile applications. The focus of this section is on various intein-related applications including the analysis of protein–protein interactions, recombinant protein production, and the utilization of intein sequences as a molecular typing tool for pathogen detection.

A. Inteins and Their Application in Protein–Protein Interaction Studies

Protein–protein interactions play key roles in various processes of biological systems, including transcriptional regulation, protein sorting, and receptor–ligand interactions. A number of techniques for the detection of protein–protein interactions, such as yeast two-hybrid, split-ubiquitin, bimolecular fluorescence complementation, or fluorescence-detected resonance energy transfer, have been invented and established in recent years (Fields and Song 1989; Hink et al. 2002; Pasch et al. 2005; Rossi et al. 1997). Several of these methods are often used in fungi, e.g., to detect or prove the interaction of fungal proteins (Frey et al. 2015; Hoff and Kück 2005; Nolting and Pöggeler 2006). All strategies used must result in the detection of a specific protein–protein interaction via conversion to a traceable signal. An intein-based method, using the reconstitution of a split-enhanced green fluorescent protein (EGFP) by splicing a split DnaE intein derived from *Synechocystis* sp. PCC6803, was developed to investigate cellular localization of proteins. In this approach, a fusion protein comprised of the IC moiety of the naturally split Ssp DnaE intein was fused to one half of the GFP protein and to a mitochondrial targeting signal. After transformation, the fusion protein was demonstrated to be translocated into the mitochondrial matrix. The other half of the GFP was fused with the IN domain of Ssp DnaE, and this construct was then fused to cDNA libraries. If a test protein contains a functional mitochondrial targeting signal, it translocates into the mitochondrial matrix, where EGFP is then formed by protein splicing. The cells harboring this reconstituted EGFP can be screened rapidly by fluorescence-activated cell sorting, and the cDNAs can be subsequently isolated and identified from the cells (Ozawa et al. 2003). This system has been also elaborated to identify proteins targeted to the endoplasmic reticulum (Ozawa et al. 2005).

In analogy to the discovery of novel antibodies using phage displays, target binders can also be identified in a yeast surface display approach. Initially established in 1997, yeast surface display systems were widely used for the identification of protein–protein interactions or the mapping of functional protein epitopes (Boder and Wittrup 1997; Gera et al. 2013). In this approach, several thousand copies of the target protein are displayed on the yeast cell surface via fusion to the cell wall-anchoring protein α-agglutinin Aga2p. A yeast clone that interacts with a candidate from a protein library is getting separated and identified. A technical advancement of this method has been recently presented that combines the yeast surface display system with the power of protein splicing that allows the release of activated proteins that can be directly linked to chemicals or other proteins in an expressed protein ligation approach (Marshall et al. 2013).

B. Intein-Mediated Protein Purification

To produce recombinant proteins in large amounts and to purify them from crude extracts has become a major task for biotechnologists in recent years. Proteins can be produced in bacterial expression systems, and the purification process can be eased by the utilization of affinity tags (Terpe 2003). Different small peptide tags (e.g., poly-His-, poly-Arg-, CBD-, or S-tags) are commonly used, as well as higher molecular weight tags (e.g., CBM3-, GST-, or MBP-tags) to induce the solubility of proteins. However, removal of the affinity tag is often a complicated challenge and accompanied by different problems. Intein-mediated protein purification systems attempt to avoid these problems. In principle, a target protein is fused at either the N- or the C-terminus to a modified intein. The other site of the intein is linked to an affinity tag and is mutated at the tag-linked splice junction to undergo inducible-specific cleavage with the site on the protein of interest. Crude protein extracts are loaded on an affinity column; the tag immobilizes the intein fusion construct, and after some washing steps, an induced splicing reaction (cleavage step) releases the desired protein from the column. Normally, the cleavage step is inducible under mild conditions, e.g., by the addition of thiolic agents, changes in pH or temperature. The first intein engineered for protein purification was the VMA1 intein from *S. cerevisiae* (Chong et al. 1997). Later, naturally occurring or artificially engineered mini-inteins and the VMA1 intein were predominantly used for protein purification systems (Ding et al. 2007; Southworth et al. 1999; Wan et al. 2011; Wood et al. 1999).

Further development in intein-mediated protein purification was achieved using naturally or artificially split inteins (Mills et al. 1998; Wu et al. 1998). The use of these trans-cleavage systems advanced the purification, because it prevented premature in vivo cleavage, a problem often observed when cis-splicing inteins are used. The trans-splicing system takes advantage of the ability of the intein halves to self-assemble. In the majority of cases, the Ssp DnaE intein is used (Chong and Xu 2005). The target protein is fused to the IN domain of Ssp DnaE, while the IC domain is mutated at the C-extein cleavage site. Furthermore, both intein halves are tagged to facilitate isolation and purification of the expressed intein fusion proteins. Co-incubation of both parts results in the reconstitution of the intein splicing activity and in the release of the target protein.

C. Intein-Mediated Production of Growth Factors and Other Pharmaceuticals

Inteins from prokaryotes and fungi were recently applied to produce and modify several pharmaceutical agents including anticoagulants, antimicrobial peptides, ribotoxins, cytokines, and hormones, such as anophelin, α-sarcin, cecropin, interferon-α, interleukin-2, oxytocin, and plectasin (Alford et al. 2014; Chen et al. 2015; Esipov et al. 2008, 2012, 2016; Wang et al. 2012; Zhou et al. 2011).

Cytokines are growth and cell differentiation factors that are highly demanded as pharmaceuticals and cell culture supplements. Since their production in heterologous hosts is a challenge, different approaches and strategies were routinely applied, including the intein-mediated production and modification. A recombinant human granulocyte–macrophage colony-stimulating factor (hGMCSF) was produced in *E. coli* and *P. pastoris* as a VMA1-intein–chitin–tag fusion variant (Srinivasa Babu et al. 2008, 2009, 2014). This precursor protein was either secreted or kept inside the cell and purified via chitin affinity chromatography. Induction of protein cleavage led to the release of hGMCSF in soluble and active form. The production outcome was comparable to the more complex and expensive production in mammalian cells.

The VMA1 intein of *S. cerevisiae* was also successfully employed to produce the human epidermal growth factor (EGF) and the human basic fibroblast growth factor (bFGF) in recombinant form (Kwong et al. 2016a, b; Kwong and Wong 2013). In these studies, the intein was used to increase the production of recombinant growth hormones in *E. coli*. Although, EGF could be produced without intein, no heterolo-

gous protein was detectable when the gene encoding bFGF was expressed in a bacterial host. Initial results indicated that the fusion of the intein between bFGF and the OmpA signal peptide increased the production level of soluble protein, but the target protein was not separated from the intein. However, a tandem fusion protein composed of OmpA–EGF–intein–bFGF not only enhanced the production of the target protein but also led to the secretion and cleavage of the precursor. Moreover, both growth factors displayed activity in a bioassay. In another approach, the authors showed that the intein and the C-terminal fusion partner could be replaced by other inteins and larger proteins without affecting the production efficiency that is dependent on the formation of the N-terminal OmpA–EGF protein.

In a further approach, the PRP8 inteins from *H. capsulatum* and *P. chrysogenum* as well as several inteins of prokaryotic origin were tested in a system to generate self-assembling light and heavy chains for the reconstitution of functional antibodies (Gion et al. 2013). Using a modular cloning system, a single open reading frame composed of secretion signals, linkers, antibody fragments, and an intein was generated that led to the transcription of the DNA to give a monocistronic mRNA in mammalian cells. Protein biosynthesis resulted in high-level production of precursor proteins that could be successfully matured in CHO and HEK293 cells.

D. Inteins as Markers to Identify Pathogenic Fungi

Inteins are encoded by highly conserved genes in fungi and were proposed to be valuable for the differentiation of closely related species (see Sect. II), especially in clinical antifungal approaches and patient care. Human pathogenic species complexes of the genera *Candida* and *Histoplasma* were recently investigated in detail (Prandini et al. 2013; Theodoro et al. 2013).

The yeast VMA intein has been established as a marker sequence to differentiate the *Candida psilosis* complex composed of the species *C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis* (Prandini et al. 2013). All members of the *Candida psilosis* complex can cause human infections, but the correct identification of species would be important for therapies. However, *C. parapsilosis* is more virulent compared to its two relatives, but induces equal levels of tissue damage than *C. orthopsilosis*. Due to the degree of relationship, accurate species identification is challenging, and multiple time-consuming and/inaccurate strategies were established including sequencing of ITS- and rDNA-regions, RFLP, or RAPD. A PCR-based identification method based on the presence or absence of the VMA intein-encoding sequence has been recently demonstrated that allows the differentiation between all three species, because *C. parapsilosis* lacks the sequence, while the full-length sequences differ in sizes in *C. metapsilosis* (1362 bps) and *C. orthopsilosis* (1590 bps), respectively.

Several members of the fungal genus *Emmonsia* were identified to be causative agents of adiaspiromycosis, a pulmonary disease. The species *E. parva* and *E. crescens* are most common in human infections, while *E. pasteuriana* was only described in a single human case. Moreover, a close relative of *E. pasteuriana* is also pathogenic to humans. This species was identified in immunocompromised adults in South Africa and India, using a combination of marker genes including PRP8 intein-encoding sequences in a molecular biology approach (Kenyon et al. 2013; Malik et al. 2016).

V. Conclusion

Inteins are internal protein domains found inside the coding region of different proteins. They are transcribed and translated together with their host protein and are removed from the unprocessed protein by protein splicing. Fungi encode large inteins comprising independent protein splicing and endonuclease domains and mini-inteins which lack the central endonuclease domain. Our knowledge on the distribution, evolution, and functionality of

fungal inteins has expanded enormously during the past 15 years. Currently, more than 200 inteins have been identified within the nuclear genomes of fungi. Most of them are embedded in homologs of the *S. cerevisiae VMA1* gene or within the *prp8* gene, but they can be also found in glutamate synthases, chitin synthases, threonyl-tRNA synthetases, and subunits of DNA-directed RNA polymerases. Genomic sequencing projects for several fungal species have been completed, and many more are under way (http://fungidb.org). This will accelerate the discovery of new fungal inteins. Biochemical and phylogenetic analysis will contribute further insight into the splicing mechanism and the evolution of fungal inteins. Ultimately, this will lead to the engineering of fungal inteins for a variety of applications.

References

Alford SC, O'Sullivan C, Obst J, Christie J, Howard PL (2014) Conditional protein splicing of α-sarcin in live cells. Mol BioSyst 10:831–837

Amitai G, Belenkiy O, Dassa B, Shainskaya A, Pietrokovski S (2003) Distribution and function of new bacterial intein-like protein domains. Mol Microbiol 47:61–73

Aranko A, Wlodawer A, Iwaï H (2014) Nature's recipe for splitting inteins. Protein Eng Des Sel 27: 263–271

Bakhrat A, Jurica MS, Stoddard BL, Raveh D (2004) Homology modeling and mutational analysis of Ho endonuclease of yeast. Genetics 166:721–728

Bakhrat A, Baranes K, Krichevsky O, Rom I, Schlenstedt G, Pietrokovski S, Raveh D (2006) Nuclear import of ho endonuclease utilizes two nuclear localization signals and four importins of the ribosomal import system. J Biol Chem 281: 12218–12226

Barzel A, Naor A, Privman E, Kupiec M, Gophna U (2011) Homing endonucleases residing within inteins: evolutionary puzzles awaiting genetic solutions. Biochem Soc Trans 39:169–173

Beachy PA, Cooper MK, Young KE, von Kessler DP, Park WJ, Hall TM, Leahy DJ, Porter JA (1997) Multiple roles of cholesterol in hedgehog protein biogenesis and signaling. Cold Spring Harb Symp Quant Biol 62:191–204

Belfort M, Bonocora RP (2014) Homing endonucleases: from genetic anomalies to programmable genomic clippers. Methods Mol Biol 1123:1–26

Belfort M, Roberts RJ (1997) Homing endonucleases: keeping the house in order. Nucleic Acids Res 25:3379–3388

Boder ET, Wittrup KD (1997) Yeast surface display for screening combinatorial polypeptide libraries. Nat Biotechnol 15:553–557

Bokor AAM, van Kan JAL, Poulter RTM (2010) Sexual mating of *Botrytis cinerea* illustrates PRP8 intein HEG activity. Fungal Genet Biol 47:392–398

Bokor AAM, Kohn LM, Poulter RTM, van Kan JAL (2012) PRP8 inteins in species of the genus Botrytis and other ascomycetes. Fungal Genet Biol 49:250–261

Burt A, Koufopanou V (2004) Homing endonuclease genes: the rise and fall and rise again of a selfish element. Curr Opin Genet Dev 14:609–615

Buskirk AR, Ong YC, Gartner ZJ, Liu DR (2004) Directed evolution of ligand dependence: small-molecule-activated protein splicing. Proc Natl Acad Sci U S A 101:10505–10510

Butler MI, Poulter RT (2005) The PRP8 inteins in Cryptococcus are a source of phylogenetic and epidemiological information. Fungal Genet Biol 42:452–463

Butler MI, Goodwin TJ, Poulter RT (2001) A nuclear-encoded intein in the fungal pathogen *Cryptococcus neoformans*. Yeast 18:1365–1370

Butler G, Kenny C, Fagan A, Kurischko C, Gaillardin C, Wolfe KH (2004) Evolution of the MAT locus and its HO endonuclease in yeast species. Proc Natl Acad Sci U S A 101:1632–1637

Butler MI, Goodwin TJ, Poulter RT (2005) Two new fungal inteins. Yeast 22:493–501

Butler MI, Gray J, Goodwin TJ, Poulter RT (2006) The distribution and evolutionary history of the PRP8 intein. BMC Evol Biol 6:42

Callahan BP, Topilina NI, Stanger MJ, Roey PV, Belfort M (2011) Structure of catalytically competent intein caught in a redox trap with functional and evolutionary implications. Nat Struct Mol Biol 18:630–633

Chen X, Shi J, Chen R, Wen Y, Shi Y, Zhu Z, Guo S, Li L (2015) Molecular chaperones (TrxA, SUMO, Intein, and GST) mediating expression, purification, and antimicrobial activity assays of plectasin in *Escherichia coli*. Biotechnol Appl Biochem 62:606–614

Chevalier BS, Stoddard BL (2001) Homing endonucleases: structural and functional insight into the catalysts of intron/intein mobility. Nucleic Acids Res 29:3757–3774

Choi JJ, Nam KH, Min B, Kim SJ, Söll D, Kwon ST (2006) Protein trans-splicing and characterization of a split family B-type DNA polymerase from the hyperthermophilic archaeal parasite *Nanoarchaeum equitans*. J Mol Biol 356:1093–1106

Chong S, Xu MQ (1997) Protein splicing of the *Saccharomyces cerevisiae* VMA intein without the endonuclease motifs. J Biol Chem 272: 15587–155890

Chong S, Xu MQ (2005) Harnessing inteins for protein purification and characterization. In: Belfort M, Derbyshire V, Stoddard BL, Wood DW (eds) Homing endoncleases and inteins. Springer, Heidelberg, pp 273–292

Chong S, Mersha FB, Comb DG, Scott ME, Landry D, Vence LM, Perler FB, Benner J, Kucera RB, Hirvonen CA, Pelletier JJ, Paulus H, Xu MQ (1997) Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. Gene 192:271–281

Cramer P, Armache KJ, Baumli S, Benkert S, Brueckner F, Buchen C, Damsma GE, Dengl S, Geiger SR, Jasiak AJ, Jawhari A, Jennebach S, Kamenski T, Kettenberger H, Kuhn CD, Lehmann E, Leike K, Sydow JF, Vannini A (2008) Structure of eukaryotic RNA polymerases. Annu Rev Biophys 37: 337–352

Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS (1997) Statistical modeling and analysis of the LAGLIDADG family of site-specific endonucleases and identification of an intein that encodes a site-specific endonuclease of the HNH family. Nucleic Acids Res 25:4626–4638

Davis EO, Jenner PJ, Brooks PC, Colston MJ, Sedgwick SG (1992) Protein splicing in the maturation of *M. tuberculosis* recA protein: a mechanism for tolerating a novel class of intervening sequence. Cell 71:201–210

Derbyshire V, Wood DW, Wu W, Dansereau JT, Dalgaard JZ, Belfort M (1997) Genetic definition of a protein-splicing domain: functional mini-inteins support structure predictions and a model for intein evolution. Proc Natl Acad Sci U S A 94:11466–11471

Ding Y, MQ X, Ghosh I, Chen X, Ferrandon S, Lesage G, Rao Z (2003) Crystal structure of a mini-intein reveals a conserved catalytic module involved in side chain cyclization of asparagine during protein splicing. J Biol Chem 278:39133–39142

Ding FX, Yan HL, Mei Q, Xue G, Wang YZ, Gao YJ, Sun SH (2007) A novel, cheap and effective fusion expression system for the production of recombinant proteins. Appl Microbiol Biotechnol 77: 483–488

Dori-Bachash M, Dassa B, Peleg O, Pineiro SA, Jurkevitch E, Pietrokovski S (2009) Bacterial intein-like domains of predatory bacteria: a new domain type characterized in *Bdellovibrio bacteriovorus*. Funct Integr Genomics 9:153–166

Duan X, Gimble FS, Quiocho FA (1997) Crystal structure of PI-SceI, a homing endonuclease with protein splicing activity. Cell 89:555–564

Dujon B (1989) Group I introns as mobile genetic elements: facts and mechanistic speculations – a review. Gene 82:91–114

Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, Marck C, Neuvéglise C, Talla E, Goffard N, Frangeul L, Aigle M, Anthouard V, Babour A, Barbe V, Barnay S, Blanchin S, Beckerich JM, Beyne E, Bleykasten C, Boisramé A, Boyer J, Cattolico L, Confanioleri F, De Daruvar A, Despons L, Fabre E, Fairhead C, Ferry-Dumazet H, Groppi A, Hantraye F, Hennequin C, Jauniaux N, Joyet P, Kachouri R, Kerrest A, Koszul R, Lemaire M, Lesur I, Ma L, Muller H, Nicaud JM, Nikolski M, Oztas S, Ozier-Kalogeropoulos O, Pellenz S, Potier S, Richard GF, Straub ML, Suleau A, Swennen D, Tekaia F, Wésolowski-Louvel M, Westhof E, Wirth B, Zeniou-Meyer M, Zivanovic I, Bolotin-Fukuhara M, Thierry A, Bouchier C, Caudron B, Scarpelli C, Gaillardin C, Weissenbach J, Wincker P, Souciet JL (2004) Genome evolution in yeasts. Nature 430:35–44

Elleuche S, Pöggeler S (2007) Trans-splicing of an artificially split fungal mini-intein. Biochem Biophys Res Commun 355:830–834

Elleuche S, Pöggeler S (2010) Inteins, valuable genetic elements in molecular biology and biotechnology. Appl Microbiol Biotechnol 87:479–489

Elleuche S, Pöggeler S (2016) Inteins and their use in protein synthesis with fungi. In: Schmoll M, Dattenböck C (eds) Gene expression systems in fungi: advancements and applications. Springer, Heidelberg, pp 289–307

Elleuche S, Nolting N, Pöggeler S (2006) Protein splicing of PRP8 mini-inteins from species of the genus Penicillium. Appl Microbiol Biotechnol 72: 959–967

Elleuche S, Döring K, Pöggeler S (2008) Minimization of a eukaryotic mini-intein. Biochem Biophys Res Commun 366:239–243

Elleuche S, Pelikan C, Nolting N, Pöggeler S (2009) Inteins and introns within the prp8-gene of four Eupenicillium species. J Basic Microbiol 49:52–57

Esipov RS, Stepanenko VN, Chupova LA, Boyarskikh UA, Filipenko ML, Miroshnikov AI (2008) Production of recombinant human epidermal growth factor using Ssp dnaB mini-intein system. Protein Expr Purif 61:1–6

Esipov RS, Stepanenko VN, Chupova LA, Miroshnikov AI (2012) Production of recombinant oxytocin through sulfitolysis of inteincontaining fusion protein. Protein Pept Lett 19:479–484

Esipov RS, Makarov DA, Stepanenko VN, Miroshnikov AI (2016) Development of the intein-mediated method for production of recombinant thymosin β4 from the acetylated in vivo fusion protein. J Biotechnol 228:73–81

Fernandes JAL, Prandini THR, Castro MCA, Arantes TD, Giacobino J, Bagagli E, Theodoro RC (2016) Evolution and application of inteins in Candida species: a review. Front Microbiol 7:1585

Fields S, Song O (1989) A novel genetic system to detect protein-protein interactions. Nature 340:245–246

Frey S, Reschka EJ, Pöggeler S (2015) Germinal center kinases SmKIN3 and SmKIN24 are associated with the *Sordaria macrospora* Striatin-interacting phosphatase and kinase (STRIPAK) complex. PLoS One 10:e0139163

Fukuda T, Nogami S, Ohya Y (2003) VDE-initiated intein homing in *Saccharomyces cerevisiae* proceeds in a meiotic recombination-like manner. Genes Cells 8:587–602

Fukuda T, Nagai Y, Ohya Y (2004) Molecular mechanism of VDE-initiated intein homing in yeast nuclear genome. Adv Biophys 38:215–232

Gera N, Hussain M, Rao BM (2013) Protein selection using yeast surface display. Methods 60:15–26

Gimble FS (2000) Invasion of a multitude of genetic niches by mobile endonuclease genes. FEMS Microbiol Lett 185:99–107

Gimble FS (2001) Degeneration of a homing endonuclease and its target sequence in a wild yeast strain. Nucleic Acids Res 29:4215–4223

Gimble FS, Stephens BW (1995) Substitutions in conserved dodecapeptide motifs that uncouple the DNA binding and DNA cleavage activities of PI-SceI endonuclease. J Biol Chem 270:5849–5856

Gimble FS, Thorner J (1992) Homing of a DNA endonuclease gene by meiotic gene conversion in *Saccharomyces cerevisiae*. Nature 357:301–306

Gimble FS, Thorner J (1993) Purification and characterization of VDE, a site-specific endonuclease from the yeast *Saccharomyces cerevisiae*. J Biol Chem 268:21844–21853

Gimble FS, Wang J (1996) Substrate recognition and induced DNA distortion by the PI-*Sce*I endonuclease, an enzyme generated by protein splicing. J Mol Biol 263:163–180

Gion WR, Davis-Taber RA, Regier DA, Fung E, Medina L, Santora LC, Bose S, Ivanov AV, Perilli-Palmer BA, Chumsae CM, Matuck JG, Kunes YZ, Carson GR (2013) Expression of antibodies using single open reading frame (sORF) vector design: demonstration of manufacturing feasibility. MAbs 5: 595–607

Goddard MR, Burt A (1999) Recurrent invasion and extinction of a selfish gene. Proc Natl Acad Sci U S A 96:13880–13885

Gogarten JP, Hilario E (2006) Inteins, introns, and homing endonucleases: recent revelations about the life cycle of parasitic genetic elements. BMC Evol Biol 6:94

Gogarten JP, Senejani AG, Zhaxybayeva O, Olendzenski L, Hilario E (2002) Inteins: structure, function, and evolution. Annu Rev Microbiol 56:263–287

Goodwin TJ, Butler MI, Poulter RT (2006) Multiple, non-allelic, intein-coding sequences in eukaryotic RNA polymerase genes. BMC Biol 4:38

Grainger RJ, Beggs JD (2005) Prp8 protein: at the heart of the spliceosome. RNA 11:533–557

Gu HH, Xu J, Gallagher M, Dean GE (1993) Peptide splicing in the vacuolar ATPase subunit A from *Candida tropicalis*. J Biol Chem 268:7372–7381

Haber JE (1998) Mating-type gene switching in *Saccharomyces cerevisiae*. Annu Rev Genet 32:561–599

Hall TM, Porter JA, Young KE, Koonin EV, Beachy PA, Leahy DJ (1997) Crystal structure of a Hedgehog autoprocessing domain: homology between Hedgehog and self-splicing proteins. Cell 91:85–97

Hink MA, Bisselin T, Visser AJ (2002) Imaging protein-protein interactions in living cells. Plant Mol Biol 50:871–883

Hirata R, Ohsumk Y, Nakano A, Kawasaki H, Suzuki K, Anraku Y (1990) Molecular structure of a gene, VMA1, encoding the catalytic subunit of H(+)-translocating adenosine triphosphatase from vacuolar membranes of *Saccharomyces cerevisiae*. J Biol Chem 265:6726–6733

Hoff B, Kück U (2005) Use of bimolecular fluorescence complementation to demonstrate transcription factor interaction in nuclei of living cells from the filamentous fungus *Acremonium chrysogenum*. Curr Genet 47:132–138

Ingham PW (2001) Hedgehog signaling: a tale of two lipids. Science 294:1879–19881

Iwai H, Züger S, Jin J, Tam P-H (2006) Highly efficient protein trans-splicing by a naturally split DnaE intein from *Nostoc punctiforme*. FEBS Lett 580:1853–1858

Jeffries TW, Grigoriev IV, Grimwood J, Laplaza JM, Aerts A, Salamov A, Schmutz J, Lindquist E, Dehal P, Shapiro H, Jin YS, Passoth V, Richardson PM (2007) Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast *Pichia stipitis*. Nat Biotechnol 25:319–326

Kane PM, Yamashiro CT, Wolczyk DF, Neff N, Goebl M, Stevens TH (1990) Protein splicing converts the yeast TFP1 gene product to the 69-kD subunit of the vacuolar H(+)-adenosine triphosphatase. Science 250:651–657

Kenyon C, Bonorchis K, Corcoran C, Meintjes G, Lockett M, Lehloenya R, Vismer HF, Naicker P, Prozesky H, van Wyk M, Bamford C, du Plooy M, Imrie G, Dlamini S, Borman AM, Colebunders R, Yansouni CP, Mendelson M, Govender NP (2013) A dimorphic fungus causing disseminated infection in South Africa. N Engl J Med 369:1416–1424

Klabunde T, Sharma S, Telenti A, Jacobs WRJ, Sacchettini JC (1998) Crystal structure of GyrA intein from *Mycobacterium xenopi* reveals structural basis of protein splicing. Nat Struct Biol 5:31–36

Koonin EV (1995) A protein splice-junction motif in hedgehog family proteins. Trends Biochem Sci 20:141–142

Koufopanou V, Burt A (2004) Degeneration and domestication of a selfish gene in yeast: molecular evolution versus site-directed mutagenesis. Mol Biol Evol 22:1535–1538

Koufopanou V, Goddard MR, Burt A (2002) Adaptation for horizontal transfer in a homing endonuclease. Mol Biol Evol 19:239–246

Kwong KWY, Wong WKR (2013) A revolutionary approach facilitating co-expression of authentic human epidermal growth factor and basic fibroblast growth factor in both cytoplasm and culture medium of *Escherichia coli*. Appl Microbiol Biotechnol 97:9071–9080

Kwong KWY, Ng AKL, Wong WKR (2016a) Engineering versatile protein expression systems mediated by inteins in *Escherichia coli*. Appl Microbiol Biotechnol 100:255–262

Kwong KWY, Sivakumar T, Wong WKR (2016b) Intein mediated hyper-production of authentic human

basic fibroblast growth factor in *Escherichia coli*. Sci Rep 6:33948
Leipe DD, Koonin EV, Aravind L (2004) STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. J Mol Biol 343:1–28
Li Y (2015) Split-inteins and their bioapplications. Biotechnol Lett 37:2121–2137
Liu XQ (2000) Protein-splicing intein: genetic mobility, origin, and evolution. Annu Rev Genet 34:61–76
Liu XQ, Yang J (2004) Prp8 intein in fungal pathogens: target for potential antifungal drugs. FEBS Lett 572:46–50
Luo HR, Moreau GA, Levin N, Moore MJ (1999) The human Prp8 protein is a component of both U2- and U12-dependent spliceosomes. RNA 5:893–908
Malik R, Capoor MR, Vanidassane I, Gogna A, Singh A, Sen B, Rudramurthy SM, Honnavar P, Gupta S, Chakrabarti A (2016) Disseminated *Emmonsia pasteuriana* infection in India: a case report and a review. Mycoses 59:127–132
Marshall CJ, Agarwal N, Kalia J, Grosskopf VA, McGrath NA, Abbott NL, Raines RT, Shusta EV (2013) Facile chemical functionalization of proteins through intein-linked yeast display. Bioconjug Chem 24:1634–1644
Mills KV, Lew BM, Jiang S, Paulus H (1998) Protein splicing *in trans* by purified N- and C-terminal fragments of the *Mycobacterium tuberculosis* RecA intein. Proc Natl Acad Sci U S A 95: 3543–3548
Mills KV, Johnson MA, Perler FB (2014) Protein splicing: how inteins escape from precursor proteins. J Biol Chem 289:14498–14505
Miyake T, Hiraishi H, Sammoto H, Ono B (2003) Involvement of the VDE homing endonuclease and rapamycin in regulation of the *Saccharomyces cerevisiae* GSH11 gene encoding the high affinity glutathione transporter. J Biol Chem 278: 39632–39636
Monier A, Sudek S, Fast NM, Worden AZ (2013) Gene invasion in distant eukaryotic lineages: discovery of mutually exclusive genetic elements reveals marine biodiversity. ISME J 7:1764–1774
Mootz HD, Muir TW (2002) Protein splicing triggered by a small molecule. J Am Chem Soc 124:9044–9045
Moure CM, Gimble FS, Quiocho FA (2002) Crystal structure of the intein homing endonuclease PI-SceI bound to its recognition sequence. Nat Struct Biol 9:764–770
Nagai Y, Nogami S, Kumagai-Sano F, Ohya Y (2003) Karyopherin-mediated nuclear import of the homing endonuclease VMA1-derived endonuclease is required for self-propagation of the coding region. Mol Cell Biol 25:1726–1736
Naor A, Altman-Price N, Soucy SM, Green AG, Mitiagin Y, Turgeman-Grott I, Davidovich N, Gogarten JP, Gophna U (2016) Impact of a homing intein on recombination frequency and organismal fitness. Proc Natl Acad Sci U S A 113:E4654–E4661
Nelson N, Harvey WR (1999) Vacuolar and plasma membrane proton-adenosinetriphosphatases. Physiol Rev 79:361–385
Nelson RT, Lodes JK (2006) *Cryptococcus neoformans* pathogenicity. In: Brown AJP (ed) The Mycota XIII. Springer, Berlin, pp 237–266
Noël AJ, Wende W, Pingoud A (2004) DNA recognition by the homing endonuclease PI-*Sce*I involves a divalent metal ion cofactor-induced conformational change. J Biol Chem 279:6794–6804
Nolting N, Pöggeler S (2006) A MADS box protein interacts with a mating-type protein and is required for fruiting body development in the homothallic ascomycete *Sordaria macrospora*. Eukaryot Cell 5:1043–1056
Noren CJ, Wang J, Perler FB (2000) Dissecting the chemistry of protein splicing and its applications. Angew Chem Int Ed Eng 39:450–466
Novikova O, Topilina N, Belfort M (2014) Enigmatic distribution, evolution, and function of inteins. J Biol Chem 289:14490–14497
Novikova O, Jayachandran P, Kelley DS, Morton Z, Merwin S, Topilina NI, Belfort M (2016) Intein clustering suggests functional importance in different domains of life. Mol Biol Evol 33:783–799
Okuda Y, Sasaki D, Nogami S, Kaneko Y, Ohya Y, Anraku Y (2003) Occurrence, horizontal transfer and degeneration of VDE intein family in Saccharomycete yeasts. Yeast 20:563–573
Ozawa T, Sako Y, Sato M, Kitamura T, Umezawa Y (2003) A genetic approach to identifying mitochondrial proteins. Nat Biotechnol 21:287–293
Ozawa T, Nishitani K, Sako Y, Umezawa Y (2005) A high-throughput screening of genes that encode proteins transported into the endoplasmic reticulum in mammalian cells. Nucleic Acids Res 33:e34
Pasch JC, Nickelsen J, Schünemann D (2005) The yeast split-ubiquitin system to study chloroplast membrane protein interactions. Appl Microbiol Biotechnol 69:440–447
Pearl EJ, Bokor AA, Butler MI, Poulter RT, Wilbanks SM (2007a) Preceding hydrophobic and beta-branched amino acids attenuate splicing by the CnePRP8 intein. Biochim Biophys Acta 1774: 995–1001
Pearl EJ, Tyndall JD, Poulter RT, Wilbanks SM (2007b) Sequence requirements for splicing by the Cne PRP8 intein. FEBS Lett 581:3000–3004
Perler FB (1998) Protein splicing of inteins and hedgehog autoproteolysis: structure, function, and evolution. Cell 92:1–4
Perler FB (2002) InBase: the intein database. Nucleic Acids Res 30:383–384

Perler FB (2005) Inteins – a historical perspective. In: Belfort M et al (eds) Homing endonucleases and inteins. Springer, Heidelberg

Perler FB, Davis EO, Dean GE, Gimble FS, Jack WE, Neff N, Noren CJ, Thorner J, Belfort M (1994) Protein splicing elements: inteins and exteins – a definition of terms and recommended nomenclature. Nucleic Acids Res 22:1125–1127

Perler FB, Olsen GJ, Adam E (1997) Compilation and analysis of intein sequences. Nucleic Acids Res 25:1087–1093

Piégu B, Bire S, Arensburger P, Bigot Y (2015) A survey of transposable element classification systems – a call for a fundamental update to meet the challenge of their diversity and complexity. Mol Phylogenet Evol 86:90–109

Pietrokovski S (1994) Conserved sequence features of inteins (protein introns) and their use in identifying new inteins and related proteins. Protein Sci 3:2340–2350

Pietrokovski S (1998) Modular organization of inteins and C-terminal autocatalytic domains. Protein Sci 7:64–71

Posey KL, Koufopanou V, Burt A, Gimble FS (2004) Degeneration and domestication of a selfish gene in yeast: molecular evolution versus site-directed mutagenesis. Nucleic Acids Res 32:3947–3956

Poulter RT, Goodwin TJ, Butler MI (2007) The nuclear-encoded inteins of fungi. Fungal Genet Biol 44:153–179

Prandini THR, Theodoro RC, Bruder-Nascimento ACMO, Scheel CM, Bagagli E (2013) Analysis of inteins in the *Candida parapsilosis* complex for simple and accurate species identification. J Clin Microbiol 51:2830–2836

Rossi F, Charlton CA, Blau HM (1997) Monitoring protein-protein interactions in intact eukaryotic cells by beta-galactosidase complementation. Proc Natl Acad Sci U S A 94:8405–8410

Saleh L, Perler FB (2006) Protein splicing in cis and in trans. Chem Rec 6:183–193

Shah NH, Muir TW (2014) Inteins: nature's gift to protein chemists. Chem Sci 5:446–461

Shingledecker K, Jiang SQ, Paulus H (1998) Molecular dissection of the *Mycobacterium tuberculosis* RecA intein: design of a minimal intein and of a trans-splicing system involving two intein fragments. Gene 207:187–195

Shingledecker K, Jiang S, Paulus H (2000) Reactivity of the cysteine residues in the protein splicing active center of the *Mycobacterium tuberculosis* RecA intein. Arch Biochem Biophys 375: 138–144

Skretas G, Wood DW (2005) Regulation of protein activity with small-molecule-controlled inteins. Protein Sci 14:523–532

Southworth MW, Adam E, Panne D, Byer R, Kautz R, Perler FB (1998) Control of protein splicing by intein fragment reassembly. EMBO J 17:918–926

Southworth MW, Amaya K, Evans TC, MQ X, Perler FB (1999) Purification of proteins fused to either the amino or carboxy terminus of the *Mycobacterium xenopi* gyrase A intein. Biotechniques 27:110–114

Srinivasa Babu K, Antony A, Muthukumaran T, Meenakshisundaram S (2008) Construction of intein-mediated hGMCSF expression vector and its purification in *Pichia pastoris*. Protein Expr Purif 57:201–205

Srinivasa Babu K, Muthukumaran T, Antony A, Prem Singh Samuel SD, Balamurali M, Murugan V, Meenakshisundaram S (2009) Single step intein-mediated purification of hGMCSF expressed in salt-inducible *E. coli*. Biotechnol Lett 31:659–664

Srinivasa Babu K, Pulicherla KK, Antony A, Meenakshisundaram S (2014) Cloning and expression of recombinant human GMCSF from *Pichia pastoris* GS115 – a progressive strategy for economic production. Am J Ther 21:462–469

Starokadomskyy PL (2007) Protein splicing. Mol Biol (Mosk) 41:314–330

Steuer S, Pingoud V, Pingoud A, Wende W (2004) Chimeras of the homing endonuclease PI-*Sce*I and the homologous *Candida tropicalis* intein: a study to explore the possibility of exchanging DNA-binding modules to obtain highly specific endonucleases with altered specificity. Chembiochem 5:206–213

Stoddard BL (2005) Homing endonuclease structure and function. Q Rev Biophys 38:49–95

Sun W, Yang J, Liu XQ (2004) Synthetic two-piece and three-piece split inteins for protein *trans*-splicing. J Biol Chem 279:35281–35286

Sun P, Ye S, Ferrandon S, Evans TC, MQ X, Rao Z (2005) Crystal structures of an intein from the split *dnaE* gene of *Synechocystis sp.* PCC6803 reveal the catalytic model without the penultimate histidine and the mechanism of zinc Ion inhibition of protein splicing. J Mol Biol 353:1093–1105

Swithers KS, Senejani AG, Fournier GP, Gogarten JP (2009) Conservation of intron and intein insertion sites: implications for life histories of parasitic genetic elements. BMC Evol Biol 9:303

Terpe K (2003) Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Appl Microbiol Biotechnol 60:523–533

Theodoro RC, Bagagli E, Oliveira C (2008) Phylogenetic analysis of PRP8 intein in *Paracoccidioides brasiliensis* species complex. Fungal Genet Biol 45:1284–1291

Theodoro RC, Volkmann G, Liu X-Q, Bagagli E (2011) PRP8 intein in Ajellomycetaceae family pathogens: sequence analysis, splicing evaluation and homing endonuclease activity. Fungal Genet Biol 48:80–91

Theodoro RC, Scheel CM, Brandt ME, Kasuga T, Bagagli E (2013) PRP8 intein in cryptic species of *Histoplasma capsulatum*: evolution and phylogeny. Infect Genet Evol 18:174–182

Topilina NI, Mills KV (2014) Recent advances in in vivo applications of intein-mediated protein splicing. Mob DNA 5:5–5

Topilina NI, Green CM, Jayachandran P, Kelley DS, Stanger MJ, Piazza CL, Nayak S, Belfort M (2015a) SufB intein of *Mycobacterium tuberculosis* as a sensor for oxidative and nitrosative stresses. Proc Natl Acad Sci U S A 112:10348–10353

Topilina NI, Novikova O, Stanger M, Banavali NK, Belfort M (2015b) Post-translational environmental switch of RadA activity by extein–intein interactions in protein splicing. Nucleic Acids Res 43:6631–6648

Volkmann G, Iwai H (2010) Protein trans-splicing and its use in structural biology: opportunities and limitations. Mol BioSyst 6:2110–2121

von der Heyde A, Lockhauserbäumer J, Uetrecht C, Elleuche S (2015) A hydrolase-based reporter system to uncover the protein splicing performance of an archaeal intein. Appl Microbiol Biotechnol 99:7613–7624

Wan W, Wang D, Gao X, Hong J (2011) Expression of family 3 cellulose-binding module (CBM3) as an affinity tag for recombinant proteins in yeast. Appl Microbiol Biotechnol 91:789–798

Wang H, Meng X-l, Xu J-p, Wang J, Wang H, Ma C-w (2012) Production, purification, and characterization of the cecropin from *Plutella xylostella*, pxCECA1, using an intein-induced self-cleavable system in *Escherichia coli*. Appl Microbiol Biotechnol 94:1031–1039

Wende W, Grindl W, Christ F, Pingoud A, Pingoud V (1996) Binding, bending and cleavage of DNA substrates by the homing endonuclease PI-*Sce*I. Nucleic Acids Res 24:4123–4132

Werner E, Wende W, Pingoud A, Heinemann U (2002) High resolution crystal structure of domain I of the Saccharomyces cerevisiae homing endonuclease PI-*Sce*I. Nucleic Acids Res 30:3962–3971

Wood DW, Wu W, Belfort G, Derbyshire V, Belfort M (1999) A genetic system yields self-cleaving inteins for bioseparations. Nat Biotechnol 17: 889–892

Woods JP (2002) *Histoplasma capsulatum* molecular genetics, pathogenesis, and responsiveness to its environment. Fungal Genet Biol 35:81–97

Wu H, Hu Z, Liu XQ (1998) Protein trans-splicing by a split intein encoded in a split DnaE gene of Synechocystis sp. PCC6803. Proc Natl Acad Sci U S A 95:9226–9231

Xu MQ, Southworth MW, Mersha FB, Hornstra LJ, Perler FB (1993) *In vitro* protein splicing of purified precursor and the identification of a branched intermediate. Cell 75:1371–1377

Yahara K, Fukuyo M, Sasaki A, Kobayashi I (2009) Evolutionary maintenance of selfish homing endonuclease genes in the absence of horizontal transfer. Proc Natl Acad Sci U S A 106:18861–18866

Zhou X, Song Z, Liu X, Jia F, Wang Y (2011) Production of recombinant porcine interferon alpha using PHB–intein-mediated protein purification strategy. Appl Biochem Biotechnol 163:981–993

Yeast Killer Toxins: Fundamentals and Applications

Raffael Schaffrath[1], Friedhelm Meinhardt[2], Roland Klassen[1]

CONTENTS

I. Introduction

A large variety of antimicrobial substances are produced by pro- and eukaryotic microorganisms in order to improve their ability to dominate a certain environmental niche by killing or inhibiting competing microorganisms. Such substances include the classic antibiotics, either low-molecular-weight secondary metabolites or peptides that are routinely synthesized non-ribosomally and contain a limited number of amino acids. Larger antimicrobial proteins synthesized by the ribosome are also produced by pro- and eukaryotic microorganisms and include the bacteriocins and yeast killer toxins. Several of these natural microbial products are of considerable interest for applied aspects in medicine, agriculture, and food industries. In this review, we focus on the large group of ribosomally synthesized antimicrobial proteins termed killer toxins from different yeast species and summarize current knowledge of toxin diversity in terms of mechanism and structure. We further present selected examples highlighting their application potential. Reviews focusing on yeast killer toxins generally or more specifically addressing specific subtypes have been published (Stark et al. 1990; Bussey 1991; Magliani et al. 1997; Schaffrath and Meinhardt 2005; Golubev 2006; Schmitt and Breinig 2002, 2006; Klassen and Meinhardt 2007; Jablonowski and Schaffrath 2007). However, continuous progress in the identification of toxin modes of action and immunity mechanisms and in the demonstration of new application potential has been made recently.

The discovery of yeast killer toxins dates back to 1963, when Bevan and Makover (1963) described the secretion of molecules by a certain isolate of brewer's yeast *Saccharomyces cerevisiae* that inhibited growth of other yeast strains. Later the secreted molecule was identified as a protein (Woods and Bevan 1968)

[1] Fachgebiet Mikrobiologie, Institut für Biologie, Universität Kassel, Kassel, Germany; e-mail: schaffrath@uni-kassel.de; roland.klassen@uni-kassel.de
[2] Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität Münster, Münster, Germany; e-mail: meinhar@uni-muenster.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

which was named killer factor or killer toxin and the producing strain termed killer yeast. Until today, production of killer toxins was identified in over 100 ascomycetous and over 50 basidiomycetous yeast species (reviewed in Klassen et al. 2017). Several of these toxins were studied extensively, either with a focus on structure and mechanisms of cell killing or to investigate their applied aspects.

Even though killer toxin production is quite common among yeasts, the toxins are as heterogenous as is the phylogenetically diverse group of fungi regarded as "yeast." Many of the yeast killer toxins are small basic proteins (<20 kDa), but other examples include multimeric protein complexes of over 100 kDa. Apart from the killer proteins which exhibit an antibiotic activity against other microorganisms, there are also other antimicrobial substances, such as toxic glycolipids that can be produced by different yeast species and confer a killer-like phenotype to the producer strain (reviewed in Golubev 2006). However, these non-proteinaceous yeast antibiotics are not considered "killer toxins" in the common sense and will not be focused on in this review.

The cellular localization of killer toxin-encoding genes varies for distinct killer types. Whereas the majority of yeast killer toxins are encoded in the nucleus, some are encoded by selfish genetic elements consisting of viral or viruslike dsDNA or dsRNA molecules that persist in the killer strain's cytoplasm. In the following parts, we will separately address the diverse group of killer toxins based on the distinct localization of the encoding genes and summarize current knowledge on killer toxins of different genera and toxic mechanisms, focusing on well-characterized killer toxins.

II. Chromosomally Encoded Killer Toxins

A. *Cyberlindnera*

The genus *Cyberlindnera* (formerly *Williopsis*, *Pichia*, and *Hansenula*) comprises several species with well-characterized chromosomally encoded killer toxins (reviewed in Klassen et al. 2017). Two extensively studied toxins from this genus are HM-1 and WmKT from *C. mrakii* (formerly known as *Williopsis saturnus* var. *mrakii* and *Hansenula mrakii*) (Ashida et al. 1983; Kasahara et al. 1994; Guyard et al. 2002a).

HM-1 (also known as HMK) is a 10.7 kDa basic protein, which is encoded by the HMK gene and exhibits remarkable thermo- and pH-stability (Yamamoto et al. 1986a, b) that is probably achieved by five intramolecular disulfide bonds (Yamamoto et al. 1986a, b; Ashida et al. 1983; Lowes et al. 2000). HM-1 resists treatment at 100 °C for 10 min and remains active in between pH 2 and 11 (Ashida et al. 1983; Lowes et al. 2000).

As for several other toxins discussed below, HM-1 is initially translated as a preprotoxin that is subsequently processed by several cleavage events during maturation (Fig. 1). Specifically, the mature, 88 amino acid (aa) spanning HM-1 toxin is formed by signal peptidase-mediated cleavage of the N-terminal signal peptide and Kex2 endopeptidase-mediated removal of a propeptide region from the 125 aa preprotoxin during secretion (Fig. 1; Kimura et al. 1993). The three-dimensional structure of HM-1 has been solved (Antuch et al. 1996; Fig. 1). The structure revealed a surprising folding similarity to γB-crystallin, a major constituent of vertebrate eye lenses, a finding that was unexpected due to the lack of detectable homology between HM-1 and γB-crystallin at the amino acid level (Antuch et al. 1996). It is assumed that eye lens crystallins and HM-1 evolved from an ancestral single-domain precursor. As for HM-1, crystallins similarily exhibit outstanding stability, likely a necessity of their structural role in vertebrate eye lenses, where almost no protein turnover takes place (Wistow and Piatigorsky 1988).

Earlier work indicated that HM-1 binds to and inhibits β-1,3-glucan synthase, a key enzyme involved in cell wall synthesis (Yamamoto et al. 1986a, b; Komiyama et al. 1996; Takasuka et al. 1995). This inhibitory action toward β-1,3-glucan synthesis was thought to impair cell wall resynthesis in zones of bud formation and to cause subsequent pore

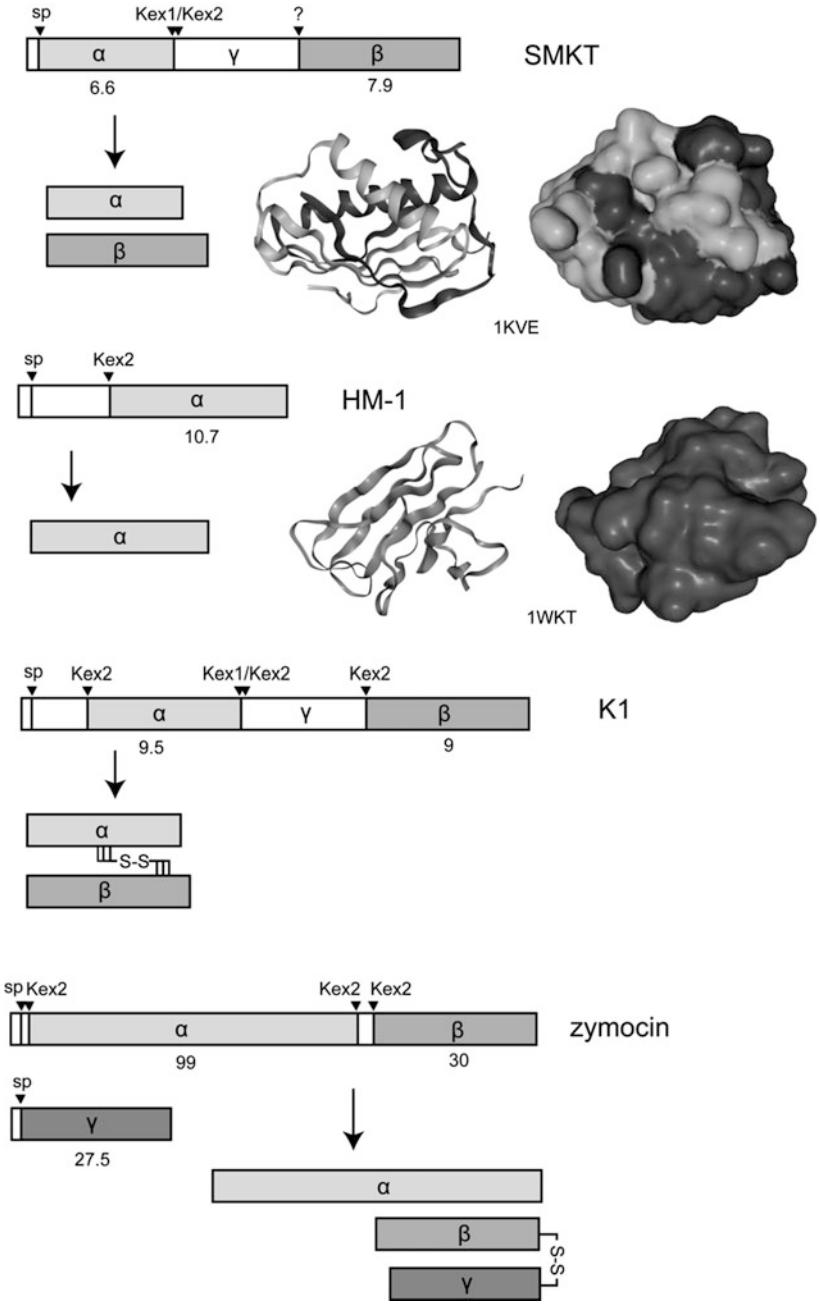


Fig. 1 Processing of toxin precursors. Toxin precursors are schematically depicted along with their mature toxins below the arrow. HM-1 and WmKT structures based on protein database entries 1KVE and 1WKT, respectively, are shown in cartoon (ribbon/coil) and surface views. Parts constituting mature toxins are given in gray, and parts being removed during processing are in white. Distinct subunits are indicated by shades of gray. Subunit sizes are given in kDa; processing sites by Kex1/Kex2 peptidases as well as signal peptidase (sp) are indicated as such. –S–S–: disulfide bridge in mature toxins; K1 contains multiple disulfide bridges

formation and cell lysis (Takasuka et al. 1995; Komiyama et al. 1996). It was further demonstrated that osmotic stabilization suppresses toxic effects of HM-1 (Komiyama et al. 1996).

Several toxins (see below) are known to initially bind to a certain cell wall receptor, followed by binding to a secondary membrane receptor. Thus, killer toxins may engage in stepwise cell wall and membrane receptor interactions preceding the actual cell-killing mechanism. HM-1, for example, is assumed to initially bind to β-1,6- and β-1,3-glucan in the cell wall since exogenously supplied β-1,6-/β-1,3-glucan can antagonize HM-1 toxicity, and *Saccharomyces cerevisiae kre6* mutants with lowered β-glucan content as well as spheroplasts are toxin resistant (Kasahara et al. 1994; Komiyama et al. 2002). In addition, HM-1 was shown to bind to an unidentified protein in the membrane fraction of yeast cells, possibly representing the secondary membrane receptor (Miyamoto et al. 2006). When using *S. cerevisiae alg3* mutants which lack the α-1,3-mannosyltransferase involved in protein glycosylation or other mutants with defects in protein glycosylation, a significantly increased HM-1 resistance was observed (Kimura et al. 1999; Miyamoto et al. 2011). At the same time, HM-1 binding efficiency to the membrane fraction is strongly reduced in the *alg3* mutant, suggesting that HM-1 first binds to a glycosylated receptor protein before inhibition of beta-glucan synthase takes place (Miyamoto et al. 2011).

Screening of the genome-wide deletion library of *S. cerevisiae* identified a number of genes that impact HM-1 sensitivity. Mutants lacking *HOG1* or *SLT2* genes are defective in signaling pathways responding to high osmotic pressure (high osmolarity glycerol pathway, *HOG*) or disturbance in cell integrity (cell wall integrity, CWI), respectively, and the mutants are hypersensitive to HM-1 (Miyamoto et al. 2011, 2012). Such mutant responses are consistent with an effect of HM-1 on osmoregulation and induction of cell wall stress, and HM-1 indeed activates both stress response pathways (Miyamoto et al. 2011, 2012). While cell wall integrity could be affected by the action of HM-1 as an inhibitor of β-1,3-glucan synthase, the role of turgor regulation in the cellular response to HM-1 involves an additional, not previously recognized aspect. In fact, the screening of the genome-wide deletion mutant collection identified the genomic *FPS1* locus of *S. cerevisiae* as being crucial for HM-1 toxicity (Miyamoto et al. 2011). Mutants lacking the gene are devoid of a porin membrane channel protein that mediates glycerol export under conditions of low osmotic pressure in order to reduce turgor (Tamás et al. 1999). In further support of a key role of Fps1 in HM-1 toxicity, it was noted that HM-1 sensitivity in distinct yeast species correlates with the presence of an Fps1 orthologue (Yamamoto et al. 1988; Miyamoto et al. 2012). Despite a defect in osmoregulation and proven induction HOG signaling by HM-1, *fps1* mutants were shown to exhibit a 17-fold increase in the IC50 dose of HM-1, suggesting that high turgor caused by the mutation is HM-1 protective. Surprisingly, however, the high turgor of *fps1* deletion mutants increases sensitivity to inhibition of β-1,3-glucan synthesis by other agents, such as echinocandins, since higher turgor likely increases the tendency of cells with injured wall to rupture (Miyamoto et al. 2012). Therefore, the HM-1-resistant response of *fps1* mutant cells was interpreted to indicate that induction of cell wall stress, rather than inhibition of β-1,3-glucan synthesis, might play a major role in the toxic effect of HM-1. Thus, additional research is needed to characterize the role of Fps1 in the HM-1 response and further define the contribution of the described inhibitory effect on β-1,3-glucan synthesis to the toxic mechanism.

The *C. mrakii* WmKT of strain MUCL4198 is, even though the producing species are identical, rather different from HM-1. The size of 85 kDa (Table 1) is significantly larger than that of HM-1, and, compared to the latter, WmKT has a substantially reduced tolerance to pH and temperature variation (Guyard et al. 2002a). Optimal activity of WmKT was observed at a pH 4.6 and temperatures between 26 and 28 °C. Such acidic pH optimum applies for a number of other killer toxins (see below) and might represent an adaptation to the environmental setting in which killer toxins serve a benefit to the producing cell by inhibiting the growth of competitors. It is assumed that the toxic principle of WmKT is mediated by hydrolysis of cell wall β-glucans (Guyard et al. 2002a). This

Table 1 Chromosomally encoded yeast killer toxins

Species	Strain	Toxin	Size (kDa)	Receptor	Toxic activity	References
Ascomycetes						
Wickerhamomyces anomalus (Pichia anomala)	NCYC434	K5/pano mycocin	49	β-1,3-glucan	Glucanase	Izgü and Altinbay (2004)
	WC65		83.3	β-1,6-glucan		Sawant et al. (1989)
	ATCC 96603; K36; UP25F	PaKT/PKT	85	β-glucan (?)		Guyard et al. (1999), Polonelli and Morace (1986)
	YF07b		47		Glucanase	Wang et al. (2007a)
	DBVPG 3003	Pikt	>3			Comitini et al. (2004a)
	BCA15/BCU24/BS91	–		β-1,3- and β-1,6-glucans	Glucanase	Muccilli et al. (2013)
Millerozyma farinosa (P. farinosa)	KK1	SMKT	α (6.6)β (7.9)		Membrane permeabilization	Suzuki and Nikkuni (1994)
P. kluyveri	1002		19		Membrane permeabilization	Middelbeek et al. (1979)
P. membranifaciens	CYC1106	PMKT	18	β-1,6-glucan	Membrane permeabilization	Santos et al. (2000)
	CYC1086	PMKT2	30	mannoprotein	Cell cycle inhibition	Santos et al. (2009, 2013)
Barnettozyma californica (Williopsis californica)	DSM 12865	Wicaltin	34			Theisen et al. (2000)
Cyberlindnera saturnus (W. saturnus)	IFO 0117	HYI	9.5		Inhibition of β-1,3-glucan synthase	Komiyama et al. (1995, 1998)
	DBVPG 4561	KT4561	62			Buzzini et al. (2004)
Cyberlindnera mrakii (W. saturnus var. mrakii)	MUCL 41968	WmKT	85	β-glucan	Glucanase/ membrane permeabilization	Guyard et al. (2002a, b)
	IFO 0895	HM-1	10.7	β-glucan	Inhibition of β-1,3-glucan synthase	Ashida et al. (1983), Kasahara et al. (1994)
Cyberlindnera mrakii (W. mrakii)	NCYC500	K500	1.8-5	β-glucan	Membrane permeabilization	Hodgson et al. (1995)
Debaryomyces hansenii	CYC1021	–	23	β-1,6-glucan		Santos et al. (2002)
Lachancea waltii (Kluyveromyces waltii)	IFO1666T	–	>10			Kono and Himeno (1997)
K. wickerhamii	DBVPG 6077	Kwkt	72			Comitini et al. (2004a)

(continued)

Table 1 (continued)

Species	Strain	Toxin	Size (kDa)	Receptor	Toxic activity	References
Tetrapisispora phaffii (K. phaffii)	DBVPG 6076	KpKt	33	β-1,6-/β-1, 3-glucan	Glucanase	Comitini et al. (2004b)
K. marxianus	NCYC587	K6	42			Izgü et al. (1999)
Schwanniomyces occidentalis	ATCC 44252		α (7.4) β (4.9)	Mannan	Membrane permeabilization	Chen et al. (2000)
Saccharomyces cerevisiae	111	KHR	20			Goto et al. (1990)
	115	KHS	75			Goto et al. (1991)
Candida noadenis		CnKT				da Silva et al. (2008)
Basidiomycetes						
Piskurozyma capsuligenum (Filobasidium capsuligenum)	IFM 40078	FC-1		β-1,6-glucan	Membrane permeabilization	Keszthelyi et al. (2006)
Vanrija humicola (Cryptococcus humicola)	VKM Y-1439	Cellobiose lipid	<1		Membrane permeabilization	Golubev and Shabalin (1994), Puchkov et al. (2001, 2002)

assumption is based on the suppression of toxic effects of WmKT by application of glucosidase inhibitors and on the detection of in vitro glucosidase activity of the killer toxin (Guyard et al. 2002a). Further, *S. cerevisiae kre1* and *kre4* mutants, defective in β-1,3- or β-1,6-glucan synthesis, display WmKT resistance (Guyard et al. 2002a, b). In addition to HM-1 and WmKT, strain NCYC500 of *C. mrakii* (formerly *W. mrakii*) produces a very small killer toxin (K500, 1.8-5 kDa) that does not exhibit the broad pH and temperature stability characteristic of HM-1 and based on its small size might rather unlikely exhibit an enzymatic activity similar to that of WmKT (Hodgson et al. 1995). Thus, different strains of the same species can produce killer toxins that may have little in common except for the overall purpose of competitor inhibition.

Another species of *Cyberlindnera* known to form a number of different toxins is *C. saturnus* (formerly *W. saturnus*). Strain IFO0117 produces a toxin termed HSK, which is similar to the above described HM-1 (Kimura et al. 1993). Strains IFO0117 and DBVPG4561 produce toxins designated HYI and KT4561 (Table 1; Komiyama et al. 1995, 1998; Buzzini et al. 2004). Of these, HYI might be similar to HM-1, both with respect to size and toxic principle as an inhibitor of the β-1,3-glucan synthase (Komiyama et al. 1995, 1998). The toxic mechanism of KT4561 is currently unknown, but similar to HM-1 it exhibits relatively good pH and temperature stability. However, with an experimentally determined size of 62 kDa, it is a much larger protein than HM-1.

B. *Pichia*

Several species of the genus *Pichia* are recognized as toxin producers. Some well-characterized *Pichia* killer species have been moved to different genera such as *Millerozyma* or *Wickerhamomyces* and will be discussed in a separate chapter. *Pichia kluyveri* (strain 1002) produces a 19 kDa killer toxin which induces toxic effects by forming ion-permeable channels (Middelbeek et al. 1979; Kagan 1983). Such ion channels were shown to result in cell shrinkage accompanied by leakage of ions, adenosine 5′-triphosphate and decrease of intracellular pH (Middelbeek et al. 1980a, b). A similar toxic principle was afterwards assigned to a number of additional toxins from diverse sources (see below). The *P. kluyveri* toxin is active at acidic conditions (pH 2.5 and 4.7) and at temperatures below 40 °C (Middelbeek et al. 1979, 1980a).

Another species of the genus, the halotolerant yeast *P. membranifaciens*, secretes a toxin termed PMKT (*P. membranifaciens* killer toxin), which exhibits a similar toxic principle as for the *P. kluyveri* toxin (Santos and Marquina 2004a). However, PMKT activity is enhanced by the presence of salt (Marquina et al. 1992; Lorente et al., 1997). PMKT is similar to the *P. kluyveri* toxin in size (18 kDa) and was found to be active against sensitive yeast cells at temperatures below 20 °C and at acidic pH (below 4.8; Santos et al. 2000). It is assumed that PMKT first binds to β-1,6-glucan as the primary receptor and subsequently interacts with Cwp2, a cell wall mannoprotein (Santos et al. 2007). Interestingly, the mature form of Cwp2 is covalently linked to β-1,6-glucan, while its precursor is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. Thus, it is assumed that interactions between PMKT and Cwp2 may assist transport of the toxin from its primary cell wall receptor to the cell membrane, where lethal ion channel formation occurs (Santos et al. 2007; Belda et al. 2017).

Transcriptional profiling of *S. cerevisiae* cells exposed to PMKT revealed the induction of genes of the high glycerol (HOG) pathway (Santos et al. 2005), resembling later observations described above for the mechanistically unrelated HM-1 (Miyamoto et al. 2011, 2012). In addition, mutants defective in Hog1 are hypersensitive to both HM-1 and PMKT (Santos et al. 2005; Miyamoto et al. 2011, 2012). Hence, PMKT and HM-1 both induce a coordinated transcriptional response in target cells resembling the response to osmotic stress which apparently counteracts the toxic effects of both toxins (Santos et al. 2005; Rep et al. 2000). Further studies are required to investigate whether both toxins have

additional mechanistic similarities not yet recognized.

Another strain of the same species (*P. membranifaciens* CYC1086) is known to produce a toxin (PMKT2) with diverse properties (Santos et al. 2009, 2013). PMKT2 is larger than PMKT (Table 1) and exhibits a different mode of action. Instead of using β-glucan as the primary receptor, PMKT2 binds to mannoproteins and stops growth of target cells by inducing an early S-phase cell cycle arrest. At low doses, PMKT2 induces apoptotic cell death, similar to a number of mechanistically unrelated killer toxins (see below) (Santos et al. 2013). For a detailed comparison of PMKT and PMKT2, we refer to a recent review (Belda et al. 2017).

C. *Wickerhamomyces* and *Millerozyma*

A variety of killer toxins have been described in different strains of *Wickerhamomyces anomalus* (formerly *Pichia anomala*) (Table 1), several of which were isolated from agricultural or food sources (Comitini et al. 2004b; Izgü and Altinbay 2004; Wang et al. 2007a; Muccilli et al. 2013). Production of killer toxins or other growth inhibitory compounds is quite common in this species as a systematic screening revealed antagonistic activities in more than 70% of *W. anomalus* strains tested from the Russian Collection of Microorganisms (VKM) (Golubev 2015). For some of the *Wickerhamomyces* killer toxins, information about their killing mechanism is available. Similar to WmKT from *C. mrakii*, several of these toxins bind to β-glucan in the target cell wall and induce toxic effects by hydrolysis of this major cell wall constituent (Table 1). In particular the glucanase killer toxins from *W. anomalus* are known to exhibit a broad antimicrobial antimicrobial activity, not restricted to yeast species. Some *W. anomalus* toxins also inhibit pathogenic bacteria or mycelial fungi and even protozoans which have raised interest in industrial applications (see Chapter IV) (Sawant et al. 1989; Walker et al. 1995; Jijakli and Lepoivre 1998; Izgü et al. 2007a, b; Wang et al. 2007a; Muccilli et al. 2013; Valzano et al. 2016).

The halotolerant yeast *Millerozyma farinosa* (formerly *Pichia farinosa*) produces the SMKT (salt-mediated killer toxin), which is expressed as a preprotoxin of 222 aa (Suzuki and Nikkuni 1994). As for HM-1, SMKT preprotoxin is processed by the signal peptidase and the Kex protease during secretion, resulting in mature α- and β-subunits of 6.6 and 7.9 kDa, respectively. In this instance, Kex processing liberates the interstitial γ-polypeptide, forming the mature αβ dimer (Fig. 1; Suzuki and Nikkuni 1994). As for many other killer toxins, SMKT is active at acidic pH (below 5). Higher pH-values induce dissociation of the toxin subunits, resulting in loss of activity (Suzuki et al. 1997). The crystal structure of SMKT has been determined (Kashiwagi et al. 1997), but the nature of its primary receptor remains unknown. Interestingly, SMKT exhibits a remarkable folding similarity to a dsRNA-encoded toxin (KP4) from a phylogenetically distinct origin (*Ustilago maydis*) (see also Sect. III). In vitro studies with purified liposomes suggest that SMKT results in membrane permeabilization, similar to PMKT and *P. kluyveri* toxins (Suzuki et al. 2001).

D. *Kluyveromyces, Lachancea, and Tetrapisispora*

Several *Kluyveromyces* species secrete toxins with different characteristics. *K. lactis* produces the well-characterized toxin zymocin, which is encoded by a cytoplasmic plasmid system described in detail in part (Sect. II.B). Chromosomally encoded toxins are known in *K. wickerhamii* (KwKt) and *K. marxianus* (K6) (Izgü et al. 1999; Comitini et al. 2004a, b; Comitini and Ciani 2011). The species *K. waltii* and *K. phaffii* are also toxin producers and were reclassified as *Lachancea waltii* and *Tetrapisispora phaffii*, respectively (Table 1) (Young and Yagiu 1978; Kono and Himeno 1997; Ciani and Fatichenti 2001).

The *T. phaffii* toxin known as KpKt (*Kluyveromyves phaffii* killer toxin) is a 33 kDa protein encoded by the *TpBGL2* gene and exhibits glucanase activity, similar to several other toxins from *W. anomalus* (Comitini et al. 2009;

Oro et al. 2014). KwKt and K6 were purified as proteins of 72 and 42 kDa; however, their mode of action remains unknown so far.

III. Extrachromosomally Encoded Toxins

A. dsRNA Virus Toxins

A number of well-characterized yeast killer toxins are encoded by killer genes with unusual cellular localization. In fact, the firstly discovered *S. cerevisiae* killer strains (Woods and Bevan 1968) were found to harbor dsRNA viruses which carry the genetic information for toxin production (Bevan et al. 1973). These *S. cerevisiae* viruses of the *Totiviridae* family exist in pairs of separately encapsulated virus-like particles in the cytoplasm (for review, see Wickner 1992, 1996; Schmitt and Breinig 2002, 2006). Strictly required for the system is the presence of the 4.6 kb L-A helper virus, which encodes the major capsid protein (Gag) and a RNA-dependent RNA polymerase (Pol). The Gag-encoding ORF1 and Pol-encoding ORF2 of L-A overlap in the -1 reading frame, and a programmed -1 ribosomal frameshift results in the formation of a Gag-Pol fusion protein, which is required for the replicative cycle of the virus (Icho and Wickner 1989; Dinman et al. 1991). The L-A type *Totivirus* may occur with or without satellite dsRNAs with the prefix "M." The M viruses termed M1, M2, M28, and Mlus encode different toxin types (K1, K2, K28, Klus) (Bevan et al. 1973; Schmitt and Breinig 2006; Rodríguez-Cousiño et al. 2011). These satellite viruses depend on the presence of an L-A-type virus, since they utilize the L-A-encoded Gag and Gag-Pol for encapsidation and replication. The capsid encoded by L-A contains 60 Gag-dimers and one or two Gag-Pol fusion proteins. The structure of the capsid contains pores to allow the exit of (+)ssRNA transcribed from the viral genome within the capsid. In addition, they allow acquisition of host metabolites but retain the dsRNA copy of the virus and exclude degradative enzymes (Castón et al. 1997). For the replicative cycle and virus gene expression, a (+)ssRNA copy is generated in the capsid by the RNA-dependent RNA polymerase activity of Gag-Pol. After release from the capsid, such (+)ssRNA copy of the virus is translated by the host ribosome into preprotoxin (M virus) or Gag and Gag-Pol fusion proteins (L-A virus). In addition, the (+)ssRNA is encapsidated into newly formed capsids, in which synthesis of the complementary (–)RNA strand occurs. These yeasts *Totiviridae* lack an extracellular route of transmission and are therefore termed viruslike particles (to distinguish from viruses with an infectious cycle). Well-characterized and functionally distinct *S. cerevisiae* toxins encoded by dsRNA viruses are K1 (encoded by M1 virus) and K28 (encoded by M28 virus) (see also Schmitt and Breinig 2002, 2006). More recently, a novel dsRNA-encoded toxin (Klus) was identified and significant progress made in the characterization of the Klus-encoding M and associated helper viruses (Rodríguez-Cousiño et al. 2011, 2013; Rodríguez-Cousiño and Esteban 2017). Also, more recent work established K2 as a toxin type with significant differences to K1 (Servienė et al. 2012; Lukša et al. 2015; Orentaite et al. 2016). Interestingly, the different types of dsRNA-encoded toxins are equally dependent on the presence of an L-A-type helper virus but generally lack conserved toxin sequences (de la Peña et al. 1981; Dignard et al. 1991; Schmitt and Tipper 1995; Suzuki and Nikkuni 1994; Rodríguez-Cousiño et al. 2011).

1. K1 and K2

As assumed for other yeast killer toxins, K1-induced target cell killing occurs in several discrete steps, involving initial contact to a primary cell wall receptor followed by binding of a distinct membrane receptor. K1 uses β-1,6-glucan in the cell wall as the primary receptor and the GPI-anchored cell wall glycoprotein Kre1 as the membrane receptor to reach the plasma membrane and ultimately forms cation-selective ion channels (Hutchins and Bussey 1983; de la Peña et al. 1981; Martinac et al. 1990; Breinig et al. 2002, 2004). The mode of action resembles the above chromosomal

PMKT which also causes membrane permeabilization, and it utilizes the same primary but a distinct membrane receptor (Cwp1). Interestingly, however, both membrane receptor proteins (Kre1 and Cwp1) are GPI-anchored and appear in a mature glucan-bound and additionally in a membrane-bound (GPI-anchored) form. Thus, the subsequent interactions of the toxins with cell wall glucan and with both mature and GPI-anchored forms of a membrane receptor may represent a common strategy to mediate transport of the toxin from the initial binding site to the actual cellular target.

The K1 toxin is a dimer with subunit sizes of 9.5 (α) and 9 kDa (β), which are covalently linked by three disulfide bridges (Bostian et al. 1984). Site-directed mutagenesis indicated that both of the subunits are involved in binding to the primary receptor, while the α-subunit alone is required for membrane interaction (Bussey 1991; Zhu and Bussey 1991). Maturation of K1 is well characterized and involves common processing steps of a preprotoxin precursor identified in other toxins. The two subunits of K1 are formed by signal peptidase and Kex1-/Kex2-dependent processing and involve the release of the γ-peptide region (Bostian et al. 1984; Zhu et al. 1992). The processing of K1 proprotoxin is strikingly similar to the above described chromosomally encoded SMKT and the viral K28 toxin, even though the toxins are diverse at the sequence level (de la Peña et al. 1981; Schmitt and Tipper 1995; Suzuki and Nikkuni 1994).

More similarities between PMKT and K1 include the significance of a functional HOG signaling pathway for toxin resistance. *S. cerevisiae* cells with a defect in HOG signaling—due to the loss of Hog1—display strong sensitivity not only to PMKT but also to K1 (Pagé et al. 2003), suggesting a transcriptional response similar to other osmotic stresses being effective in suppressing K1 toxicity.

Other than the chromosomally encoded toxins, which typically target distinct species, dsRNA-encoded toxins are usually active against *S. cerevisiae* strains that do not carry the L-A- and M-type *Totiviridae*, while toxin producers are immune against their own, but not other K-type toxins (Schmitt and Breinig 2006). For K1, the immunity mechanism is known to be mediated by the K1 toxin precursor (preprotox). It was demonstrated, that expression of a cDNA copy of M1 in mutants lacking Kex2 confers K1 immunity in the absence of toxin production. Further, expression of the α-subunit together with 31 N-terminal residues of γ is sufficient for K1 immunity, indicating that the presence of a part of the preprotoxin confers K1 protection (Zhu et al. 1993).

The K2 toxin exhibits similarities to K1 in terms of the cell killing strategy causing membrane permeabilization (Orentaite et al. 2016). Like K1, K2 binds to β-1,6-glucan and apparently also uses Kre1 as the plasma membrane receptor; however, the primary K2 sequence is unrelated to K1, and K2 displays a more acidic pH optimum (Young and Yagiu 1978; Pfeiffer and Radler 1984; Dignard et al. 1991; Schmitt and Breinig 2002; Novotná et al. 2004; Lukša et al. 2015). In addition, K2 killers remain susceptible to K1 (and vice versa), and screening of the *S. cerevisiae* genome-wide deletion collection revealed 332 genes changing susceptibility to K2, the majority of which not influencing K1 susceptibility (Dignard et al. 1991; Meskauskas and Citavicius 1992; Servienė et al. 2012). Thus, even though K1 and K2 may utilize similar cell wall and membrane receptors and target the plasma membrane to induce cell killing, functional differences exist between these toxins with respect to target cell interaction and immunity mechanism (Dignard et al. 1991; Meskauskas and Citavicius 1992; Novotná et al. 2004; Servienė et al. 2012).

2. K28

Another well-characterized toxin encoded by a *S. cerevisiae* dsRNA is K28. Similar to K1, it consists of two small subunits (α, β) of around 10 kDa (Table 2), which are covalently linked by a disulfide bridge (Schmitt and Tipper 1995). K28 is encoded by a single ORF and initially translated as preprotoxin which is processed during secretion by signal peptidase and Kex1/Kex2 to form two toxin subunits from a single polypeptide (Schmitt and Tipper 1990,

Table 2 Extrachromosomally encoded yeast killer toxins

Species	Strain	Toxin	Size	Receptor	Toxic activity	References
Ascomycetes						
dsRNA encoded						
S. cerevisiae	KL88	K1	α (9.5)β (9)	β-1,6-glucan	Membrane permeabilization	Young and Yagiu (1978)
	M471	K2	α/β (21.5)	β-1,6-glucan	Membrane permeabilization	Dignard et al. (1991); Lukša et al. (2015), Orentaite et al. (2016)
	CBS8112	K28	α (10)β (11)	Mannoprotein	Blocking DNA synthesis	Schmitt and Tipper (1990)
		Klus				
Hanseniaspora uvarum	470		18	β-1,6-glucan		Schmitt and Neuhausen (1994)
Zygosaccharomyces bailii	412	Zygocin	10	Mannoprotein	Membrane permeabilization	Schmitt and Neuhausen (1994)
dsDNA encoded						
Kluyveromyces lactis	IFO1267	Zymocin	α (99)β (30)γ (28)	Chitin	$tRNA^{Glu}$-specific tRNase	Gunge et al. (1981)
Babjeviella inositovora (Pichia inositovora)	NRRL Y-18709		>100	Chitin	rRNA cleavage	Hayman and Bolen (1991); Kast et al. (2014)
Millerozyma acaciae (P. acaciae)	NRRL Y-18665	PaT	α (110)β (39)γ (38)	Chitin	$tRNA^{Gln}$-specific tRNase	Worsham and Bolen (1990)
Debaryomyces robertsiae (Wingea robertsiae)	CBS6693	DrT	>100	Chitin	$tRNA^{Gln}$-specific tRNase	Klassen and Meinhardt (2002), Klassen et al. (2014)
Basidiomycetes						
dsRNA encoded						
Ustilago maydis	P1	KP1	13.4			Park et al. (1996a)
	P4	KP4	13.6		Blocking calcium uptake	Park et al. (1994)
	P6	KP6	α (8.6)β (9.1)		K^+ depletion	Tao et al. (1990)
Mrakia aquatica (Cryptococcus aquaticus)	VKM Y-2428					Pfeiffer et al. (2004)
Cystofilobasidium infirmominiatum	VKM Y-2897		>15			Golubev et al. (2003)
Tausonia pullulans (Trichosporon pullulans)	VKM Y-2303		>15			Golubev et al. (2002)
T. insectorum	CBS10422					Fuentefria et al. (2008)

1995; Riffer et al. 2002; Schmitt and Breinig 2006). As for K1, the protoxin contains a γ-peptide region intervening the α-β sequences. This γ-peptide is deleted by processing with the Kex2 endopeptidase (Schmitt and Tipper 1995). Despite this striking similarity in toxin maturation between K1 and K28, target cell interaction and killing mechanisms are completely different for both of the toxins.

In fact, K28 is so far the only example of a yeast killer toxin gaining access to the target cell by endocytosis and subsequent retrograde passage of the secretory pathway, followed by exit from the endoplasmic reticulum (ER), a strategy followed by several bacterial toxins of the so-called A/B type. For K28, the toxic α-subunit subsequently enters the nucleus, where it blocks DNA replication (Fig. 2; Schmitt et al. 1996; Eisfeld et al. 2000; Heiligenstein et al. 2006).

The K28 toxin uses cell wall mannoprotein as the primary receptor and subsequently interacts with Erd2 as the membrane receptor (Schmitt and Radler 1987; Schmitt and Breinig 2006; Eisfeld et al. 2000; Becker et al. 2016). Erd2 is a H/KDEL receptor protein which localizes mainly to the Golgi/endoplasmic reticulum and mediates retention of ER-resident proteins, but a minor fraction is present in the cytoplasmic membrane (Semenza et al. 1990; Becker et al. 2016). This minor fraction of Erd2 was shown to bind to the HDEL motif present in the K28 β-subunit (Schmitt and Breinig 2006; Becker et al. 2016), followed by endocytosis and retrograde transport of K28 to the ER (Eisfeld et al. 2000; Becker et al. 2016). The HDEL motif recognized by Erd2 is uncovered upon Kex1 processing of K28 β at the C-terminus (HDELR) and is a strict requirement for toxin function, due to its crucial role in mediating the reentry of the toxin into the secretory pathway of the target cell (Eisfeld et al. 2000).

The exit of the toxin from the ER to the cytoplasm occurs via the the Sec61 translocon (Eisfeld et al. 2000; Heiligenstein et al. 2006). The Sec61 complex mediates bidirectional translocation of protein across the ER membrane. This includes secretory proteins entering the ER and misfolded proteins, which are removed from the secretory pathway for subsequent degradation via the ERAD (ER-associated protein degradation) pathway (reviewed in Nakatsukasa and Brodsky 2008). K28 apparently mimics an ERAD substrate, and the ER chaperones Kar2, Pdi1, Scj1, Jem1, and Pmr1, which normally mediate the ER-specific unfolded protein response, assist exit of partially unfolded but covalently linked α-β subunits from the ER (Heiligenstein et al. 2006). Once in the cytosol, the α-β dimer is split by a toxin-intrinsic mechanism to reduce the disulfide bond between the two subunits, releasing the toxic α-subunit (Suzuki et al. 2017). The free β-subunit gets ubiquitinated and is subsequently degraded by the proteasome, while the α-subunit enters the nucleus and ultimatively inhibits DNA synthesis as the final growth inhibitory event (Schmitt et al. 1996; Heiligenstein et al. 2006). Screening collections of over 5000 mutants with deletions in nonessential or temperature-sensitive alleles of essential genes revealed a number of processes protecting the cells from K28, such as Hog1, vacuolar proteins, and the proteasome (Carroll et al. 2009). Also, the same study identified the AP2 clathrin adaptor subunits as crucial for K28 toxicity, by performing an important function in endocytosis of this toxin (Carroll et al. 2009).

Similar to K1, the preprotoxin of K28 also mediates toxin immunity specifically against K28, and again K28 immunity is also established in a *kex2* mutant, which is unable to release active toxin (Schmitt and Tipper 1992; Zhu et al. 1992). While the precise mechanism of K1 immunity is still unknown, details for K28 are established (Breinig et al. 2006). K28 killer cells were shown to reinternalize mature K28 toxin, and an interaction with unprocessed K28 preprotoxin in the cytoplasm is the key step for immunity. Mechanistically, this involves ubiquitination and selective proteasomal degradation of the mature re-internalized K28 (Breinig et al. 2006). Notably, partial immunity is already conferred by the α-subunit alone when present in the cytoplasm and full immunity required only a nonspecific sequence extension to the α-subunit, which strikingly resembles the situation in K1 immunity (Breinig et al. 2006).

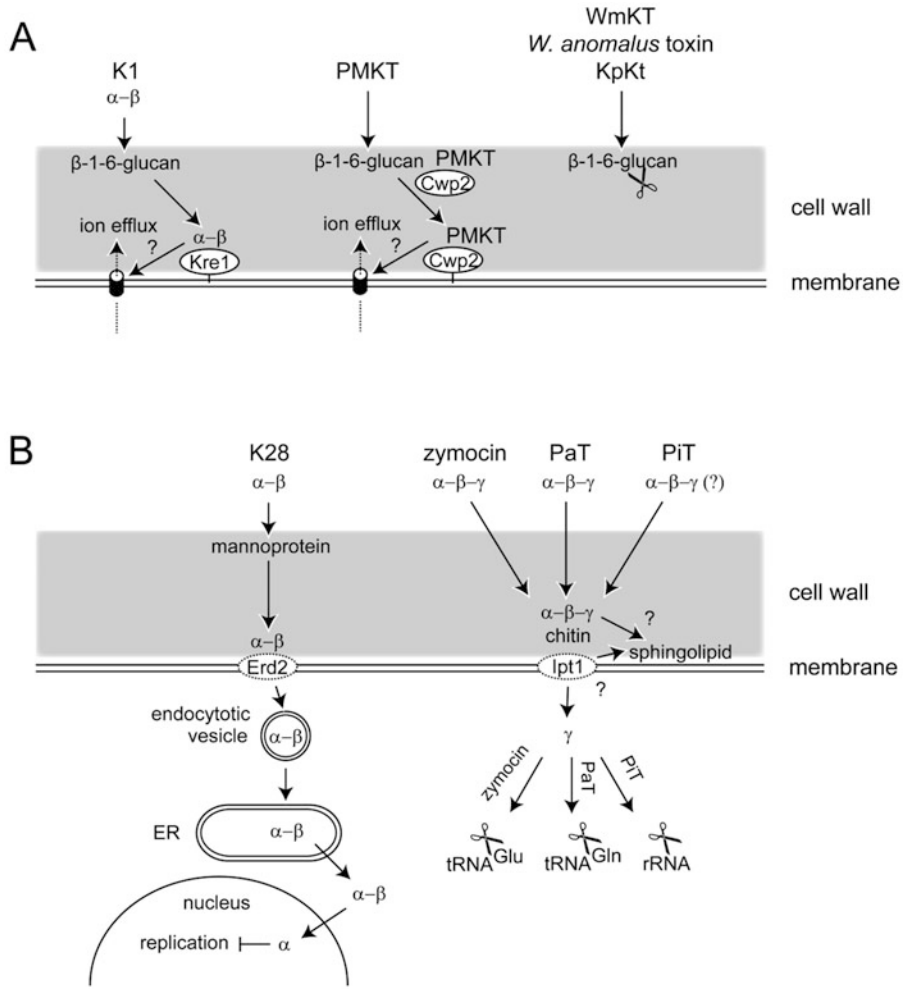


Fig. 2 Schematic representation of killer toxin mode of action. (**a**) Toxins targeting cell wall or membrane. β-1,6-glucan is the primary cell wall receptor for chromosomally encoded PMKT and dsRNA-encoded K1. PMKT and K1 utilize GPI-anchored Kre1 and Cwp2 as secondary membrane receptors and induce membrane channel formation. WmKT, W. anomalus toxin, and KpKt act as glucanases. (**b**) Toxins with intracellular targets. Mannoprotein represents the cell wall receptor for dimeric K28. Chitin is the proposed cell wall receptor for zymocin, PaT, and PiT. Erd2 acts as the secondary membrane receptor for K28, which gains access to the target cell's cytoplasm by retrograde transport and finally inhibits DNA synthesis in the nucleus. The secondary membrane receptor for zymocin and related toxins PaT and PiT is unknown; however, Ipt1-synthesized sphingolipid is required for cellular uptake of the zymocins' γ-subunit which cleaves cellular tRNAGlu. In analogy to zymocin γ, toxic subunits of PaT and PiT also enter the target cell to cleave tRNAGln or rRNA, respectively. See text for details and references

3. Other dsRNA Virus Toxins

Similar to *S. cerevisiae*, the yeasts *Hanseniaspora uvarum* and *Zygosaccharomyces bailii* are also known to carry L-A- and M-type dsRNA viruses of the *Totiviridae* family (Radler et al. 1990; Zorg et al. 1988; Schmitt and Neuhausen 1994; Schmitt et al. 1997; Weiler et al. 2002). The *H. uvarum* toxin is a 18 kDa monomer which utilizes cell wall β-1,6-glucan as the primary receptor and displays a relatively broad killing spectrum as compared to the *S. cerevisiae* K1 or K28 toxins (Radler et al. 1990; Schmitt and Neuhausen 1994; Schmitt et al.

1997). The 10 kDa *Z. bailii* toxin zygocin also exhibits a broad spectrum of sensitive yeasts, which includes human and phytopathogenic fungi (Weiler and Schmitt 2003). While the lethal mechanism of the *H. uvarum* toxin remains unknown, zygocin was shown to induce cell permeabilization, possibly due to membrane channel formation (Weiler and Schmitt 2003; Schmitt and Breinig 2006). In contrast to the other dsRNA-encoded toxins, the zygocin preprotoxin is not required for immunity of the producer cell. It was demonstrated that *Z. bailii* whole cells and protoplasts are naturally resistant to zygocin (Weiler et al. 2002), a situation that was observed for chromosomally encoded toxins as well (Weiler et al. 2002).

dsRNA viruses encoding killer toxins are not even restricted to the ascomycetous yeast species. They were also identified in the dimorphic fungus *Ustilago maydis* (Puhalla 1968), a basidiomycete which has budding yeast like and filamentous growth stages (reviewed in Steinberg and Perez-Martin 2008). Three different immunity specificities (P1, P4, and P6) have been found in this species (Koltin and Day 1976; Tipper and Bostian 1984). In contrast to the *S. cerevisiae* dsRNA-encoded toxins, which are routinely small dimers, KP1 and KP4 are monomeric proteins of 13.4 and 13.6 kDa, respectively (Park et al. 1994, 1996a); KP6, however, is a dimer with subunits of 8.6 and 9.1 kDa (Tao et al. 1990). The structure of KP4 and KP6 were determined (Gu et al. 1995; Li et al. 1999; Allen et al. 2013). KP4 was found to exhibit structural similarities to scorpion toxins, which are known to act on Na^+ channels (Gu et al. 1995). Since KP4's toxic effects could be suppressed by exogenous Ca^{2+}, it was suggested that KP4 may act by inhibition of Ca^{2+} channels (Gu et al. 1995). This was further substantiated by demonstrating KP4-mediated inhibition of voltage-gated Ca^{2+} channels in mammalian neuronal cells (Gu et al. 1995). However, KP4 was also realized to be a close structural homologue of the chromosomally encoded toxin SMKT from *M. farinosa* (Kashiwagi et al. 1997), which is thought to directly induce membrane permeabilization (Suzuki et al. 2001). KP6 is structurally distinct from KP4; its two subunits exhibit remarkable structural similarity to each other but not to other known toxins (Allen et al. 2013). Again, α- and β-subunits of the toxin are formed by Kex processing of the protoxin and removal of a γ-peptide from the center of the protoxin, located between the α- and β-regions (Tao et al. 1990; Allen et al. 2013). Although it was suggested that KP6 may induce target cell killing by pore formation leading to leakage of cell contents (Peery et al. 1987; Li et al. 1999; Steinlauf et al. 1988), the presence of intramolecular disulfide bonds in the α- and β-subunits of KP6 were interpreted to be more consistent with an indirect induction of cell lysis by the toxin, for example, by activation of membrane channels (Allen et al. 2013).

A number of additional basidiomyceteous yeasts were identified to carry viruslike particles with dsRNA genomes, which are associated with killer phenotypes (Table 2) (Golubev et al. 2002, 2003; Pfeiffer et al. 2004; Fuentefria et al. 2008). Some characteristics of the encoded toxins are described, but their modes of action and structural details remain to be investigated.

B. Toxins Encoded by Viruslike Elements (VLEs)

A second type of extranuclear genetic information associated with killer toxin production is represented by the dsDNA elements previously termed linear plasmids, killer plasmids, but more recently, due to evidences for viral ancestry viruslike elements (VLE) (reviewed in Satwika et al. 2012a; Meinhardt and Schaffrath 2001; Schaffrath and Meinhardt 2005; Klassen and Meinhardt 2007). Reminiscent of the *S. cerevisiae* L-A *Totivirus* being associated with satellite M viruses that confer the killer phenotype, dsDNA killer elements (Fig. 3) can be distinguished into autonomous and nonautonomous traits as well. They occur in pairs or triplets in a number of different ascomycetous genera such as *Debaryomyces*, *Millerozyma*, *Babjeviella*, *Saccharomycopsis*, *Schwanniomyces*, and *Botryoascus* but also in the basidiomycete *Tausonia* (Trichosporon) *pullulans* (Kitada and Hishinuma 1987; Ligon et al.

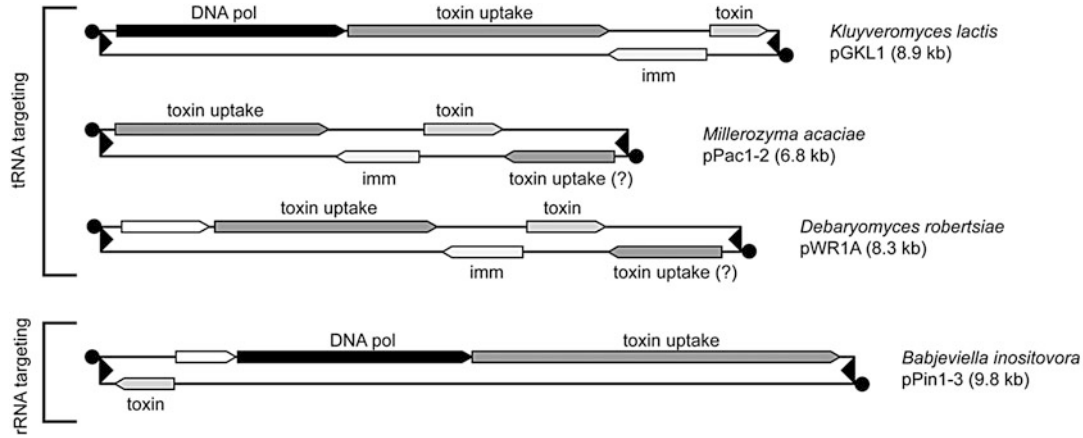


Fig. 3 Schematic representation of viruslike dsDNA plasmids from yeasts encoding killer toxins. dsDNA plasmids are grouped according to the target molecules. Arrows indicate ORFs and their transcriptional direction; terminal proteins are depicted as filled circles; terminal inverted repeats correspond to filled triangles. Known or proposed functions of encoded proteins are indicated. Toxin, intracellular toxic subunit; imm, immunity proteins. The predicted toxin uptake protein is homologous in all systems

1989; Worsham and Bolen 1990; Hayman and Bolen 1991; Bolen et al. 1992; Cong et al. 1994; Fukuhara 1995; Chen et al. 2000).

As for the *Totiviridae*, most of these dsDNA molecules reside in the cytoplasm of the host, and the autonomous element provides key functions to facilitate cytoplasmic DNA replication and transcription, which the associated nonautonomous elements depend on (reviewed in Jeske et al. 2006; Klassen and Meinhardt 2007).

Cytoplasmic replication is initiated using a free –OH group of the VLE-encoded terminal protein (TP) (protein priming). The TP is expressed as a fusion protein with a viral B-type DNA polymerase, and after completion of replication, it remains covalently bound to the 5′ ends of the plasmids (Tommasino et al. 1988; Hishinuma and Hirai 1991; Hishinuma et al. 1984; Stark et al. 1984; Sor and Fukuhara 1985; Klassen et al. 2001; Klassen and Meinhardt 2003; Jeske and Meinhardt 2006). Related B-type DNA polymerases involved in a similar mode of replication are also found in adenoviruses and certain bacteriophages (reviewed in Klassen and Meinhardt 2007).

For cytoplasmic transcription, a uniquely structured VLE-encoded RNA polymerase and a mRNA capping enzyme are employed which are both encoded by the autonomous element as well (Wilson and Meacock 1988; Larsen et al. 1998; Schaffrath et al. 2000). The capping enzyme is most closely related to the one from cytoplasmic vaccinia virus (Larsen et al. 1998; Tiggemann et al. 2001; Klassen and Meinhardt 2007). The genes on the killer elements are equipped with unique cytoplasmic promoters characterized by a short 6 nt consensus sequence that is recognized by the plasmid-encoded RNA polymerase (Kämper et al. 1989a, b; Kämper et al. 1991; Romanos and Boyd 1988; Schaffrath et al. 1996; Schickel et al. 1996). Due to the unique promoter structure of cytoplasmic genes, nuclear genes cannot be expressed on the cytoplasmic dsDNA elements unless their promoter is exchanged, and—vice versa—plasmid genes cannot be expressed in the nucleus without modification (Romanos and Boyd 1988; Schaffrath and Meacock 1996; Stark et al. 1990; Meinhardt et al. 1994; Schaffrath et al. 1995; Schründer and Meinhardt 1995; Schickel et al. 1996). As for the satellite M viruses, killer toxin production is in all cases known exclusively associated with the nonautonomous elements (Fig. 3), but only some of the described nonautonomous elements are in fact associated with a killer phenotype. A number of nonautonomous elements

are apparently cryptic, but several of them show remnants of killer toxin genes, suggesting loss of functional toxin genes during evolution (Klassen et al. 2002; Klassen and Meinhardt 2007; Satwika et al. 2012b).

The currently recognized dsDNA-encoded killer toxins are found in *Kluyveromyces lactis* (zymocin), *Millerozyma* (*Pichia*) *acaciae* (PaT), *Debaryomyces* (*Wingea*) *robertsiae* (DrT), and *Babjevia* (*Pichia*) *inositovora* (PiT). They were found to target distinct tRNA species in target cells, such as tRNA or rRNA.

1. tRNA-Targeting Toxins

a) Zymocin

The best studied instance of the tRNA-targeting toxins is zymocin from *K. lactis*, which is encoded by the dsDNA element pGKL1 that is associated with the autonomous pGKL2 (Stark and Boyd 1986; Stark et al. 1990). The toxin is a heterotrimer (αβγ) with subunit sizes of 99, 30, and 28 kDa, which are encoded by 2 separate genes on pGKL1 (Jablonowski and Schaffrath 2007). As for various other toxins (see above), the polypeptide encoded by the larger ORF (ORF2) is processed by signal peptidase and the *K. lactis* Kex1 (*S. cerevisiae* Kex2 homologue) endopeptidase during secretion to form α- and β-subunits (Hishinuma et al. 1984; Stark et al. 1984, 1990; Sor and Fukuhara 1985; Stark and Boyd 1986; Tokunaga et al. 1987). The γ-subunit is encoded by a separate gene and becomes covalently linked to β via a disulfide bond (Stark and Boyd 1986; Stark et al. 1990; Wemhoff et al. 2014). Zymocin utilizes target cell wall chitin as the primary receptor for target cell binding. This step is mediated by the α-subunit, which is characterized by the presence of a chitin-binding and chitinase domain (Stark et al. 1990; Butler et al. 1991a; Jablonowski et al. 2001). Unlike other known killer toxins not targeting the cell wall itself, the receptor-binding part of the toxin can also hydrolyze the receptor, and the ability to do so appears to be essential for toxin function (Butler et al. 1991a; Wemhoff et al. 2014). Following binding and possibly hydrolysis of chitin, the γ-subunit is imported into the target cell in a poorly understood process. This is thought to involve the aid of the hydrophobic β-subunit and depends on a particular membrane sphingolipid ($M(IP)_2C$) as well as a proton gradient generated by the plasma membrane ATPase Pma1 (Mehlgarten and Schaffrath 2004; Zink et al. 2005).

In addition to the chitinase activity of the α-subunit, also the disulfide bond between β and γ is essential for the killing activity of zymocin (Butler et al. 1991a; Wemhoff et al. 2014). However, toxicity of the complex absolutely requires the presence of the γ-subunit and this protein alone, when conditionally expressed inside the cell mimics toxic effects of the trimeric complex (Tokunaga et al. 1989; Stark et al. 1990; Butler et al. 1991b; Frohloff et al. 2001; Wemhoff et al. 2014). The actual mechanism of cell killing is the selective enzymatic cleavage of $tRNA^{Glu}_{UUC}$ by hydrolyzing the phosphodiester bond between the wobble nucleoside (U34) and the 3′ nucleoside (U35) (Lu et al. 2005; Jablonowski et al. 2006). Cleavage of this tRNA is dependent on the presence of the eukaryotic form of the conserved xm^5U modification 5-methoxy-carbonyl-methyl-2-thiouridine (mcm^5s^2U) at the wobble position (Butler et al. 1994; Frohloff et al. 2001; Huang et al. 2005; Lu et al. 2005). The six-subunit Elongator complex (Elp1–Elp6, for a recent review see Schaffrath and Leidel 2017) and the tRNA methyltransferase Trm9 are required for the synthesis of the methoxy-carbonyl-methyl side chain (mcm^5U), and a separate sulfur transfer pathway facilitates the thiolation at position 2 (s^2U) of the uracil base (Kalhor and Clarke 2003; Huang et al. 2005, 2008; Lu et al. 2005; Noma et al. 2009; Leidel et al. 2009). Consistent with the importance of mcm^5s^2U for cell killing by the tRNase, loss of *ELP1-ELP6* or *TRM9* prevents, and loss of any member of the thiolation pathway genes reduces zymocin toxicity (Frohloff et al. 2001; Fichtner et al. 2003; Lu et al. 2005; Jablonowski et al. 2006). This dependency of the toxin on the presence of the complex tRNA modification has been utilized to identify further loci with a previously unknown role in tRNA mcm^5s^2U modification (Fichtner and Schaffrath 2002; Mehlgarten and Schaffrath 2003; Jablonowski and Schaffrath 2007; Fichtner et al. 2002;

Huang et al. 2005; Lu et al. 2005; Bär et al. 2008; Zabel et al. 2008; Studte et al. 2008).

When the γ-subunit was purified and its tRNAse activity was analyzed in vitro, two other mcm^5s^2U-modified tRNAs ($tRNA^{Gln}_{UUG}$ and $tRNA^{Lys}_{UUU}$) were cleaved, although the efficiency was much lower compared to $tRNA^{Glu}_{UUC}$ (Lu et al. 2005). Since combined overexpression of $tRNA^{Glu}$, $tRNA^{Gln}$, and $tRNA^{Lys}$ increased zymocin resistance compared to overexpression of $tRNA^{Glu}$ alone, all three tRNAs were assumed to represent in vivo targets, but $tRNA^{Glu}$ is the preferred one (Lu et al. 2005; Jablonowski et al. 2006). Indeed, intracellular induction of the tRNAse subunit resulted in depletion of $tRNA^{Glu}$, but no reduction in the abundance of $tRNA^{Lys}$ or $tRNA^{Gln}$ was observed (Lu et al. 2005). In fact, it could be demonstrated that the anticodon sequence U34U35C36 and an adenosine in position 37 and a cytidine in position 38 are required for efficient cleavage by γ-toxin in vitro, a requirement that is met by $tRNA^{Glu}$ but not $tRNA^{Gln}$ or $tRNA^{Lys}$ (Lu et al. 2008). Consistent with differential zymocin resistance phenotypes of Elongator/tRNA thiolation mutants, it was shown that the presence of the mcm^5 side chain provides a strong stimulatory effect, while the presence of the s^2U group revealed a weak positive effect on the cleavage efficiency (Lu et al. 2008). Interestingly, it was further demonstrated that the presence of a chemically slightly distinct form of the xm^5 modification, the bacterial 5-methylaminomethyl (mnm^5) group, is a negative determinant for cleavage (Lu et al. 2008).

Strinkingly, γ lacks sequence similarity to other ribonucleases. Site-directed mutagenesis identified Glu9, Arg151, and His209 of the γ-subunit as the probable catalytic residues (Keppetipola et al. 2009; Jain et al. 2011). Despite the absence of primary sequence similarity to other known ribonucleases, it is assumed that γ-toxin cleaves its target by an RNase A-like chemical mechanism of transesterification involving His209 and Glu9 as general acid-base catalysts with a stabilization of the transition state by Arg151 (Keppetipola et al. 2009; Jain et al. 2011). As for other RNases using such transesterification mechanism, γ also produces 2′3′cyclic phosphate and 5′OH ends that require specific end-healing enzymatic activities before repair by ligation is possible (Lu et al. 2005; Nandakumar et al. 2008). Eukaryotic cells, such as *S. cerevisiae*, carry a tRNA ligase enzyme (Trl1) that is capable of tRNA end healing and sealing and normally operates in the process of tRNA splicing. Therefore, it was somewhat surprising that the zymocin-induced tRNA cleavage products are evidently not efficiently repaired by Trl1, even though this enzyme can fix a very similar tRNA damage in the splicing reaction (Nandakumar et al. 2008). It was demonstrated that specifically the yeast tRNA ligase is inhibited by the presence of the mcm^5s^2U modification at the cleavage site, whereas other RNA ligases (plant and phage) are capable of repairing zymocin-induced tRNA damage and confer toxin resistance (Nandakumar et al. 2008).

b) PaT and DrT

The VLE-encoded killer toxins from *Millerozyma acaciae* (*Pichia acaciae*) and *D. robertsiae* (formerly *Wingea robertsiae*) display similarities but also differences to zymocin (Worsham and Bolen 1990; Meinhardt and Schaffrath 2001; Klassen and Meinhardt 2002; Klassen et al. 2004, 2008, 2014). In both cases, nonautonomous elements associated with autonomous elements were found to carry genes with similarity to the pGKL1 gene encoding the zymocin αβ precursor protein, while at the same time a gene encoding a γ-subunit homologue is absent (Klassen et al. 2004). PaT and DrT are thought to share initial steps of target cell interaction with zymocin: They both bind cell wall chitin and subsequently import a toxic subunit in a common process involving the conserved αβ-like protein with chitin-binding (and likely chitinase) activity (Klassen et al. 2004). However, the intracellularly active toxin subunits of PaT and DrT target a distinct tRNA species ($tRNA^{Gln}_{UUG}$) for cleavage compared to zymocin (Klassen et al. 2008, 2014). PaT was shown to cleave $tRNA^{Gln}_{UUG}$ at position 34 as does zymocin but, unlike the latter, does not require the presence of mcm^5s^2U (Klassen et al. 2008). In vitro cleavage experiments using total yeast tRNA with or without the modification suggest that PaT may utilize an additional

cleavage site upstream of U34, likely U32 (Klassen et al. 2008; Meineke et al. 2012). This assumption is based on the detection of two closely migrating cleavage protducts with fully modified $tRNA^{Gln}$ and detection of only the faster migrating one in the absence of mcm^5s^2U (Klassen et al. 2008). It is assumed that in the presence of mcm^5s^2U, both sites may be cleaved, which could lead to the excision of a dinucleotide. This interpretation is further supported by the analysis of RNA repair enzymes on in vivo. Other than for zymocin (see above), plant and phage tRNA ligases were unable to suppress PaT toxicity in the presence of endogenous yeast *TRL1* and mcm^5s^2U modification (Nandakumar et al. 2008; Meineke et al. 2012). Interestingly, however, plant and phage tRNA ligase were capable of suppressing toxic effects in the absence of mcm^5s^2U, a condition where available evidence suggests that only the U32 site is cleaved. A likely explanation for the differential rescue of toxic effects of the two distinct killer endonucleases is that PaT, but not zymocin can cleave two sites in its target tRNA, which may result in the excision of a dinucleotide and hence, may damage the target in a non-repairable fashion (Meineke et al. 2012). It should be noted, however, that dinucleotide excision could not yet directly be demonstrated in fully modified $tRNA^{Gln}_{UUG}$ and was undetectable when using synthetic unmodified substrates in in vitro cleavage studies with purified PaT PaOrf2 (Chakravarty et al. 2014). Thus, a discrepancy between results with unmodified stem-loop substrates and fully modified tRNA exists, and it could also be possible that other reasons than dual cleavage sites account for the detection of duplet bands in in vitro cleavage reactions with PaT. The crystal structure of the toxic subunit of PaT has been determined and indicated a novel type of folding pattern and active site arrangement distinct from any other ribonuclease (Chakravarty et al. 2014).

Apart from direct inhibition of translation, PaT was found to induce cellular effects resembling those induced by DNA-damaging agents (Klassen et al. 2004, 2008, 2011; Wemhoff et al. 2016a, b). PaT induces S-phase cell cycle arrest and *S. cerevisiae* mutants defective in DNA repair pathways base excision repair and homologous recombination exhibit strongly enhanced toxin susceptibility, suggesting a link between translational integrity and genome surveillance. Ribonucleotide reductase (RNR) was recently identified as a potential mediator of the DNA-damaging effect of PaT. RNR is periodically expressed and induced in early S-phase to satisfy the massively increased demand for ribonucleotide to desoxyribonucleotide conversion when genome replication is initiated. In the presence of sublethal doses of PaT, the induction of RNR in early S-phase is impaired, which likely accounts for the observed stalling of replication forks in the toxin mediated S-phase arrest. Impaired RNR formation and subsequent dNTP pool depletion will also impair repair of endogenous DNA lesions via pathways requiring dNTP and could indirectly increase DNA damage by inhibiting endogenous repair. In support of a general connection between inhibition of translation and DNA damage, specific DNA repair pathways were demonstrated to protect cells not only from PaT but also from zymocin and the ribosome inhibitor hygromycin B (Klassen et al. 2011; Wemhoff et al. 2016a, b).

c) PiT

A third dsDNA-encoded toxin related to zymocin, PaT, and DrT is produced in *B. inositovora* (formerly *P. inositovora*, *Yamadazyma inositovora*) (Hayman and Bolen 1991; Klassen and Meinhardt 2003; Kast et al. 2014). Again, a precursor protein similar to zymocin αβ is encoded by a nonautonomous plasmid, and a separate gene encodes a subunit that is imported into the target cell and induces the actual toxic effects (Klassen and Meinhardt 2003; Kast et al. 2014). As for the other dsDNA-encoded toxins, conditional expression of the toxic subunit devoid of its signal peptide mimics toxic effects of the holotoxin. In contrast to zymocin, PaT, and DrT, however, PiT apparently targets ribosomal RNA (rRNA) instead of tRNA, as the toxic subunit was shown to induce fragmentation of the 18S and 25S rRNAs (Kast et al. 2014). Positions of PiT-induced cleavage sites were approximately mapped using Northern hybridizations, and multiple positions were identified that are

cleaved after induction of the toxic subunit. One of the cleavage sites in 18S rRNA was mapped at the nucleotide level and found to reside in a small loop of the 18S rRNA that exhibits some sequence similarity to the anticodon loop of $tRNA^{Glu}_{UUC}$ (Kast et al. 2014). Hence, PiT might exhibit a distant relationship to zymocin, which specifically cleaves this tRNA. It remains to be determined whether the toxic subunit indeed exhibits rRNA-specific ribonuclease activity and whether cleavage occurs in the context of assembled ribosomes. In marked contrast to zymocin, however, loss of tRNA modification mcm5s2U only modestly increases toxin resistance (Kast et al. 2014). It is not known if RNA modifications, which occur in rRNA as well as in tRNA act as modulators of PiT-induced cleavage.

d) Immunity Against dsDNA-Encoded Toxins

All killer toxin-producing yeasts utilize a strategy to exclude themselves from the effects of their own toxin. One strategy is the production of toxins that utilize receptors not present in the producer strain. However, both the dsRNA- and dsDNA-encoded toxins are routinely active against strains of the same species devoid of the killer virus/viruslike element. It is assumed that this toxin specificity for other strains of the same species creates a strong positive selection to maintain the toxin-encoding genetic element. For the dsRNA viruses, the preprotoxin is often associated with immunity as well (see above). In contrast, there are separate immunity genes in case of the nonautonomus elements encoding zymocin, PaT, and DrT (Tokunaga et al. 1987; Paluszynski et al. 2007; Kast et al. 2015). An immunity gene is apparently lacking in the nonautonomous plasmid-encoding PiT (Hayman and Bolen 1991; Klassen and Meinhardt 2003). The immunity genes of PaT and DrT display detectable sequence similarity as do the corresponding tRNAse subunits of the toxins (Klassen et al. 2004, 2014; Paluszynski et al. 2007), and while each mediates full protection against the cognate toxin, at least the PaT immunity factor can provide detectable cross protection against DrT as well (Klassen et al. 2014). Since DrT and PaT toxic subunits are not detectably similar to zymocin γ either at the sequence level or with respect to the target tRNA, no cross protection between PaT/DrT and zymocin was observable (Kast et al. 2015). Based on these observations, it was concluded that these immunity proteins directly recognize the cognate toxin and protect against its toxic RNA-cleaving activity (Klassen et al. 2014; Kast et al. 2015). Interestingly, all three immunity factors entirely prevent toxic action of intrcellularly expressed tRNAse subunits (Paluszynski et al. 2007; Klassen et al. 2014; Chakravarty et al. 2014; Kast et al. 2015), indicating that immunity factors neutralize the reimported toxin subunit in the producer cell, rather than blocking its uptake. For PaT, direct inhibition of the in vitro tRNAse activity by the immunity protein was demonstrated (Chakravarty et al. 2014). A unusually high A/T content of PaT, DrT, and zymocin immunity genes was recently demonstrated to ensure exclusive gene expression in the cytoplasm (Kast et al. 2015). Even when equipped with a nuclear promoter, these genes cannot be functionally expressed in the nucleus due to recognition of A/T rich motifs within the immunity gene transcripts by the nuclear polyadenylation machinery. As a result, such transcripts become internally cleaved and polyadenylated. This mechanism is thought to prevent successful nuclear capture of immunity genes, which would undermine the autoselection principle imposed by VLE-encoded toxin and immunity gene combinations (Kast et al. 2015).

IV. Applications

A. Antifungals for Human Therapy

Some of the yeast killer toxins exhibit activity against human pathogens causing severe systemic infections, such as *Candida albicans* or *Cryptococcus neoformans* that are difficult to treat with conventional antimycotics. Based on this activity, they have been suggested to be potentially useful for therapy of human infections (Yamamoto et al. 1988; Walker et al. 1995; Weiler and Schmitt 2003; Buzzini et al. 2004; Theisen et al. 2000; Magliani et al. 1997; Izgü et al. 2007a). However, direct application of

killer toxins is of limited practical importance because many of these proteins are unstable or inactive at temperatures around 37 °C or neutral pH. In addition, antigenicity and toxicity may prohibit application in the human bloodstream and therefore cannot directly be considered as a therapeutic option to treat severe systemic mycoses (Magliani et al. 2004). However, some specific killer toxins display a broad temperature stability which might facilitate their use as topical applications on superficial skin lesions (Buzzini et al. 2004). For example, *W. anomalus* K5 toxin, which exhibits stability at 37 °C was studied against dermatophytes and several pathogenic *Candida* species. All clinical isolates tested as well as type strains belonging to the genera *Trichosporon*, *Microsporum*, and *Candida* were found to be susceptible to K5, suggesting this toxin might indeed be applicable as a topical antifungal agent (Izgü et al. 2007a, b).

To overcome the abovementioned problems associated with the direct application of killer toxins, a strategy of using antibodies with killer activity has been initiated by Polonelli and Morace (1988). A monoclonal antibody (mAbKT4) which neutralized the in vitro activity of *W. anomalus* UCSC 25F (=ATCC 96603; Table 1) PaKT (Polonelli and Morace 1987) was used to raise anti-idiotypic antibodies, which display an internal image of the toxin's active site. Strikingly, such natural polyclonal and subsequently developed monoclonal antibodies or single-chain variable fragments (scFv) derived from a phage display library were able to interact with the cell wall and kill yeast cells susceptible to the original *W. anomala* toxin (Polonelli and Morace 1988; Polonelli et al. 1990, 1997; Magliani et al. 1997, 2004). Vaccination with mAbKT4 in the mouse model resulted in the production of killer toxin-like antibodies, which conferred significant protection against experimental candidiasis (Polnelli et al. 1993, 1994). These antibodies displaying the activity of a killer toxin were termed antibiobodies (antibiotic-like antibodies) and are considered a significant addition to the repertoire of antifungals for the treatment of invasive fungal infections (reviewed in Magliani et al. 2012).

A further improvement in the field was achieved by development of fungicidal killer peptides (KPs) that are derived from antibiobodies displaying *W. anomalus* toxin-like activity. Such KPs are decapeptides, the sequence of which was originally derived from the active antibiobodies and was further optimized by alanine scanning, resulting in further improved stability and broad target spectrum (Polonelli et al. 2003; Magliani et al. 2012). Such *W. anomalus* killer toxin-derived KPs are active against pathogenic microorganisms which are known to induce severe systemic mycoses that are difficult to treat with conventional antimycotics (Cenci et al. 2004; Travassos et al. 2004). In addition, they are active against a variety of pathogenic prokaryotic microorganisms, such as *Mycobacteria*, *Staphylococcus*, or *Streptococcus* species, and plant pathogenic *Pseudomonas* strains (reviewed in Magliani et al. 2004). It is assumed that glucan or glucan-like molecules in the cell wall of susceptible pro- and eukaryotic microogranisms constitute the basis for the broad spectrum of toxin activity observed for idiotypic antibodies and decapeptides derived thereof (Magliani et al. 2004).

In addition to *W. anomalus* toxin, HM-1 was also used to produce toxin-neutralizing antibodies that were subsequently employed in idiotypic vaccination and production of killer toxin-like antibodies, which display an internal image of HM-1's active site and inhibit target cell's glucan synthase activity (Selvakumar et al. 2006a, b, c). As for the *W. anomalus* toxin, killer peptides could be derived from such killer activity bearing antibodies and may have application potential in the treatment of human fungal infections (Kabir et al. 2011). Small peptides derived from antibiobodies are of special interest since they can be produced much more economically when compared to the antibiobodies.

B. Antifungals in Agriculture, Food, and Feed Industry

The ability of preventing growth of competing microorganisms by secreting inhibitory killer toxins has raised interest in application of

such strains as bioprotective agents in agriculture and food industries. In particular, fermented food and beverage products are often at risk to lose product quality due to the development of spoilage yeasts, fungi, or bacteria. For example, wine fermentations and post-fermentative aging processes can get spoiled by *Dekkera/Brettanomyces* or *Kloeckera/Hanseniaspora* yeast species, which results in loss of sensory product quality due to unpleasant odor and taste development (Comitini et al. 2004a; Wedral et al. 2010). Hence, there is an application potential for yeast killer toxins capable of inhibiting growth of spoilage yeasts and the specific conditions of wine fermentation and aging (low pH of ~3.5 and low temperatures). For example, KpKt from *Tetrapisispora phaffii* is active under such conditions against apiculate yeast species, including *Hanseniaspora uvarum*, which dominate on grapes and grape juice (Ciani and Fatichenti 2001). During experimental wine fermentation, KpKt was found to display inhibitory activity against *H. uvarum* comparable to the routinely applied SO_2. It was suggested that KpKt could substitute for SO_2, thereby eliminating undesired or harmful residual traces of SO_2 in the final product (Ciani and Fatichenti 2001; Comitini et al. 2004b). As an alternative of using the killer strain, a production strain for production of recombinant KpKt (rKpKt) was recently developed (Chessa et al. 2017). As a further benefit, rKpKt was found to exhibit a broadened spectrum of target yeasts, killing not only *Kloeckera/Hanseniaspora* and *Zygosaccharomyces* but also *D. bruxellensis* (Chessa et al. 2017).

Other killer toxins such as PiKt (*Wickerhamomyces anomalus*), KwKt (*Kluyveromyces wickerhamii*), and CpKT1/CpKT2 (*Candida pyralidae*) and a KP6-related toxin (*Ustilago maydis*) were also shown to be active and stable in wine environment and are capable of inhibiting *Dekkera/Brettanomyces* spoilage yeasts, indicating an application potential in wine industry for these toxins as well (Comitini et al. 2004a; Santos et al. 2011; Mehlomakulu et al. 2014, 2017).

In addition to the mentioned non-*Saccharomyces* killer toxins, also the virus-encoded K1/K2 toxins of *S. cerevisiae* have application potential in wine industry. Wine fermentation is typically started using defined *S. cerevisiae* strains optimized for fermentation performance and able to dominate native yeasts in the grape must (Pretorius 2000). Specific starter yeast strains were engineered by cytoduction to possess the L-A and M viruses and the corresponding killer phenotype (Ouchi and Akiyama 1976; Hara et al. 1980; Seki et al. 1985; Boone et al. 1990; Sulo et al. 1992; Sulo and Michalcáková 1992; Michalcáková et al. 1994). Since K2 displays a higher activity at wine pH (~3.5) compared to K1 (Pfeiffer and Radler 1984), it is considered to be most suitable for biocontrol in the wine environment. As an alternative to strain engineering, fermentation starters which naturally express the K2-type killer phenotype as well as desired fermentation characteristics can also be directly selected from the population of indigenous yeasts (Lopes et al. 2007). Such selected or engineered *S. cerevisiae* killer strains typically retain desired flavor and fermentation characteristics and are able to suppress indigenous *S. cerevisiae* strains due to toxin production. Since K2 killer strains are frequent among the natural population on grape surfaces, the use of defined K2 killer-positive fermentation starters, which also display K2 immunity, additionally prevents overgrowth of the starter strain by the indigenous killer (Jacobs and Van Vuuren 1991). A limitation of the *S. cerevisiae* killer toxins in wine and fermentation industry, however, is the relatively narrow spectrum of sensitive target yeast species for these toxins. In particular, non-*Saccharomyces* yeasts present at grape surfaces are routinely insensitive to the *S. cerevisiae* killer toxins (Young and Yagiu 1978) and thus are largely restricting the biocontrol potential to *Saccharomyces* contaminants.

Prevention of spoilage in other fermentation products by killer toxins was also investigated. *K. lactis* zymocin and *Cyb. mrakii* HM-1 can potentially be used in controlling silage spoilage (Kitamoto et al. 1993, 1999; Lowes et al. 2000).

Besides application in fermentation industries, killer yeasts are also attractive agents for biocontrol purposes in agriculture. Several of

the the glucanase toxins from different strains of *Wickerhamomyces anomalus* (formerly *Pichia anomala*) are characterized by a broad antimicrobial activity which is directed not only against other yeasts but also inhibits pathogenic bacteria or mycelial fungi and even protozoans (Sawant et al. 1989; Walker et al. 1995; Jijakli and Lepoivre 1998; Izgü et al. 2007a, b; Wang et al. 2007a; Muccilli et al. 2013; Valzano et al. 2016). The activity against mycelial fungi has been exploited for biocontrol of postharvest diseases caused by plant pathogenic fungi on commercially important fruits (Walker et al. 1995; Santos et al. 2004; Santos and Marquina 2004b; Platania et al. 2012; Aloui et al. 2015; Perez et al. 2016). In particular, green mold disease caused by *Penicillium digitatum* developing on citrus fruit during postharvest storage could be controlled by *W. anomalus* toxin (Platania et al. 2012; Perez et al. 2016). Currently, efforts are undertaken to embed killer yeasts in edible coatings made of sodium alginate and locust bean gum, which results in high retention of the killer strain on the fruit surface and was shown to strongly reduce green mold development (Aloui et al. 2015). In a related application, *P. membranifaciens* toxin was shown to be applicable against *Botrytis cinerea*, the causal agent of gray mold disease on grapes. Treatment of *Vitis vinifera* plants with either purified toxin or the *P. membranifaciens* killer strain protected against *B. cinerea* (Santos and Marquina 2004b). The strains of this killer species were also active in suppressing *B. cinerea* growth on apples or pears following harvest, identifying a general application potential for *P. membranifaciens* killer toxins or strains in biocontrol agent of gray mold disease (Santos et al. 2004; Lutz et al. 2013). It was suggested that biocontrol efficiency is not only determined by the production of killer toxin but influenced by the ability to colonize wounds, production of other hyrolytic enzymes such as chitinase or protease, and the inhibition of spore germination (Lutz et al. 2013).

A *W. anomalus* strain isolated from marine environment was shown to be of potential use in the biocontrol of a crab pathogenic yeast, *Metschnikowia bicuspidata*. Infection of the commercially important crab species *Portunus trituberculatus* by the pathogenic yeast has caused severe economic losses in aquacultures of this species in China. The identification and preliminary characterization of the toxin suggested that it could be used for inhibiting growth of *M. bicuspidata* in aquaculture (Wang et al. 2007a, b).

More recently, *W. anomalus* glucanase killer strains were isolated from *Anopheles* mosquitoes. Since the toxin has demonstrated activity against the rodent malaria parasite *Plasmodium berghei*, novel strategies to utilize such strains to control the spread of plasmodium infection in malaria mosquitoes were proposed (Valzano et al. 2016).

Lastly, killer toxins were expressed in transgenic plants, leading to disease resistant crops. For example, transgenic maize plants were constructed expressing KP4 toxin from *Ustilago maydis*, leading to robust resistance against infection by *U. maydis* (Allen et al. 2011). Similar approaches were also followed in other plants or using differents toxins (*U. maydis* KP6, *W. anomala* KP) (Kinal et al. 1995; Park et al. 1996b; Donini et al. 2005).

V. Concluding Remarks

Yeast killer toxins are thought to serve the purpose of competitor killing and thereby provide a selective advantage to the producing species. However, since a number of such toxins are encoded on selfish genetic elements of viral origin, they may also serve the purpose of genetic stabilization of the viruslike element in the cell. Even though known killer toxins are most heterogenous with respect to protein primary and tertiary structures, some common features can be recognized. This includes common toxin maturation principles involving processing in the ER of the producer cell and the utilization of similar mechanisms to first interact with the target cell and subsequently target an essential biological process either inside or outside of it. Several strategies are currently followed to exploit such natural antimicrobials acting on eukaryotic target cells for

application in medicine or agriculture and food industries.

References

Allen A, Islamovic E, Kaur J, Gold S, Shah D, Smith TJ (2011) Transgenic maize plants expressing the Totivirus antifungal protein, KP4, are highly resistant to corn smut. Plant Biotechnol J 9:857–864

Allen A, Chatt E, Smith TJ (2013) The atomic structure of the virally encoded antifungal protein, KP6. J Mol Biol 425:609–621

Aloui H, Licciardello F, Khwaldia K, Hamdi M, Restuccia C (2015) Physical properties and antifungal activity of bioactive films containing *Wickerhamomyces anomalus* killer yeast and their application for preservation of oranges and control of postharvest green mold caused by *Penicillium digitatum*. Int J Food Microbiol 200:22–30

Antuch W, Güntert P, Wüthrich K (1996) Ancestral beta gamma-crystallin precursor structure in a yeast killer toxin. Nat Struct Biol 3:662–665

Ashida S, Shimazaki T, Kitano K, Hara S (1983) New killer toxin of *Hansenula mrakii*. Agric Biol Chem 47:2953–2955

Bär C, Zabel R, Liu S, Stark MJ, Schaffrath R (2008) A versatile partner of eukaryotic protein complexes that is involved in multiple biological processes: Kti11/Dph3. Mol Microbiol 69:1221–1123

Becker B, Blum A, Gießelmann E, Dausend J, Rammo D, Müller NC, Tschacksch E, Steimer M, Spindler J, Becherer U, Rettig J, Breinig F, Schmitt MJ (2016) H/KDEL receptors mediate host cell intoxication by a viral A/B toxin in yeast. Sci Rep 6:31105

Belda I, Ruiz J, Alonso A, Marquina D, Santos A (2017) The biology of *Pichia membranifaciens* killer toxins. Toxins (Basel) 9:pii: E112

Bevan EA, Makower M (1963) The physiological basis of the killer character in yeast. Proc XIth Int Congr Genet 1:202–203

Bevan EA, Herring AJ, Mitchell DJ (1973) Preliminary characterization of two species of dsRNA in yeast and their relationship to the "killer" character. Nature 245:81–86

Bolen PL, Kurtzman CP, Ligon JM, Mannarelli BM, Bothast RJ (1992) Physical and genetic characterization of linear DNA plasmids from the heterothallic yeast *Saccharomycopsis crataegensis*. Antonie Van Leuwenhoek 61:195–295

Boone C, Sdicu AM, Wagner J, Degré R, Sanchez C, Bussey H (1990) Integration of the yeast K1 killer toxin gene into the genome of marked wine yeasts and its effect on vinifcation. Am J Enol Vitic 41:37–42

Bostian KA, Elliott Q, Bussey H, Burn V, Smith A, Tipper DJ (1984) Sequence of the preprotoxin dsRNA gene of type I killer yeast: multiple processing events produce a two-component toxin. Cell 36:741–751

Breinig F, Tipper DJ, Schmitt MJ (2002) Kre1p, the plasma membrane receptor for the yeast K1 viral toxin. Cell 108:395–405

Breinig F, Schleinkofer K, Schmitt MJ (2004) Yeast Kre1p is GPI-anchored and involved in both cell wall assembly and architecture. Microbiology 150:3209–3218

Breinig F, Sendzik T, Eisfeld K, Schmitt MJ (2006) Dissecting toxin immunity in virus-infected killer yeast uncovers an intrinsic strategy of self-protection. Proc Natl Acad Sci U S A 103:3810–3815

Bussey H (1991) K1 killer toxin, a pore-forming protein from yeast. Mol Microbiol 5:2339–2343

Butler AR, O'Donnell RW, Martin VJ, Gooday GW, Stark MJ (1991a) *Kluyveromyces lactis* toxin has an essential chitinase activity. Eur J Biochem 199:483–488

Butler AR, Porter M, Stark MJR (1991b) Intracellular expression of *Kluyveromyces lactis* toxin γ subunit mimics treatment with exogenous toxin and distinguishes two classes oftoxin-resistant mutant. Yeast 7:617–625

Butler AR, White JH, Folawiyo Y, Edlin A, Gardiner D, Stark MJ (1994) Two *Saccharomyces cerevisiae* genes which control sensitivity to G1 arrest induced by *Kluyveromyces lactis* toxin. Mol Cell Biol 14:6306–6316

Buzzini P, Corazzi L, Turchetti B, Buratta M, Martini A (2004) Characterization of the in vitro antimycotic activity of a novel killer protein from *Williopsis saturnus* DBVPG 4561 against emerging pathogenic yeasts. FEMS Microbiol Lett 238:359–365

Carroll SY, Stirling PC, Stimpson HE, Giesselmann E, Schmitt MJ, Drubin DG (2009) A yeast killer toxin screen provides insights into a/b toxin entry, trafficking, and killing mechanisms. Dev Cell 17 (4):552–560

Castón JR, Trus BL, Booy FP, Wickner RB, Wall JS, Steven AC (1997) Structure of L-A virus: a specialized compartment for the transcription and replication of double-stranded RNA. J Cell Biol 138:975–985

Cenci E, Bistoni F, Mencacci A, Perito S, Magliani W, Conti S, Polonelli L, Vecchiarelli A (2004) A synthetic peptide as a novel anticryptococcal agent. Cell Microbiol 6:953–961

Chakravarty AK, Smith P, Jalan R, Shuman S (2014) Structure, mechanism, and specificity of a eukaryal tRNA restriction enzyme involved in self-nonself discrimination. Cell Rep 7:339–347

Chen WB, Han JF, Jong SC, Chang SC (2000) Isolation, purification, and characterization of a killer protein from *Schwanniomyces occidentalis*. Appl Environ Microbiol 66:5348–5352

Chessa R, Landolfo S, Ciani M, Budroni M, Zara S, Ustun M, Cakar ZP, Mannazzu I (2017) Biotechnological

exploitation of *Tetrapisispora phaffii* killer toxin: heterologous production in *Komagataella phaffii* (*Pichia pastoris*). Appl Microbiol Biotechnol 101:2931–2942

Ciani M, Fatichenti F (2001) Killer toxin of *Kluyveromyces phaffii* DBVPG 6076 as a biopreservative agent to control apiculate wine Yeasts. Appl Environ Microbiol 67:3058–3063

Comitini F, Ciani M (2011) *Kluyveromyces wickerhamii* killer toxin: purification and activity towards *Brettanomyces/Dekkera* yeasts in grape must. FEMS Microbiol Lett 316:77–82

Comitini F, De Ingeniis J, Pepe L, Mannazzu I, Ciani M (2004a) *Pichia anomala* and *Kluyveromyces wickerhamii* killer toxins as new tools against *Dekkera/Brettanomyces* spoilage yeasts. FEMS Microbiol Lett 238:235–240

Comitini F, Di Pietro N, Zacchi L, Mannazzu I, Ciani M (2004b) *Kluyveromyces phaffii* killer toxin active against wine spoilage yeasts: purification and characterization. Microbiology 150:2535–2541

Comitini F, Mannazzu I, Ciani M (2009) *Tetrapisispora phaffii* killer toxin is a highly specific beta-glucanase that disrupts the integrity of the yeast cell wall. Microb Cell Factories 8:55

Cong YS, Yarrow D, Li YY, Fukuhara H (1994) Linear DNA plasmids from *Pichia etchellsii*, *Debaryomyces hansenii* and *Wingea robertsiae*. Microbiology 140:1327–1335

da Silva S, Calado S, Lucas C, Aguiar C (2008) Unusual properties of the halotolerant yeast Candida nodaensis Killer toxin, CnKT. Microbiol Res 163:243–251

de la Peña P, Barros F, Gascón S, Lazo PS, Ramos S (1981) Effect of yeast killer toxin on sensitive cells of *Saccharomyces cerevisiae*. J Biol Chem 256:10420–10425

Dignard D, Whiteway M, Germain D, Tessier D, Thomas DY (1991) Expression in yeast of a cDNA copy of the K2 killer toxin gene. Mol Gen Genet 227:127–136

Dinman JD, Icho T, Wickner RB (1991) A -1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a gag-pol fusion protein. Proc Natl Acad Sci U S A 88:174–178

Donini M, Lico C, Baschieri S, Conti S, Magliani W, Polonelli L, Benvenuto E (2005) Production of an engineered killer peptide in *Nicotiana benthamiana* by using a potato virus X expression system. Appl Environ Microbiol 71:6360–6367

Eisfeld K, Riffer F, Mentges J, Schmitt MJ (2000) Endocytotic uptake and retrograde transport of a virally encoded killer toxin in yeast. Mol Microbiol 37:926–940

Fichtner L, Schaffrath R (2002) *KTI11* and *KTI13*, *Saccharomyces cerevisiae* genes controlling sensitivity to G1 arrest induced by *Kluyveromyces lactis* zymocin. Mol Microbiol 44:865–875

Fichtner L, Frohloff F, Burkner K, Larsen M, Breunig KD, Schaffrath R (2002) Molecular analysis of *KTI12/TOT4*, a *Saccharomyces cerevisiae* gene required for *Kluyveromyces lactis* zymocin action. Mol Microbiol 43:783–791

Fichtner L, Jablonowski D, Schierhorn A, Kitamoto HK, Stark MJR, Schaffrath R (2003) Elongator's toxin-target (TOT) function is nuclear localization sequence dependent and suppressed by post-translational modification. Mol Microbiol 49:1297–1307

Frohloff F, Fichtner L, Jablonowski D, Breuning KD, Schaffrath R (2001) *Saccharomyces cerevisiae* elongator mutations confer resistance to the *Kluyveromyces lactis* zymocin. EMBO J 20:1993–2003

Fuentefria AM, Suh SO, Landell MF, Faganello J, Schrank A, Vainstein MH, Blackwell M, Valente P (2008) Trichosporon insectorum sp. nov., a new anamorphic basidiomycetous killer yeast. Mycol Res 112:93–99

Fukuhara H (1995) Linear DNA plasmids of yeasts. FEMS Microbiol Lett 131:1–9

Golubev WI (2006) Antagonistic interactions among yeasts. In: Rosa CA, Péter G (eds) Biodiversity and ecophysiology of yeasts, The yeast handbook. Springer, Berlin, pp 197–219

Golubev WI (2015) Intraspecific and intrageneric antagonistic activity of *Wickerhamomyces anomalus*. Microbiol (Moscow) 84:193–193

Golubev W, Shabalin Y (1994) Microcin production by the yeast *Cryptococcus humicola*. FEMS Microbiol Lett 119:105–110

Golubev WI, Pfeifer I, Golubeva E (2002) Mycocin production in *Trichosporon pullulans* populations colonizing tree exudates in the spring. FEMS Microbiol Ecol 40:151–157

Golubev WI, Pfeiffer I, Churkina LG, Golubeva EW (2003) Double-stranded RNA viruses in a mycocinogenic strain of *Cystofilobasidium infirmominiatum*. FEMS Yeast Res 3:63–68

Goto K, Iwatuki Y, Kitano K, Obata T, Hara S (1990) Cloning and nucleotide sequence of the KHR killer gene of *Saccharomyces cerevisiae*. Agric Biol Chem 54:979–984

Goto K, Fukuda H, Kichise K, Kitano K, Hara S (1991) Cloning and nucleotide sequence of the KHS killer gene of *Saccharomyces cerevisiae*. Agric Biol Chem 55:1953–1958

Gu F, Khimani A, Rane SG, Flurkey WH, Bozarth RF, Smith TJ (1995) Structure and function of a virally encoded fungal toxin from *Ustilago maydis*: a fungal and mammalian $Ca2^{+}$ channel inhibitor. Structure 3:805–814

Gunge N, Tamaru A, Ozawa F, Sakaguchi K (1981) Isolation and characterization of linear deoxyribonucleic acid plasmids from *Kluyveromyces lactis* and the plasmid-associated killer character. J Bacteriol 145:382–390

Guyard C, Séguy N, Lange M, Ricard I, Polonelli L, Cailliez JC (1999) First steps in the purification and characterization of a *Pichia anomala* killer toxin. J Eukaryot Microbiol 46:144S

Guyard C, Séguy N, Cailliez JC, Drobecq H, Polonelli L, Dei-Cas E, Mercenier A, Menozzi FD (2002a) Characterization of a *Williopsis saturnus* var. *mrakii* high molecular weight secreted killer toxin with broad-spectrum antimicrobial activity. J Antimicrob Chemother 49:961–971

Guyard C, Dehecq E, Tissier JP, Polonelli L, Dei-Cas E, Cailliez JC, Menozzi FD (2002b) Involvement of β-glucans in the wide-spectrum antimicrobial activity of *Williopsis saturnus* var. *mrakii* MUCL 41968 killer toxin. Mol Med 8:686–694

Hara S, Iimura Y, Otsuka K (1980) Breeding of useful killer wine yeasts. Am J Enol Vitic 31(1):28–33

Hayman GT, Bolen BL (1991) Linear DNA plasmids of *Pichia inositovora* are associated with a novel killer toxin activity. Curr Genet 19:389–393

Heiligenstein S, Eisfeld K, Sendzik T, Jimenéz-Becker N, Breinig F, Schmitt MJ (2006) Retrotranslocation of a viral A/B toxin from the yeast endoplasmic reticulum is independent of ubiquitination and ERAD. EMBO J 25:4717–4727

Hishinuma F, Hirai K (1991) Genome organization of the linear plasmid, pSKL, isolated from *Saccharomyces kluyveri*. Mol Gen Genet 226:97–106

Hishinuma F, Nakamura K, Hirai K, Nishizawa R, Gunge N, Maeda T (1984) Cloning and nucleotide sequence of the DNA killer plasmids from yeast. Nucleic Acids Res 12:l7581–l7597

Hodgson VJ, Button D, Walker GM (1995) Anti-Candida activity of a novel killer toxin from the yeast *Williopsis mrakii*. Microbiology 141:2003–2012

Huang B, Johansson MJ, Bystrom AS (2005) An early step in wobble uridine tRNA modification requires the Elongator complex. RNA 11:424–436

Huang B, Lu J, Byström AS (2008) A genome-wide screen identifies genes required for formation of the wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine in *Saccharomyces cerevisiae*. RNA 14:2183–2194

Hutchins K, Bussey H (1983) Cell wall receptor for yeast killer toxin: involvement of (1-6)-β-D-glucan. J Bacteriol 154:161–169

Icho T, Wickner RB (1989) The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. J Biol Chem 264:6716–6723

Izgü F, Altinbay D (2004) Isolation and characterization of the K5-type yeast killer protein and its homology with an exo-β-1,3-glucanase. Biosci Biotechnol Biochem 68:685–693

Izgü F, Altinbay D, Sağiroğlu AK (1999) Isolation and characterization of the K6 type yeast killer protein. Microbios 99:161–172

Izgü F, Altinbay D, Türeli AE (2007a) In vitro susceptibilities of *Candida* spp. to Panomycocin, a novel exo-β-1,3-glucanase isolated from *Pichia anomala* NCYC 434. Microbiol Immunol 51:797–803

Izgü F, Altinbay D, Türeli AE (2007b) In vitro activity of panomycocin, a novel exo-β-1,3-glucanase isolated from *Pichia anomala* NCYC 434, against dermatophytes. Mycoses 50:31–34

Jablonowski D, Schaffrath R (2007) Zymocin, a composite chitinase and tRNase killer toxin from yeast. Biochem Soc Trans 35:1533–1537

Jablonowski D, Fichtner L, Martin VJ, Klassen R, Meinhardt F, Stark MJR, Schaffrath R (2001) *Saccharomyces cerevisiae* cell wall chitin, the potential *Kluyveromyces lactis* zymocin receptor. Yeast 18:1285–1299

Jablonowski D, Zink S, Mehlgarten C, Daum G, Schaffrath R (2006) $tRNA^{Glu}$ wobble uridine methylation by Trm9 identifies Elongator's key role for zymocin-induced cell death in yeast. Mol Microbiol 59:677–688

Jacobs CJ, Van Vuuren HJJ (1991) Effects of different killer yeasts on wine fermentations. Am J Enol Vitic 42(4):295–300

Jain R, Poulos MG, Gros J, Chakravarty AK, Shuman S (2011) Substrate specificity and mutational analysis of *Kluyveromyces lactis* gamma-toxin, a eukaryal tRNA anticodon nuclease. RNA 17:1336–1343

Jeske S, Meinhardt F (2006) Autonomous cytoplasmic linear plasmid pPac1-1 of *Pichia acaciae*: molecular structure and expression studies. Yeast 23: 479–486

Jeske S, Meinhardt F, Klassen R (2006) Extranuclear inheritance: virus-like DNA-elements in yeast. In: Esser K, Lüttge U, Kadereit J, Beyschlag W (eds) Progress in botany, vol 68. Springer, Berlin, pp 98–129

Jijakli MH, Lepoivre P (1998) Characterization of an exo-β-1,3-glucanase produced by *Pichia anomala* strain K, antagonist of *Botrytis cinerea* on apples. Phytopathology 88:335–343

Kabir ME, Karim N, Krishnaswamy S, Selvakumar D, Miyamoto M, Furuichi Y, Komiyama T (2011) Peptide derived from anti-idiotypic single-chain antibody is a potent antifungal agent compared to its parent fungicide HM-1 killer toxin peptide. Appl Microbiol Biotechnol 92:1151–1160

Kagan B (1983) Mode of action of yeast killer toxins: channel formation in lipid bilayer membranes. Nature 302:709–711

Kalhor HR, Clarke S (2003) Novel methyltransferase for modified uridine residues at the wobble position of tRNA. Mol Cell Biol 23:9283–9292

Kämper J, Meinhardt F, Gunge N, Esser K (1989a) New recombinant linear DNA-elements derived from *Kluyveromyces lactis* killer plasmids. Nucleic Acids Res 17:1781

Kämper J, Meinhardt F, Gunge N, Esser K (1989b) In vivo construction of linear vectors based on killer plasmids from *Kluyveromyces lactis*: selection of a nuclear gene results in attachment of telomeres. Mol Cell Biol 9:3931–3937

Kämper J, Esser K, Gunge N, Meinhardt F (1991) Heterologous gene expression on the linear DNA killer plasmid from *Kluyveromyces lactis*. Curr Genet 19:109–118

Kasahara S, Ben Inoue S, Mio T, Yamada T, Nakajima T, Ichishima E, Furuichi Y, Yamada H (1994) Involvement of cell wall β-glucan in the action of HM-1 killer toxin. FEBS Lett 348:27–32

Kashiwagi T, Kunishima N, Suzuki C, Tsuchiya F, Nikkuni S, Arata Y, Morikawa K (1997) The novel acidophilic structure of the killer toxin from halotolerant yeast demonstrates remarkable folding similarity with a fungal killer toxin. Structure 5:81–94

Kast A, Klassen R, Meinhardt F (2014) rRNA fragmentation induced by a yeast killer toxin. Mol Microbiol 91:606–617

Kast A, Voges R, Schroth M, Schaffrath R, Klassen R, Meinhardt F (2015) Autoselection of cytoplasmic yeast virus like elements encoding toxin/antitoxin systems involves a nuclear barrier for immunity gene expression. PLoS Genet 11:e1005005

Keppetipola N, Jain R, Meineke B, Diver M, Shuman S (2009) Structure-activity relationships in Kluyveromyces lactis γ-toxin, a eukaryal tRNA anticodon nuclease. RNA 15:1036–1044

Keszthelyi A, Ohkusu M, Takeo K, Pfeiffer I, Litter J, Kucsera J (2006) Characterisation of the anticryptococcal effect of the FC-1 toxin produced by *Filobasidium capsuligenum*. Mycoses 49:176–183

Kimura T, Kitamoto N, Matsuoka K, Nakamura K, Iimura Y, Kito Y (1993) Isolation and nucleotide sequences of the genes encoding killer toxins from *Hansenula mrakii* and *H. saturnus*. Gene 137:265–270

Kimura T, Komiyama T, Furuichi Y, Iimura Y, Karita S, Sakka K, Ohmiya K (1999) N-Glycosylation is involved in the sensitivity of *Saccharomyces cerevisiae* to HM-1 killer toxin secreted from *Hansenula mrakii* IFO 0895. Appl Microb Biotechnol 51:176–184

Kinal H, Park CM, Berry JO, Koltin Y, Bruenn JA (1995) Processing and secretion of a virally encoded antifungal toxin in transgenic tobacco plants: evidence for a Kex2p pathway in plants. Plant Cell 7:677–688

Kitada K, Hishinuma H (1987) A new linear plasmid isolated from the yeast *Saccharomyces kluyveri*. Mol Gen Genet 206:377–381

Kitamoto HK, Ohmomo S, Nakahara T (1993) Selection of killer yeasts (*Kluyveromyces lactis*) to prevent aerobic deterioration in silage making. J Dairy Sci 76:803–811

Kitamoto HK, Hasebe A, Ohmomo S, Suto EG, Muraki M, Iimura Y (1999) Prevention of aerobic spoilage of maize silage by a genetically modified killer yeast, *Kluyveromyces lactis*, defective in the ability to grow on lactic acid. Appl Environ Microbiol 65:4697–4700

Klassen R, Meinhardt F (2002) Linear plasmids pWR1A and pWR1B of the yeast *Wingea robertsiae* are associated with a killer phenotype. Plasmid 48:142–148

Klassen R, Meinhardt F (2003) Structural and functional analysis of the killer element pPin1-3 from *Pichia inositovora*. Mol Gen Genomics 270:190–199

Klassen R, Meinhardt F (2007) Linear protein-primed replicating plasmids in eukaryotic microbes. In: Meinhardt F, Klassen R (eds) Microbial linear plasmids, microbiology monographs, vol 7. Springer, Berlin, pp 187–226

Klassen R, Tontsidou L, Larsen M, Meinhardt F (2001) Genome organization of the linear cytoplasmic element pPE1B from *Pichia etchellsii*. Yeast 18:953–961

Klassen R, Jablonowski D, Schaffrath R, Meinhardt F (2002) Genome organization of the linear *Pichia etchellsii* plasmid pPE1A: evidence for expression of an extracellular chitin-binding protein homologous to the alpha-subunit of the *Kluyveromyces lactis* killer toxin. Plasmid 47:224–233

Klassen R, Teichert S, Meinhardt F (2004) Novel yeast killer toxins provoke S-phase arrest and DNA damage checkpoint activation. Mol Microbiol 53:263–273

Klassen R, Paluszynski J, Wemhoff S, Pfeiffer A, Fricke J, Meinhardt F (2008) The primary target of the killer toxin from *Pichia acaciae* is $tRNA^{Gln}$. Mol Microbiol 69:681–697

Klassen R, Wemhoff S, Krause J, Meinhardt F (2011) DNA repair defects sensitize cells to anticodon nuclease yeast killer toxins. Mol Gen Genomics 285:185–195

Klassen R, Kast A, Wünsche G, Paluszynski J, Meinhardt F (2014) Immunity factors for two related $tRNA^{Gln}$ targeting killer toxins distinguish cognate and non-cognate toxic subunits. Curr Genet 60:213–222

Klassen R, Schaffrath R, Buzzini P, Philip Ganter PF (2017) Antagonistic interactions and killer yeasts. In: Buzzini P, Lachance MA, Yurkov A (eds) *Yeasts in natural ecosystems: ecology*. Springer, Cham, pp 229–275

Koltin Y, Day PR (1976) Inheritance of killer phenotypes and double-stranded RNA in *Ustilago maydis*. Proc Natl Acad Sci U S A 73:594–598

Komiyama T, Ohta T, Furuichi Y, Ohta Y, Tsukada Y (1995) Structure and activity of HYI killer toxin from *Hansenula saturnus*. Biol Pharm Bull 18:1057–1059

Komiyama T, Ohta T, Urakami H, Shiratori Y, Takasuka T, Satoh M, Watanabe T, Furuichi Y (1996) Pore formation on proliferating yeast *Saccharomyces cerevisiae* cell buds by HM-1 killer toxin. J Biochem 119:731–736

Komiyama T, Shirai T, Ohta T, Urakami H, Furuichi Y, Ohta Y, Tsukada Y (1998) Action properties of HYI killer toxin from *Williopsis saturnus* var. *saturnus*, and antibiotics, aculeacin A and papulacandin B. Biol Pharm Bull 21:1013–1019

Komiyama T, Kimura T, Furuichi Y (2002) Round shape enlargement of the yeast spheroplast of *Saccharomyces cerevisiae* by HM-1 toxin. Biol Pharm Bull 25:959–965

Kono I, Himeno K (1997) A novel killer yeast effective on *Schizosaccharomyces pombe*. Biosci Biotechnol Biochem 61:563–564

Larsen M, Gunge N, Meinhardt F (1998) *Kluyveromyces lactis* killer plasmid pGKL2: evidence for a viral-like capping enzyme encoded by OFR3. Plasmid 40:243–246

Leidel S, Pedrioli PG, Bucher T, Brost R, Costanzo M, Schmidt A, Aebersold R, Boone C, Hofmann K, Peter M (2009) Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. Nature 458:228–232

Li N, Erman M, Pangborn W, Duax WL, Park CM, Bruenn J, Ghosh D (1999) Structure of Ustilago maydis killer toxin KP6 alpha-subunit. A multimeric assembly with a central pore. J Biol Chem 4:20425–20431

Ligon JM, Bolen PL, Hill DS, Bothast RJ, Kurtzman CP (1989) Physical and biological characterization of linear DNA plasmids of the yeast *Pichia inositovora*. Plasmid 2:185–194

Lopes CA, Rodríguez ME, Sangorrín M, Querol A, Caballero AC (2007) Patagonian wines: the selection of an indigenous yeast starter. J Ind Microbiol Biotechnol 34:539–546

Lowes KF, Shearman CA, Payne J, MacKenzie D, Archer DB, Merry RJ, Gasson MJ (2000) Prevention of yeast spoilage in feed and food by the yeast mycocin HMK. Appl Environ Microbiol 66:1066–1076

Lu J, Huang B, Esberg A, Johansson MJ, Bystrom AS (2005) The *Kluyveromyces lactis* γ-toxin targets tRNA anticodons. RNA 11:1648–1654

Lu J, Esberg A, Huang B, Byström AS (2008) *Kluyveromyces lactis* gamma-toxin, a ribonuclease that recognizes the anticodon stem loop of tRNA. Nucleic Acids Res 36:1072–1080

Lukša J, Podoliankaité M, Vepštaite I, Strazdaité-Žieliene Z, Urbonavičius J, Serviené E (2015) Yeast-1,6-glucan is a primary target for the *Saccharomyces cerevisiae* K2 toxin. Eukaryot Cell 14:406–414

Lutz MC, Lopes CA, Rodriguez ME, Sosa MC, Sangorrín MP (2013) Efficacy and putative mode of action of native and commercial antagonistic yeasts against postharvest pathogens of pear. Int J Food Microbiol 164:166–172

Magliani W, Conti S, Gerloni M, Bertolotti D, Polonelli L (1997) Yeast killer systems. Clin Microbiol Rev 10:369–400

Magliani W, Conti S, Salati A, Vaccari S, Ravanetti L, Maffei DL, Polonelli L (2004) Therapeutic potential of yeast killer toxin-like antibodies and mimotopes. FEMS Yeast Res 5:11–18

Magliani W, Conti S, Giovati L, Zanello PP, Sperindè M, Ciociola T, Polonelli L (2012) Antibody peptide based antifungal immunotherapy. Front Microbiol 3:190

Marquina D, Peres C, Caldas FV, Marques JF, Peinado JM, Spencer-Martins I (1992) Characterization of the yeast populations in olive brines. Lett Appl Microbiol 14:279–283

Martinac B, Zhu H, Kubalski A, Zhou XL, Culbertson M, Bussey H, Kung C (1990) Yeast K1 killer toxin forms ion channels in sensitive yeast spheroplasts and in artificial liposomes. Proc Natl Acad Sci U S A 87:6228–6232

Mehlgarten C, Schaffrath R (2003) Mutant casein kinase I (Hrr25p/Kti14p) abrogates the G1 cell cycle arrest induced by *Kluyveromyces lactis* zymocin in budding yeast. Mol Gen Genomics 269:188–196

Mehlgarten C, Schaffrath R (2004) After chitin docking, toxicity of *Kluyveromyces lactis* zymocin requires *Saccharomyces cerevisiae* plasma membrane H+-ATPase. Cell Microbiol 6:569–580

Mehlomakulu NN, Setati ME, Divol B (2014) Characterization of novel killer toxins secreted by wine-related non-*Saccharomyces yeasts* and their action on *Brettanomyces* spp. Int J Food Microbiol 188:83–91

Mehlomakulu NN, Prior KJ, Setati ME, Divol B (2017) *Candida pyralidae* killer toxin disrupts the cell wall of *Brettanomyces bruxellensis* in red grape juice. J Appl Microbiol 122:747–758

Meineke B, Kast A, Schwer B, Meinhardt F, Shuman S, Klassen R (2012) A fungal anticodon nuclease ribotoxin exploits a secondary cleavage site to evade tRNA repair. RNA 18:1716–1724

Meinhardt F, Schaffrath R (2001) Extranuclear inheritance: cytoplasmic linear double-stranded DNA killer elements of the dairy yeast *Kluyveromyces lactis*. In: Esser K, Lüttge U, Kadereit JW, Beyschlag W (eds) Progress in botany, vol 62. Springer, Berlin, pp 51–70

Meinhardt F, Larsen M, Wodara C, Schickel J (1994) A novel approach to express a heterologous gene on linear killer plasmids: expression of the bacterial aph gene from a cytoplasmic promoter fragment without in-phase fusion to the plasmid open reading frame. Plasmid 32:318–327

Meskauskas A, Citavicius D (1992) The K2-type killer toxin- and immunity-encoding region from *Saccharomyces cerevisiae*: structure and expression in yeast. Gene 111:135–139

Michalcáková S, Sturdík E, Sulo P (1994) Construction and properties of K2 and K3 type killer

Saccharomyces wine yeasts. Wein-Wissenschaft 49:130–132

Middelbeek EJ, Hermans JM, Stumm C (1979) Production, purification and properties of a *Pichia kluyveri* killer toxin. Antonie Van Leeuwenhoek 45:437–450

Middelbeek EJ, van de Laar HH, Hermans JM, Stumm C, Vogels GD (1980a) Physiological conditions affecting the sensitivity of *Saccharomyces cerevisiae* to a *Pichia kluyveri* killer toxin and energy requirement for toxin action. Antonie Van Leeuwenhoek 46:483–497

Middelbeek EJ, Crützen QH, Vogels GD (1980b) Effects of potassium and sodium ions on the killing action of a *Pichia kluyveri* toxin in cells of *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 18:519–524

Miyamoto M, Onozato N, Selvakumar D, Kimura T, Furuichi Y, Komiyama T (2006) The role of the histidine-35 residue in the cytocidal action of HM-1 killer toxin. Microbiology 152:2951–2958

Miyamoto M, Furuichi Y, Komiyama T (2011) Genome-wide screen of *Saccharomyces cerevisiae* for killer toxin HM-1 resistance. Yeast 28:27–41

Miyamoto M, Furuichi Y, Komiyama T (2012) The high-osmolarity glycerol- and cell wall integrity-MAP kinase pathways of *Saccharomyces cerevisiae* are involved in adaptation to the action of killer toxin HM-1. Yeast 29:475–485

Muccilli S, Wemhoff S, Restuccia C, Meinhardt F (2013) Exoglucanase-encoding genes from three *Wickerhamomyces anomalus* killer strains isolated from olive brine. Yeast 30:33–43

Nakatsukasa K, Brodsky JL (2008) The recognition and retrotranslocation of misfolded proteins from the endoplasmic reticulum. Traffic 9:861–870

Nandakumar J, Schwer B, Schaffrath R, Shuman S (2008) RNA repair: an antidote to cytotoxic eukaryal RNA damage. Mol Cell 31:278–286

Noma A, Sakaguchi Y, Suzuki T (2009) Mechanistic characterization of the sulfur-relay system for eukaryotic 2-thiouridine biogenesis at tRNA wobble positions. Nucleic Acids Res 37:1335–1352

Novotná D, Flegelová H, Janderová B (2004) Different action of killer toxins K1 and K2 on the plasma membrane and the cell wall of *Saccharomyces cerevisiae*. FEMS Yeast Res 4:803–813

Orentaite I, Poranen MM, Oksanen HM, Daugelavicius R, Bamford DH (2016) K2 killer toxin-induced physiological changes in the yeast *Saccharomyces cerevisiae*. FEMS Yeast Res 16:fow003

Oro L, Zara S, Fancellu F, Mannazzu I, Budroni M, Ciani M, Comitini F (2014) *TpBGL2* codes for a *Tetrapisispora phaffii* killer toxin active against wine spoilage yeasts. FEMS Yeast Res 14:464–471

Ouchi K, Akiyama H (1976) Breeding of useful killer sake yeasts by repeated back-crossing. J Ferment Technol 54:615

Pagé N, Gérard-Vincent M, Ménard P, Beaulieu M, Azuma M, Dijkgraaf GJ, Li H, Marcoux J, Nguyen T, Dowse T, Sdicu AM, Bussey H (2003) A *Saccharomyces cerevisiae* genome-wide mutant screen for altered sensitivity to K1 killer toxin. Genetics 163:875–894

Paluszynski JP, Klassen R, Meinhardt F (2007) *Pichia acaciae* killer system: genetic analysis of toxin immunity. Appl Environ Microbiol 73:4373–4378

Park CM, Bruenn JA, Ganesa C, Flurkey WF, Bozarth RF, Koltin Y (1994) Structure and heterologous expression of the *Ustilago maydis* viral toxin KP4. Mol Microbiol 11:155–164

Park CM, Banerjee N, Koltin Y, Bruenn JA (1996a) The *Ustilago maydis* virally encoded KP1 killer toxin. Mol Microbiol 20:957–963

Park CM, Berry JO, Bruenn JA (1996b) High-level secretion of a virally encoded anti-fungal toxin in transgenic tobacco plants. Plant Mol Biol 30:359–366

Peery T, Shabat-Brand T, Steinlauf R, Koltin Y, Bruenn J (1987) Virus-encoded toxin of *Ustilago maydis*: two polypeptides are essential for activity. Mol Cell Biol 7:470–477

Perez MF, Contreras L, Garnica NM, Fernández-Zenoff MV, Farías ME, Sepulveda M, Ramallo J, Dib JR (2016) Native killer yeasts as biocontrol agents of postharvest fungal diseases in lemons. PLoS One 11(10):e0165590

Pfeiffer P, Radler F (1984) Comparison of the killer toxin of several yeasts and the purification of a toxin of type K2. Arch Microbiol 137:357–361

Pfeiffer I, Golubev WI, Farkas Z, Kucsera J, Golubev N (2004) Mycocin production in *Cryptococcus aquaticus*. Antonie Van Leeuwenhoek 86:369–375

Platania C, Restuccia C, Muccilli S, Cirvilleri G (2012) Efficacy of killer yeasts in the biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*). Food Microbiol 30:219–225

Polonelli L, Morace G (1986) Reevaluation of the yeast killer phenomenon. J Clin Microbiol 24:866–869

Polonelli L, Morace G (1987) Production and characterization of yeast killer toxin monoclonal antibodies. J Clin Microbiol 25:460–462

Polonelli L, Morace G (1988) Yeast killer toxin-like anti-idiotypic antibodies. J Clin Microbiol 26:602–604

Polonelli L, Fanti F, Conti S, Campani L, Gerloni M, Castagnola M, Morace G, Chezzi C (1990) Detection by immunofluorescent anti-idiotypic antibodies of yeast killer toxin cell wall receptors of *Candida albicans*. J Immunol Methods 132:205–209

Polonelli L, Lorenzini R, De Bernardis F, Gerloni M, Conti S, Morace G, Magliani W, Chezzi C (1993) Idiotypic vaccination: immunoprotection mediated by anti-idiotypic antibodies with antibiotic activity. Scand J Immunol 37:105–110

Polonelli L, De Bernardis F, Conti S, Boccanera M, Gerloni M, Morace G, Magliani W, Chezzi C, Cassone A (1994) Idiotypic intravaginal vaccination to

protect against candidal vaginitis by secretory, yeast killer toxin-like anti-idiotypic antibodies. J Immunol 152:3175–3182

Polonelli L, Séguy N, Conti S, Gerloni M, Bertolotti D, Cantelli C, Magliani W, Cailliez JC (1997) Monoclonal yeast killer toxin-like candidacidal anti-idiotypic antibodies. Clin Diagn Lab Immunol 4:142–146

Polonelli L, Magliani W, Conti S, Bracci L, Lozzi L, Neri P, Adriani D, De Bernardis F, Cassone A (2003) Therapeutic activity of an engineered synthetic killer antiidiotypic antibody fragment against experimental mucosal and systemic candidiasis. Infect Immun 71:6205–6212

Pretorius IS (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16:675–729

Puchkov EO, Wiese A, Seydel U, Kulakovskaya TV (2001) Cytoplasmic membrane of a sensitive yeast is a primary target for *Cryptococcus humicola* mycocidal compound (microcin). Biochim Biophys Acta 1512:239–250

Puchkov EO, Zähringer U, Lindner B, Kulakovskaya TV, Seydel U, Wiese A (2002) The mycocidal, membrane-active complex of *Cryptococcus humicola* is a new type of cellobiose lipid with detergent features. Biochim Biophys Acta 1558:161–170

Puhalla JE (1968) Compatibility reactions on solid medium and interstrain inhibition in *Ustilago maydis*. Genetics 60:461–474

Radler F, Schmitt MJ, Meyer B (1990) Killer toxin of *Hanseniaspora uvarum*. Arch Microbiol 154:175–178

Rep M, Krantz M, Thevelein JM, Hohmann S (2000) The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. J Biol Chem 275:8290–8300

Riffer F, Eisfeld K, Breinig F, Schmitt MJ (2002) Mutational analysis of K28 preprotoxin processing in the yeast *Saccharomyces cerevisiae*. Microbiology 148:1317–1328

Rodríguez-Cousiño N, Esteban R (2017) Relationships and evolution of double-stranded RNA totiviruses of yeasts inferred from analysis of L-A-2 and L-BC variants in wine yeast strain populations. Appl Environ Microbiol 83:pii: e02991-16

Rodríguez-Cousiño N, Maqueda M, Ambrona J, Zamora E, Esteban R, Ramírez M (2011) A new wine *Saccharomyces cerevisiae* killer toxin (Klus), encoded by a double-stranded rna virus, with broad antifungal activity is evolutionarily related to a chromosomal host gene. Appl Environ Microbiol 77:1822–1832

Rodríguez-Cousiño N, Gómez P, Esteban R (2013) L-A-lus, a new variant of the L-A totivirus found in wine yeasts with Klus killer toxin-encoding Mlus double-stranded RNA: possible role of killer toxin-encoding satellite RNAs in the evolution of their helper viruses. Appl Environ Microbiol 79:4661–4674

Romanos M, Boyd A (1988) A transcriptional barrier to expression of cloned toxin genes of the linear plasmid k1 of *Kluyveromyces lactis*: evidence that native k1 has novel promoters. Nucleic Acids Res 16:7333–7350

Santos A, Marquina D (2004a) Ion channel activity by *Pichia membranifaciens* killer toxin. Yeast 21:151–162

Santos A, Marquina D (2004b) Killer toxin of *Pichia membranifaciens* and its possible use as a biocontrol agent against grey mould disease of grapevine. Microbiology 150:2527–2534

Santos A, Marquina D, Leal JA, Peinado JM (2000) (1-6)-β-D-glucan as cell wall receptor for *Pichia membranifaciens* killer toxin. Appl Environ Microbiol 66:1809–1813

Santos A, Marquina D, Barroso J, Peinado JM (2002) (1-6)-β-D-glucan as the cell wall binding site for *Debaryomyces hansenii* killer toxin. Lett Appl Microbiol 34:95–99

Santos A, Sánchez A, Marquina D (2004) Yeasts as biological agents to control *Botrytis cinerea*. Microbiol Res 159:331–338

Santos A, Del Mar Alvarez M, Mauro MS, Abrusci C, Marquina D (2005) The transcriptional response of *Saccharomyces cerevisiae* to *Pichia membranifaciens* killer toxin. J Biol Chem 280:41881–41892

Santos A, San Mauro M, Abrusci C, Marquina D (2007) Cwp2p, the plasma membrane receptor for *Pichia membranifaciens* killer toxin. Mol Microbiol 64:831–843

Santos A, San Mauro M, Bravo E, Marquina D (2009) PMKT2, a new killer toxin from *Pichia membranifaciens*, and its promising biotechnological properties for control of the spoilage yeast *Brettanomyces bruxellensis*. Microbiology 155:624–634

Santos A, Navascués E, Bravo E, Marquina D (2011) *Ustilago maydis* killer toxin as a new tool for the biocontrol of the wine spoilage yeast *Brettanomyces bruxellensis*. Int J Food Microbiol 145:147–154

Santos A, Alonso A, Belda I, Marquina D (2013) Cell cycle arrest and apoptosis, two alternative mechanisms for PMKT2 killer activity. Fungal Genet Biol 50:44–54

Satwika D, Klassen R, Meinhardt F (2012a) Anticodon nuclease encoding virus like elements in yeast. Appl Microbiol Biotechnol 96:345–356

Satwika D, Klassen R, Meinhardt F (2012b) Repeated capture of a cytoplasmic linear plasmid by the host nucleus in *Debaryomyces hansenii*. Yeast 29:145–154

Sawant AD, Abdelal AT, Ahearn DG (1989) Purification and characterization of the anti-*Candida* toxin of

Pichia anomala WC 65. Antimicrob Agents Chemother 33:48–52

Schaffrath R, Leidel SA (2017) Wobble uridine modifications – a reason to live, a reason to die?! RNA Biol 14:1209–1222

Schaffrath R, Meacock PA (1996) A cytoplasmic gene shuffle system in *Kluyveromyces lactis*: use of epitope-tagging to detect a killer plasmid-encoded gene product. Mol Microbiol 19:545–554

Schaffrath R, Meinhardt F (2005) *Kluyveromyces lactis* zymocin and other plasmid-encoded yeast killer toxins. In: Schmitt MJ, Schaffrath R (eds) Microbial protein toxins, vol 11. Springer, Berlin, pp 133–155

Schaffrath R, Soond SM, Meacock PA (1995) Cytoplasmic gene expression in yeast: a plasmid-encoded transcription system in *Kluyveromyces lactis*. Biochem Soc Trans 23:128

Schaffrath R, Meinhardt F, Meacock PA (1996) Yeast killer plasmid pGKL2: molecular analysis of UCS5, a cytoplasmic promoter element essential for ORF5 gene function. Mol Gen Genet 250:286–294

Schaffrath R, Sasnauskas K, Meacock PA (2000) Use of gene shuffles to study the cytoplasmic transcription system operating on *Kluyveromyces lactis* linear DNA plasmids. Enzym Microb Technol 26:664–670

Schickel J, Helmig C, Meinhardt F (1996) *Kluyveromyces lactis* killer system. Analysis of cytoplasmic promoters of linear plasmids. Nucleic Acids Res 24:1879–1886

Schmitt MJ, Breinig F (2002) The viral killer system in yeast: from molecular biology to application. FEMS Microbiol Rev 26:257–276

Schmitt MJ, Breinig F (2006) Yeast viral killer toxins: lethality and self-protection. Nat Rev Microbiol 4:212–221

Schmitt MJ, Neuhausen F (1994) Killer toxin-secreting double-stranded RNA mycoviruses in the yeasts *Hanseniaspora uvarum* and *Zygosaccharomyces bailii*. J Virol 68:1765–1772

Schmitt M, Radler F (1987) Mannoprotein of the yeast cell wall as primary receptor for the killer toxin of *Saccharomyces cerevisiae* strain 28. J Gen Microbiol 133:3347–3354

Schmitt MJ, Tipper DJ (1990) K28, a unique double-stranded RNA killer virus of *Saccharomyces cerevisiae*. Mol Cell Biol 10:4807–4815

Schmitt MJ, Tipper DJ (1992) Genetic analysis of maintenance and expression of L and M double-stranded RNAs from yeast killer virus K28. Yeast 8:373–384

Schmitt MJ, Tipper DJ (1995) Sequence of the M28 dsRNA: preprotoxin is processed to an α/β heterodimeric protein toxin. Virology 213:341–351

Schmitt MJ, Klavehn P, Wang J, Schönig I, Tipper DJ (1996) Cell cycle studies on the mode of action of yeast K28 killer toxin. Microbiology 142:2655–2662

Schmitt MJ, Poravou O, Trenz K, Rehfeldt K (1997) Unique double-stranded RNAs responsible for the anti-Candida activity of the yeast Hanseniaspora uvarum. J Virol 71:8852–8855

Schründer J, Meinhardt F (1995) An extranuclear expression system for analysis of cytoplasmic promoters of yeast linear killer plasmids. Plasmid 33:139–151

Seki T, Choi EH, Ryu D (1985) Construction of killer wine yeast strain. Appl Environ Microbiol 49:1211–1215

Selvakumar D, Zhang QZ, Miyamoto M, Furuichi Y, Komiyama T (2006a) Identification and characterization of a neutralizing monoclonal antibody for the epitope on HM-1 killer toxin. J Biochem 139:399–406

Selvakumar D, Miyamoto M, Furuichi Y, Komiyama T (2006b) Inhibition of fungal β-1,3-glucan synthase and cell growth by HM-1 killer toxin single-chain anti-idiotypic antibodies. Antimicrob Agents Chemother 50:3090–3097

Selvakumar D, Miyamoto M, Furuichi Y, Komiyama T (2006c) Inhibition of β-1,3-glucan synthase and cell growth of *Cryptococcus* species by recombinant single-chain anti-idiotypic antibodies. J Antibiot (Tokyo) 59:73–79

Semenza JC, Hardwick KG, Dean N, Pelham HR (1990) *ERD2*, a yeast gene required for the receptor-mediated retrieval of luminal ER proteins from the secretory pathway. Cell 61:1349–1357

Servienė E, Lukša J, Orentaitė I, Lafontaine DL, Urbonavičius J (2012) Screening the budding yeast genome reveals unique factors affecting K2 toxin susceptibility. PLoS One 7:e50779

Sor F, Fukuhara H (1985) Structure of a linear plasmid of the yeast *Kluyveromyces lactis*: compact organization of the killer genome. Curr Genet 9:147–155

Stark MJR, Boyd A (1986) The killer toxin of *Kluyveromyces lactis:* characterization of the toxin subunits and identification of the genes which encode them. EMBO J 5:1995–2002

Stark MJ, Mileham AJ, Romanos MA, Boyd A (1984) Nucleotide sequence and transcription analysis of a linear DNA plasmid associated with the killer character of the yeast *Kluyveromyces lactis*. Nucleic Acids Res 12:6011–6030

Stark MJR, Boyd A, Mileham AJ, Romanos MA (1990) The plasmid encoded killer system of *Kluyveromyces lactis:* a review. Yeast 6:1–29

Steinberg G, Perez-Martin J (2008) *Ustilago maydis*, a new fungal model system for cell biology. Trends Cell Biol 18:61–67

Steinlauf R, Peery T, Koltin Y, Bruenn J (1988) The *Ustilago maydis* virus encoded toxin—effect of KP6 on cells and spheroplasts. Exp Mycol 12:264–274

Studte P, Zink S, Jablonowski D, Bär C, von der Haar T, Tuite MF, Schaffrath R (2008) tRNA and protein methylase complexes mediate zymocin toxicity in yeast. Mol Microbiol 69:1266–1277

Sulo P, Michalcáková S (1992) The K3 type killer strains of genus *Saccharomyces* for wine production. Folia Microbiol (Praha) 37:289–294

Sulo P, Michalcakova S, Reiser V (1992) Construction and properties of K1 type killer wine yeasts. Biotechnol Lett 14:55–60

Suzuki C, Nikkuni S (1994) The primary and subunit structure of a novel type killer toxin produced by a halotolerant yeast, *Pichia farinosa*. J Biol Chem 269:3041–3046

Suzuki C, Kashiwagi T, Tsuchiya F, Kunishima N, Morikawa K, Nikkuni S, Arata Y (1997) Circular dichroism analysis of the interaction between the α and β subunits in a killer toxin produced by a halotolerant yeast, *Pichia farinosa*. Protein Eng 10:99–101

Suzuki C, Ando Y, Machida S (2001) Interaction of SMKT, a killer toxin produced by *Pichia farinosa*, with the yeast cell membranes. Yeast 18:1471–1478

Suzuki Y, Schwartz SL, Mueller NC, Schmitt MJ (2017) Cysteine residues in a yeast viral A/B toxin crucially control host cell killing via pH-triggered disulfide rearrangements. Mol Biol Cell 28:1123–1131

Takasuka T, Komiyama T, Furuichi Y, Watanabe T (1995) Cell wall synthesis specific cytocidal effect of *Hansenula mrakii* toxin-1 on *Saccharomyces cerevisiae*. Cell Mol Biol Res 41:575–581

Tamás MJ, Luyten K, Sutherland FC, Hernandez A, Albertyn J, Valadi H, Li H, Prior BA, Kilian SG, Ramos J, Gustafsson L, Thevelein JM, Hohmann S (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. Mol Microbiol 31:1087–1104

Tao J, Ginsberg I, Banerjee N, Held W, Koltin Y, Bruenn JA (1990) *Ustilago maydis* KP6 killer toxin: structure, expression in *Saccharomyces cerevisiae*, and relationship to other cellular toxins. Mol Cell Biol 10:1373–1381

Theisen S, Molkenau E, Schmitt MJ (2000) Wicaltin, a new protein toxin secreted by the yeast *Williopsis californica* and its broad-spectrum antimycotic potential. J Microbiol Biotechnol 10:547–550

Tiggemann M, Jeske S, Larsen M, Meinhardt F (2001) *Kluyveromyces lactis* cytoplasmic plasmid pGKL2: heterologous expression of Orf3p and prove of guanylyltransferase and mRNA-triphosphatase activities. Yeast 18:815–825

Tipper DJ, Bostian KA (1984) Double-stranded ribonucleic acid killer systems in yeasts. Microbiol Rev 48:125–156

Tokunaga M, Wada N, Hishinuma F (1987) Expression and identification of immunity determinants on linear DNA killer plasmids pGKL1 and pGKL2 in *Kluyveromyces lactis*. Nucleic Acids Res 15:1031–1046

Tokunaga M, Kawamura A, Hishinuma F (1989) Expression of pGKL killer 28K subunit in *Saccharomyces cerevisiae*: identification of 28K subunit as a killer protein. Nucleic Acids Res 17:3435–3446

Tommasino M, Ricci S, Galeotti C (1988) Genome organization of the killer plasmid pGKL2 from *Kluyveromyces lactis*. Nucleic Acids Res 16:5863–5978

Travassos LR, Silva LS, Rodrigues EG, Conti S, Salati A, Magliani W, Polonelli L (2004) Therapeutic activity of a killer peptide against experimental paracoccidioidomycosis. J Antimicrob Chemother 54:956–958

Valzano M, Cecarini V, Cappelli A, Capone A, Bozic J, Cuccioloni M, Epis S, Petrelli D, Angeletti M, Eleuteri AM, Favia G, Ricci I (2016) A yeast strain associated to Anopheles mosquitoes produces a toxin able to kill malaria parasites. Malar J 15:21

Walker GM, McLeod AH, Hodgson VJ (1995) Interactions between killer yeasts and pathogenic fungi. FEMS Microbiol Lett 127:213–222

Wang X, Chi Z, Yue L, Li J, Li M, Wu L (2007a) A marine killer yeast against the pathogenic yeast strain in crab (*Portunus trituberculatus*) and an optimization of the toxin production. Microbiol Res 162:77–85

Wang X, Chi Z, Yue L, Li J (2007b) Purification and characterization of killer toxin from a marine yeast *Pichia anomala* YF07b against the pathogenic yeast in crab. Curr Microbiol 55:396–401

Wedral D, Shewfelt R, Frank J (2010) The challenge of *Brettanomyces* in wine. LWT-Food Sci Technol 43:1474–1479

Weiler F, Schmitt MJ (2003) Zygocin, a secreted antifungal toxin of the yeast *Zygosaccharomyces bailii*, and its effect on sensitive fungal cells. FEMS Yeast Res 3:69–76

Weiler F, Rehfeldt K, Bautz F, Schmitt MJ (2002) The *Zygosaccharomyces bailii* antifungal virus toxin zygocin: cloning and expression in a heterologous fungal host. Mol Microbiol 46:1095–1105

Wemhoff S, Klassen R, Meinhardt F (2014) Site-directed mutagenesis of the heterotrimeric killer toxin zymocin identifies residues required for early steps in toxin action. Appl Environ Microbiol 80:6549–6559

Wemhoff S, Klassen R, Meinhardt F (2016a) DNA damage induced by the anticodon nuclease from a *Pichia acaciae* killer strain is linked to ribonucleotide reductase depletion. Cell Microbiol 18:211–222

Wemhoff S, Klassen R, Beetz A, Meinhardt F (2016b) DNA damage responses are induced by tRNA anticodon nucleases and hygromycin B. PLoS One 11: e0157611

Wickner RB (1992) Double-stranded and single-stranded RNA viruses of *Saccharomyces cerevisiae*. Annu Rev Microbiol 46:347–375

Wickner RB (1996) Double-stranded RNA viruses of *Saccharomyces cerevisiae*. Microbiol Rev 60:250–265

Wilson DW, Meacock PA (1988) Extranuclear gene expression in yeast: evidence for a plasmid encoded RNA-polymerase of unique structure. Nucleic Acids Res 16:8097–8112

Wistow GJ, Piatigorsky J (1988) Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. Annu Rev Biochem 57:479–504

Woods DR, Bevan EA (1968) Studies on the nature of the killer factor produced by *Saccharomyces cerevisiae*. J Gen Microbiol 51:115–126

Worsham PL, Bolen PL (1990) Killer toxin production in *Pichia acaciae* is associated with linear DNA plasmids. Curr Genet 18:77–80

Yamamoto T, Iratani T, Hirata H, Imai M, Yamaguchi H (1986a) Killer toxin from *Hansenula mrakii* selectively inhibits cell wall synthesis in a sensitive yeast. FEBS Lett 197:50–54

Yamamoto T, Imai M, Tachibana K, Mayumi M (1986b) Application of monoclonal antibodies to the isolation and characterization of a killer toxin secreted by *Hansenula mraki*i. FEBS Lett 195:253–257

Yamamoto T, Uchida K, Hiratani T, Miyazaki T, Yagiu J, Yamaguchi H (1988) In vitro activity of the killer toxin from yeast *Hansenula mrakii* against yeasts and molds. Antibiot (Tokyo) 41:398–403

Young TW, Yagiu M (1978) A comparison of the killer character in different yeasts and its classification. Antonie Van Leeuwenhoek 44(1):59–77

Zabel R, Bär C, Mehlgarten C, Schaffrath R (2008) Yeast alpha-tubulin suppressor Ats1/Kti13 relates to the Elongator complex and interacts with Elongator partner protein Kti11. Mol Microbiol 69:175–187

Zhu H, Bussey H (1991) Mutational analysis of the functional domains of yeast K1 killer toxin. Mol Cell Biol 11:175–181

Zhu YS, Zhang XY, Cartwright CP, Tipper DJ (1992) Kex2-dependent processing of yeast K1 killer preprotoxin includes cleavage at ProArg-44. Mol Microbiol 6:511–520

Zhu YS, Kane J, Zhang XY, Zhang M, Tipper DJ (1993) Role of the gamma component of preprotoxin in expression of the yeast K1 killer phenotype. Yeast 9:251–266

Zink S, Mehlgarten C, Kitamoto HK, Nagase J, Jablonowski D, Dickson RC, Stark MJR, Schaffrath R (2005) Mannosyl-diinositolphospho-ceramide, the major yeast plasma membrane sphingolipid, governs toxicity of *Kluyveromyces lactis* zymocin. Eukaryot Cell 4:879–889

Zorg J, Kilian S, Radler F (1988) Killer toxin producing strains of the yeasts *Hanseniaspora uvarum* and *Pichia kluyveri*. Arch Microbiol 149:261–267

The Fungal MCC/Eisosome Complex: An Unfolding Story

Krisztina Kolláth-Leiß[1], Frank Kempken[1]

CONTENTS

I. Introduction

Filamentous fungi provide simple experimental systems and thus are model systems for higher eukaryotes (Roche et al. 2014). However, there are also cellular structures present in fungi which are unique to them. In this review, we discuss one of these unique features, namely, the fungal MCC/eisosomal complex.

The plasma membrane acts as a barrier to separate the cell from its environment, but it also provides an interface for communication processes between the intra- and extracellular space. In connection with latter function, it regulates the exchange of metabolites and signal molecules, and therefore it plays a role in several physiological processes like growth, development and stress response (Truong-Quang and Lenne 2014). It consists of a lipid bilayer and of proteins, which are integrated in the lipid bilayer or associated with its surface (Yadeta et al. 2013). Originally, according to the fluid mosaic model (Singer and Nicolson 1972), the distribution of lipids and proteins in the plasma membrane was postulated to be homogenous. Nowadays, it is clear that **the plasma membrane is** highly **asymmetric**; there are enormous differences between the composition of the inner and outer leaflet of the lipid bilayer (Daleke 2003). Furthermore, **the plasma membrane is** also **laterally compartmentalised**. In the baker's yeast, the localisation of 46 integral membrane proteins was investigated, and none of them showed a homogenous distribution but accumulated at distinct membrane areas (Spira et al. 2012). According to the methods of visualisation, several distinct **micro-domains** of various size, composition and mobility can be identified (Kusumi et al. 2012). In the fungal plasma membrane, the following micro-domains have been described: **membrane compartment of Can1 (MCC)**, **membrane compartments of Pma1 (MCP)**, **membrane compartment of Torc2 (MCT)**, **endocytic sites**, **sterol-rich domains** and **cell division and polarity nodes** (Malinsky and Opekarova 2016). This article focusses on the description of the **MCC domain** and its **associated protein complex** called **eisosome** (Table 1).

II. The MCC/Eisosome Complex in *Saccharomyces cerevisiae*

Although the existence of **furrow-like invaginations in the yeast** *Saccharomyces cerevisiae* **plasma membrane** has already been investigated in the early 1960s by freeze-fracture electron microscopy (Moor and Mühlethaler 1963), the composition, formation and function of

[1] Botanisches Institut, Christian-Albrechts-Universität zu Kiel, Kiel, Germany; e-mail: kkollath@bot.uni-kiel.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

Table 1 Description of proteins

Protein	Data base name[a]	Species	Name description[a]	Localisation
Abp1	YCR088W	*S. cerevisiae*	Actin-binding protein	Actin patch
Avo2	YMR068W	*S. cerevisiae*	Adheres voraciously (to TOR2)	MCT
Bem46	NCU03276	*N. crassa*	Bud emergence 46-like	Eisosome, ER
Can1	YEL063C	*S. cerevisiae*	Canavanine resistance	MCC
Lsp1	YPL004C	*S. cerevisiae*	Long-chain bases stimulate phosphorylation	Eisosome
Mtr	NCU06619	*N. crassa*	Methyltryptophan resistant	Eisosome
Nce102	YPR149W	*S. cerevisiae*	Nonclassical export	MCC
Pil1	YGR086C	*S. cerevisiae*	Phosphorylation inhibited by long-chain bases	Eisosome
Pkc1	YBL105C	*S. cerevisiae*	Protein kinase C	Cytoplasm
Pkh1	YDR490C	*S. cerevisiae*	Pkb-activating kinase homolog	Eisosome
Pkh2	YOL100W	*S. cerevisiae*	Pkb-activating kinase homolog	Eisosome
Rho2	YNL090W	*S. cerevisiae*	Ras homolog	Membrane
Rvs161	YCR009C	*S. cerevisiae*	Reduced viability on starvation	Actin patch
Rvs167	YDR388W	*S. cerevisiae*	Reduced viability on starvation	Actin patch
Seg1	YMR086W	*S. cerevisiae*	Stability of eisosomes guaranteed	MCC/eisosome
Sjl1/Inp51	YIL002C	*S. cerevisiae*	Inositol polyphosphate 5-phosphatase	Membrane/ endocytic sites
Sjl2/Inp52	YNL106C	*S. cerevisiae*	Inositol polyphosphate 5-phosphatase	Membrane/ endocytic sites
Sle1/Seg1	SPAC1A6.07	*S. pombe*	Seg1-like eisosome protein	MCC/eisosome
Slm1	YIL105C	*S. cerevisiae*	Synthetic lethal with Mss4	Eisosome
Slm2	YNL047C	*S. cerevisiae*	Synthetic lethal with Mss4	Eisosome
Ste3	YKL178C	*S. cerevisiae*	Sterile	Membrane
Sur7	YML052W	*S. cerevisiae*	Suppressor of Rvs167 mutation	MCC
Syj1	SPBC2G2.02	*S. pombe*	Synaptojanin homolog 1	Cytoplasm
Tat2	YOL020W	*S. cerevisiae*	Tryptophan amino acid Transporter	Eisosome
Tax4/Irs4	SPAC1687.09	*S. pombe*	–	Cytoplasm
Tor2	YKL203C	*S. cerevisiae*	Target of rapamycin	MCT
Xrn1	YGL173C	*S. cerevisiae*	Exoribonuclease	Eisosome/ cytoplasm
Ypk1	YKL126W	*S. cerevisiae*	Yeast protein kinase	Membrane/ cytoplasm

[a]According to *Saccharomyces* Genome Database (www.yeastgenome.org), PomBase (www.pombase.org) and FungiDB (www.fungidb.org)

these remained unknown for a long time. Finally, in 2009, the invaginations were reported to correspond to distinct lateral plasma membrane domains known as **membrane compartment of Can1** or **MCC** (Strádalová et al. 2009).

The high-affinity arginine permease Can1/YEL063C accumulates exclusively in MCC patches. Its localisation to the plasma membrane is essentially dependent on ethanolamine (Opekarová et al. 2002). *Can1* mutants are resistant against the toxic arginine analogue L-canavanine (Whelan et al. 1978).

MCC domains of the yeast plasma membrane (Fig. 1) are characterised as furrow-like invaginations of 300 nm in length and 50 nm in depth (Douglas et al. 2011). They consist of a few integral membrane proteins, which are essential for MCC formation. The core of the MCC domain consists of the integral membrane proteins **Sur7** and **Nce102**.

Sur7/YML052W is a protein with three transmembrane helices, an extracellular N-terminal and a cytoplasmic C-terminal part (Sivadon et al. 1997). Overexpression of the protein suppresses defects in actin polarisation,

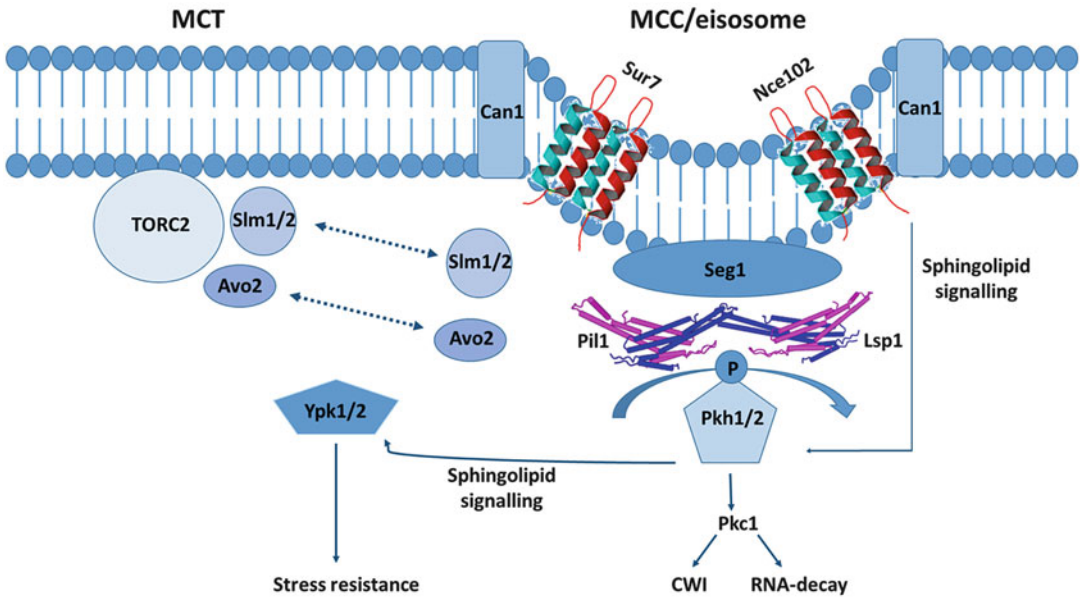


Fig. 1 Composition of the yeast MCC/eisosome complex. Continuous arrows indicate correlation without distinguishing positive or negative influences. Spotted arrows show spatial movement of proteins, and thick arrow stays for phosphorylation event by Pkh1/2. See main text for further explanation. Figure modified from Douglas and Konopka (2014)

bud-site selection and growth caused by mutations in the *rvs161* and/or *rvs167* genes by influencing the sphingolipid biosynthesis of *S. cerevisiae* (Young et al. 2002). *Sur7* is differentially expressed in the late G_2/M phase of the cell cycle (Spellman et al. 1998).

A sphingolipid-sensing protein, Nce102 (nonclassical export protein 2)/YPR149W, is crucial for the formation of MCC patches as well as furrow-like invaginations (Fröhlich et al. 2009). Knockout mutants exhibit flattened and elongated MCC domains (Strádalová et al. 2009). The protein consists of two transmembrane domains with cytoplasmic-oriented C- and N-termini. Six amino acid residues of the C-terminus are responsible for the correct formation of MCC patches and the corresponding invaginations (Loibl et al. 2010).

The eisosomal protein Pil1 is responsible for the correct localisation of Nce102 in forming MCC patches (Moreira et al. 2008). Nce102 and its homologs seem to act as sphingolipid sensors in the plasma membrane. They play a role in the regulation of plasma membrane functions by influencing the Pkh signalling, according to the sphingolipid level (Fröhlich et al. 2009).

Two F-BAR domain containing proteins, **Slm1** (synthetic lethal with Mss4) and **Slm2**, are needed for the proper targeting of the MCC domain to the furrow-like invaginations. Both proteins are able to bind phosphatidylinositol-4,5-bisphosphate (PI4,5P2) (Audhya et al. 2004). As **downstream effectors of the TOR complex 2** (TORC2), they play a role in the regulation of the actin cytoskeleton in stress response reactions. Mutation of *slm1* and its homolog *slm2* leads to lethal growth damages due to the depolarisation of the actin cytoskeleton (Fadri et al. 2005). *Slm* mutants also show defect localisation of the eisosomal core protein Pil1 (Kamble et al. 2011).

The **formation of the furrows**, corresponding with the MCC area of the yeast plasma membrane, is **promoted by** an interacting protein complex called **eisosome** (Olivera-Couto and Aguilar 2012; Douglas and Konopka 2014). Eisosomes (Greek eiso = into, soma = body) are immobile protein complexes of uniform size interacting with the inner part of the MCC domains of the plasma membrane. Several

membrane-associated and cytoplasmic proteins are reported to be localised to these structures; some of them are facultative, and others are essential components for structure and/or function. The core of the eisosome complex consists of two proteins: **Pil1** and **Lsp1** (Douglas et al. 2011). Although their amino acid sequences show a similarity of 74%, the functions of the two proteins are distinct (Olivera-Couto et al. 2015).

Two different pools in yeast cells could be identified by fluorescence fluctuation analyses: a cytoplasmic pool with free diffusion of the proteins and an eisosomal pool, which shows slow dynamics similar to a binding-unbinding homeostasis (Olivera-Couto et al. 2015). The ratio of the Pil1:Lsp1 content of this fraction is nearly 1:1.

Pil1 and Lsp1 proteins undergo self-assembly and build heterodimers, which seem to be the minimal building blocks for eisosome formation (Ziółkowska et al. 2011). As a first step in this process, the MCC-localised **Seg1/** Ymr086w protein **establishes a platform** on the inner surface of the plasma membrane for the eisosomal core proteins Pil1 and Lsp1 (Moreira et al. 2012). Once recruited, the **Pil1/Lsp1 heterodimers build a scaffold** below the MCC area.

On one hand, this scaffold stabilises the Seg1 platform, and—more importantly—on the other hand, it promotes the bending of the plasma membrane, resulting in the furrow-like invaginations. The membrane-bending ability of the Pil1/Lsp1 proteins is mediated by their Bin/Amphiphysin/Rvs (BAR) domains (Ziółkowska et al. 2011). The banana-shaped BAR domains are similar to the membrane-sculpting amphiphysin, arfaptin or endophilin of mammals. Moreover, overexpression of Pil1 or Lsp1 in mammal cells leads to the tubulation of liposomes and the formation of tubules (Olivera-Couto et al. 2011). Yeast cells, expressing the Pil1 protein without functional BAR domain, are defect in eisosome assembly and consequently in plasma membrane organisation. Positively charged patches on the surface of the Pil1 protein are reported to be responsible for plasma membrane binding and liposome tubulation, whereupon Pil1 shows a preference for binding phosphatidylinositol-containing membranes. Clustering of lipids and proteins in the plasma membrane, mediated by BAR-domain-containing proteins, seems to be an overall principle in the eukaryotic kingdom (Zhao et al. 2013).

The fact that Pil1 deletion mutants are unable to build proper eisosomes, but show clustering of eisosomal invaginations in large eisosome remnants (Walther et al. 2006), suggests that Pil1 is the essential component for eisosome formation. Its **BAR-domain-mediated** ability for **membrane binding and bending** has already been explained above. There is one more aspect that is crucial for eisosome formation: **phosphorylation**. Many putative phosphorylation sites were investigated in Pil1, and at least five of them (Ser-6, [26STT28], Thr-233, Ser-41 and Ser-265) were shown to be involved in eisosome formation (Luo et al. 2008). Both Lsp1 and Pil1 proteins are phosphorylated by the Pkh1 and Pkh2 kinases, and due to the fact that *pkh1* and *2* double knockouts show abnormal eisosome formation, the phosphorylation event is essential for this process.

Moreover, the rate of phosphorylation is crucial for the regulation of eisosome assembly and disassembly (Deng et al. 2009). Under in vivo normal growth condition, Pil1 and Lsp1 proteins are existent as phosphorylated species B. Increased Pkh activity leads to hyperphosphorylation of species B to species C, which results in eisosome disassembly. In contrast, decreased Pkh activity leads to hypophosphorylation and the assembly of eisosomes (Luo et al. 2008). As Pkh is a part of the long-chain base (LCB) signalling, Pil1 and Lsp1 are considered to be targets of this pathway. Indeed, the LCB content of the cells also influences eisosome formation, similar to Pkh activity: High LCB concentrations result in hypophosphorylation of Lsp1/Pil1 and therefore in eisosome assembly. Low LCB content induces the opposite effects, namely, hyperphosphorylation and eisosome disassembly (Walther et al. 2007).

By combining fluorescence microscopy and mass spectroscopy, the composition of protein complexes was analysed (Deng et al. 2009). Forty-two diverse eisosomal proteins could be identified, amongst others several transporter, sterol and signalling molecules. It was also shown that **reversible phosphorylation/dephosphorylation of proteins led to eisosomal assembly/disassembly.**

Eisosomes are involved in several different processes in the yeast cells; however their exact **function remains** somewhat **elusive.** At first, they were considered to be sites of endocytosis (Walther et al. 2006) due to the fact that eiso-

somal disruption leads to the formation of large, aberrant invaginations instead of distinct endocytic sites. Though this hypothesis has already been disproven, there is a striking **link between eisosomes and endocytosis.**

Pil1 influences the endocytosis of the receptor protein for the peptide pheromone factor Ste3 (Hagen et al. 1986; Murphy et al. 2011). In Δ*pil1* mutants, endocytic patches with the Abp1 protein persist longer, and endocytic sites containing GFP-coupled Rvs161 and 167 show an altered scission efficiency and frequency of formation. Moreover, in the same mutants, the PI(4,5)P2 phosphatases Sjl1 and 2, which are involved in endocytic membrane trafficking (Singer-Krüger et al. 1998), are mistargeted to the cytoplasm, which leads to the conclusion that Pil1 is required for correct recruitment of Sjl to endocytic sites.

As any plasma membrane domain, eisosomes play an important role in the **clustering of membrane proteins**. Furthermore, eisosomes are crucial for **PI(4,5)P2 regulation of the plasma membrane**. PI(4,5)P2 is a phospholipid component of the plasma membrane, which acts as a substrate for diverse proteins, included in signalling pathways.

As already mentioned above, Pil1 shows a preference to bind PI(4,5)P2-containing membranes. In *S. pombe* the hydrolysis of PI(4,5)P2 takes place over the Sle1-Syj1-Tax4 pathway, in which Syj1 is a synaptojanin-like lipid phosphatase with Tax4 as its ligand (Kabeche et al. 2014). Defective PI(4,5)P2 regulation leads to eisosomal defects. It is indicated that there is an interdependence between the two plasma membrane domains MCC and MCT (membrane compartment of TORC2), concerning the regulation of PI(4,5)P2 (Bartlett et al. 2015). TORC2 mutants show morphology and growth effects that can be suppressed by mutations in the Pil1-Sle1-Syj1-Tax4 pathway. It is also shown that the MCT marker protein Avo2 partially co-localises with Pil1. Formation of MCT depends on eisosome integrity and on the PI(4,5)P2 concentration of the plasma membrane. Altogether, there seems to be a connection between signal transduction over TORC and the eisosome-dependent regulation of PI(4,5)P2.

In the bud of the dividing yeast cells, the de novo formation and disassembly of eisosomes are regulated by the sphingolipid-Pkh1,2-Ypk pathway (Luo et al. 2008). Inactivation of Ypk by the sphingolipid-Pkh signalling pathway or chemically by myriocin results in eisosome disassembly. Pil1 acts as a central regulator in this process, which determines both size and location of newly built eisosomes (Moreira et al. 2008). The Pil1 expression level is cell cycle dependent and therefore synchronised with the plasma membrane growth. Higher expression of Pil1 leads to the formation of the same number of larger eisosomes, and lower expression levels result in lower number of eisosomes which still have normal size.

Thus, eisosomes play a role in controlling endocytosis. In this process Pkh1 and Pkh2 respond to changes in the sphingolipid level of the plasma membrane. The signal is then transmitted to the eisosomes via Pil1 phosphorylation.

In some cases eisosomes act as flexible **regulatory sites in response to stress reactions** or to varying environmental conditions by accumulating the key enzyme(s) of distinct pathways in eisosomes, spatially separated from the rest of the machinery (Grousl et al. 2015; Malinsky and Opekarova 2016). Some prominent examples for this kind of regulation are the Pkh1/Pkh2 kinases, a flavodoxin-like quinone oxidoreductase (Grossmann et al. 2008; Li et al. 2015) and the 5→3 exoribonucleases Xrn1 (Grousl et al. 2015), which is a key enzyme of the mRNA decay (Nagarajan et al. 2013). It has been shown to be eisosomal localised in post-diauxic yeast cells.

In this case the eisosomal localisation of Xrn1 separates the key enzyme from the other proteins of the pathway, which remains inactive until a proper signal effects the release of the key protein to the cytoplasmic counterparts and therefore to the activation of the pathway. Which signal is crucial for the release of the protein from the eisosomes and how this signal is transmitted are still elusive. Under nutrient starvation, the Pkh1-Pkc1 cascade is required for mRNA processing body (P-body) formation as a response to stress conditions (Cowart et al. 2010). This result suggests the involvement of the nutrition signalling TOR pathway in stress-dependent RNA degradation and additional to this the transmission of the signal over phosphorylation cascades.

Analyses of the phosphoproteome of protein kinase C (Pkc1) overexpressing yeast cells identified five **eisosomal localised targets of the cell wall integrity** (CWI) **pathway** (Mascaraque et al.

2013). CWI is activated under diverse conditions by Pkc1. Overexpression of Pkc1 leads to activation of CWI independently from external conditions. Although both Pil1 and Lsp1 proteins were shown to be phosphorylated under these circumstances, the eisosomal core proteins are not direct targets of the Pkc1 kinase.

Eisosomes could play a role as multipotent regulatory sites under diverse stress conditions, according to their composition, to their connection to diverse plasma membrane domains and signal transduction pathways, as well as to their ability for both receiving external and internal signals and to react to those flexible by reversible phosphorylation/dephosphorylation. As a special aspect of this topic, eisosomes seem to act as a membrane reservoir under hypoosmotic stress (Kabeche et al. 2015). Under these conditions, expansion of the plasma membrane is required. The membrane reservoir for this development is in the invaginations, built by the eisosomes. It was reported that eisosomes of yeast protoplast, under hypoosmotic stress, disappeared and the corresponding invaginations became flattened.

III. The MCC/Eisosome Complex in Other Fungi

A. *Schizosaccharomyces pombe*

Investigations on the Pil1 homolog (SpPil1) in the fission yeast (*Schizosaccharomyces pombe*) demonstrated its unique role in the spatial organisation of the cell cortex (Kabeche et al. 2011). As well as the purified protein in vitro, SpPil1 assembles to linear, cortical filaments in vivo. These stable filaments are absent at the active regions of cell growth, and their assembly is independent of actin cables and microtubules. Knockout mutants of SpPil1 do not exhibit obvious growth effects, as reported for *Ashbya gossypii* (Seger et al. 2011); however the overexpression of the protein leads to irregular septum formation and deficient localisation of cortical proteins. These together result in defects of cell polarity and cytokinesis. Overexpressed SpPil1 proteins are organised in long cytoplasmic rods with characteristic ultrastructure. The SpPil1 tubules form a regular, hexagonal pattern (Kabeche et al. 2015). These structures are not exclusively built of proteins but also contain lipids, as positive filipin staining demonstrates. All together the **SpPil1** protein is considered as **a novel component of the yeast cytoskeleton** that maintains the spatial organisation at the cell cortex.

Interestingly, there seem to be some differences in the grade of functional conservation between the eisosomal components in different organisms. The interspecies transfer of distinct MCC/eisosome proteins leads to different results (Vaskovicova et al. 2015). The SpPil1 protein is fully functional in *S. cerevisiae*. SpPil1 recognises both Sur7 and Seg1 proteins correctly, and furrow-like invaginations form normally. In contrast to this, as long as the Seg1 protein of *S. cerevisiae* recognises both Pil1 and SpPil1, the SpSle1 protein, which is a functional homolog of the Seg1 protein, is only able to recognise its own SpPil1.

As well as in the case of the baker's yeast, fission yeast eisosomes are functionally linked to the cell integrity pathway (Kabeche et al. 2015), which is a conserved MAPK signalling cascade that is activated by various environmental stresses. Eisosomes play a key role in this process by promoting the hydrolysis of PI (4,5)P2 and influencing their spatial organisation into clusters at the plasma membrane. The latter contains, amongst others, two proteins that are both part of the integrity pathway: The PI5-kinase Its3 is required for the cluster formation and the GTPase Rho2 plays a key role in the activation of the MAPK cascade.

B. *Candida albicans*

Core components of MCC/eisosome complex in *C. albicans* are identical to those described in the yeast model. However, there are some functional divergences, probably due to different growth conditions and development.

The *Δsur7* strain shows altered septin and actin localisation as well as defective morphogenesis. Additionally, cell wall synthesis is also impaired in the mutants, which form abnormal cytoplasmic cell wall invaginations, similar to

the structures that can be detected under cell wall stress condition (Alvarez et al. 2009).

Moreover, *Δsur7* mutants are more sensitive to detergent, chitin-binding stains like calcofluor and congo red, inhibitors of chitin synthesis (nikkomycin Z) and also inhibitors of the β-1,3-glucan synthesis (caspofungin). The mutants are less tolerant to lysis conditions, which is due to their decreased β-glucan content; however the β-glucan synthase is not localised in the stable MCC/eisosome complex, but it is mobile and associates with actin patches (Wang et al. 2011). Hence *Candida albicans* is a human pathogen, it is of special importance that MCC/eisosome is critical for the virulence of the fungus. *Δsur7* mutants are more sensitive to oxidation and to copper, and this leads to defective intraphagosomal growth in macrophages. Both initial infection and invasive growth are impaired by *sur7* deletion, which leads to inhibited pathogenesis in mice (Douglas et al. 2011).

Another MCC core protein Nce102 is also crucial for normal pathogenicity in *C. albicans*. Deletion of the protein leads to the inhibition of hyphae formation and to an additional unique phenotype: Mutants are able to invade in high-concentrated agar but have problems in invading low agar concentrations. This is due to defects in actin organisation and leads finally to decreased virulence in mice (Douglas et al. 2013). Considering the fact that correct **MCC/eisosome formation is crucial for pathogenicity of *C. albicans***, it increases its importance as a novel target of antifungal drug development.

In the yeast model, two kinases Pkh1 and Pkh2 are responsible for phosphorylation events in MCC/eisosome core components, which are crucial for formation and stability of the structure. In *C. albicans*, only one ortholog CaPkh2 has been identified, although an additional kinase CaPkh3 also influences, at least, the furrow formation of the plasma membrane (Pastor-Flores et al. 2016; Wang et al. 2016). The C-terminal part of the protein is crucial for both kinase activity and lipid binding. Moreover, these two functions seem to be interconnected. CaPkh2 is able to interact with structural and signal lipids. It possesses a pleckstrin homology (PH) domain (Lemmon 2007), with the ability to bind sulfatide-like lipids at the C-terminus. Deletion of CaPkh2 leads to abnormal formation of furrow-like invaginations (Wang et al. 2016).

The two main eisosomal core components Pil1 and Lsp1 co-localise in eisosomes in all developmental stages in *C. albicans* (Reijnst et al. 2011). Simultaneous deletion of both proteins leads to loss of eisosome formation and finally to defective plasma membrane organisation and morphogenesis (Wang et al. 2016). Double knockout mutants develop cytoplasmic cell wall invaginations, similar to those described for the *Δsur7* mutant. In both cases, accumulation of PI(4,5)P2 at the furrow site can be detected. Both *Δsur7* and *Δpil1Δlsp1* mutants show increased sensitivity in diverse stress conditions. Although the overexpression of Sur7 rescues many of the *Δpil1Δlsp1* phenotypes, the direct role of the three proteins seems to be distinct.

C. *Ashbya gossypii*

The **formation of eisosomes, during the asexual development**, is best described in the filamentous Saccharomycete *Ashbya gossypii* (Seger et al. 2011). In this fungus, eisosomes form, during the whole cycle, from germinating conidiospores to mature hyphae, though the density of eisosomes is much higher in early germination stages than later on. This also corresponds with the tenfold higher mRNA levels of eisosome components in spores compared to hyphae. New eisosomes are built at the first 30 μm of the hyphal tip but always subapical from the region of endocytosis, which clearly shows spatial separation of the two processes. The rate of the formation is 1.6 (±0.5) eisosomes per minute.

The core components of eisosomes are similar to those in *S. cerevisiae*, with some functional divergences. The Pil1 homologous protein in *A. gossypii* is essential for polar growth, which seems to be unique in all investigated species. Additionally to this, *A. gossypii* eisosomes are not stabilised by the Nce102 homolog, but by a novel eisosomal component, which is homologous to the yeast protein Ymr086w.

D. *Aspergillus nidulans*

The eisosomal core components Pil1 and Lsp1 and the integral membrane protein Sur7 are conserved in all Ascomycetes. However,

according to an independent duplication event in the subphylum, there are two identified homologous proteins for Pil1/Lsp1, namely, **PilA** and **PilB** (Vangelatos et al. 2010). PilA is functionally more convergent to Pil1. In *Aspergillus nidulans,* several Sur7 proteins have been identified; amongst them **SurG** shows the highest homology. While eisosomes are present during the whole life cycle of the yeasts *S. cerevisiae*, *S. pombe* and *C. albicans*, **eisosome formation in filamentous fungi is temporally concentrated on the early stages of spore germination**. In the filamentous Saccharomycete *C. albicans*, eisosome density is much higher in conidiospores than in hyphae (Seger et al. 2011). In the Ascomycete *A. nidulans*, no eisosomes are present in mycelia.

PilA shows a punctuate localisation close to the plasma membrane during the whole life cycle of the fungus. However, PilB and SurG are co-localising with these patches in mature conidio- and ascospores only (Vangelatos et al. 2010; Athanasopoulos et al. 2013). During germination, PilB and SurG are released from the eisosomes. They move to the cytoplasm and to vacuoles and endosomes, respectively. Deletion of *pilA*, *pilB* and *surG* obviously affects neither the asexual nor the sexual development of *A. nidulans*.

The Nce102 homologous protein in *A. nidulans* (AnNce102) is localised to the eisosomes and regulates their number and density in early germination stages (Athanasopoulos et al. 2015). Together with PilA, it also plays a role in the regulation of sphingolipid biosynthesis. Interestingly, the Nce102 homolog of the closely related human pathogen *Aspergillus fumigatus* (AfuNce102) does not seem to be localised in the eisosomes but in the endoplasmic reticulum (Khalaj et al. 2012). Unlike *C. albicans*, the protein does not play a crucial role in the pathogenicity of the fungus, although (*afu*)*nce102* deletion leads to conidiation defects.

E. *Neurospora crassa*

In filamentous fungi, characterisation of the core components of the eisosome is in the main focus. However the localisation of other proteins could provide important information for functional characterisation of the MCC/eisosome complex. In the filamentous Ascomycete *Neurospora crassa*, the PilA homolog accumulates in discrete patches near to the plasma membrane. Two proteins, the **bud emergency 46 (Bem46)** and the neutral amino acid permease (**Mtr**), have been reported to co-localise with the PilA homolog [Fig. 2, (Kollath-Leiß et al. 2014)]. Mtr is homologous to the H+-driven tryptophan and tyrosine permease of the baker's yeast. It is one of the few transporter proteins, which are eisosomal localised in *S. cerevisiae* (Grossmann et al. 2008).

The Bem46 protein is an αβ-hydrolase, which is conserved in the eukaryotic kingdom (Kumar et al. 2013). Bem46 homologous proteins are involved in the maintenance of cell polarity; however their exact function is still elusive (Valencik and Pringle 1995; Mochizuki et al. 2005). Due to an unusual ER retention signal in *N. crassa,* Bem46 is localised to the perinuclear ER and in the eisosomes.

Neurospora crassa strains that overexpress the Bem46 protein show delayed conidiospore germination and reduced growth of vegetative hyphae compared to the wild type. Over 99% of the ascospores of these strains are unable to germinate; however single ascospores do build germination tubes, which are apparently unable to develop properly and therefore end as bubble-like structures (Mercker et al. 2009; Kollath-Leiß et al. 2014). Bem46 interacts directly with the anthranilate synthase, which is a key enzyme of the tryptophan biosynthesis.

Bem46 not only co-localises with the tryptophan transporter Mtr in the eisosomes, but in *bem46* up- or downregulated strains, gene expression of *Mtr* is modified. This suggests the involvement of **Bem46** in the **regulation of the intracellular tryptophan level**. Probably due to this regulatory role, Bem46 has an **impact on the tryptophan-dependent auxin biosynthesis** of the fungus, which may explain the **developmental defects** of *bem46* up- or downregulated strains. Altogether, the Bem46 protein of *N. crassa* seems to act regulatory in certain developmental steps of the fungus. Hence, its eisosomal localisation might be crucial for this process.

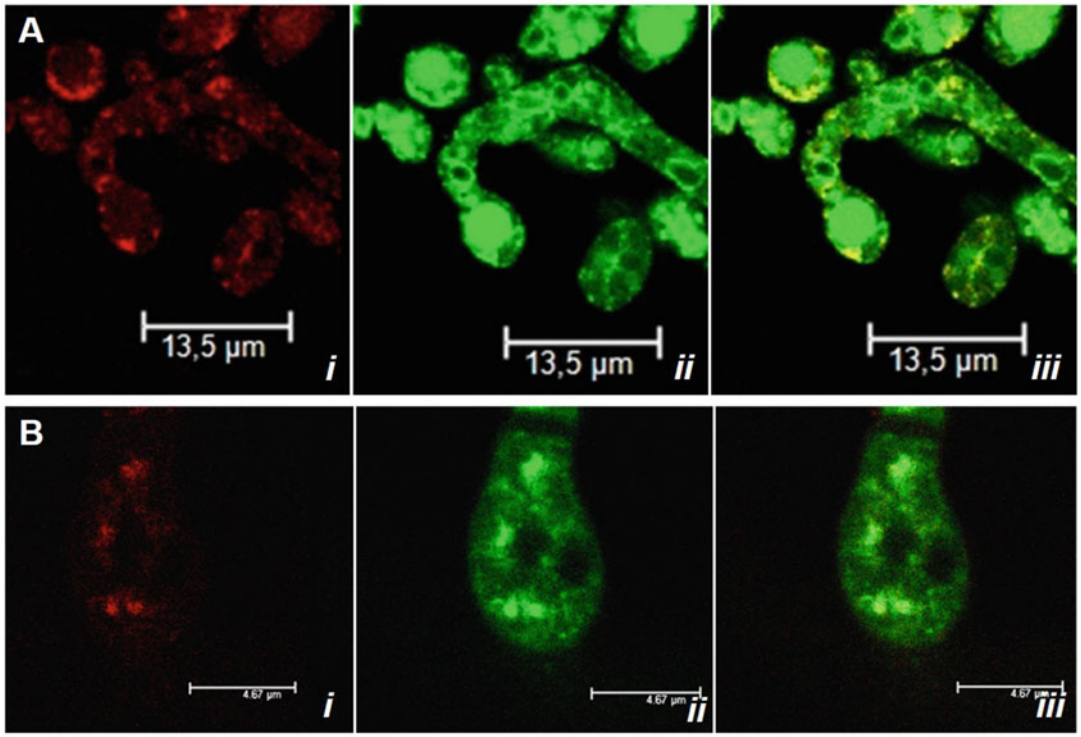


Fig. 2 Confocal laser scanning images of germinating conidiospores co-expressing the PilA::tRFP protein (*i*) with the Bem46::eGFP (**a***ii*) or the Mtr::eGFP (**b***ii*) proteins. (*iii*) merge

IV. Conclusion

The patchwork-like **organisation of the plasma membrane in** several different **micro-domains** leads to a heterogeneous distribution of both lipid and protein components. This heterogeneity does not seem to be random, but accumulation of distinct protein or lipid molecules in distinct micro-domains is **regulated by multiple signalling networks**, involved in diverse developmental, metabolic or signalling pathways. The **unique fungal micro-domain MCC/eisosome** not only provides protection from endocytosis for its lipid and protein components, but it also regulates different processes by accumulating key enzymes separately from the rest of the pathways. Moreover, the MCC/eisosome complex is interconnected with other micro-domains, and it seems to **play a crucial role in the spatial organisation of the plasma membrane.**

Further investigations on eisosomal localised proteins in different developmental stages could allow us to connect the MCC/eisosome complex as a regulator to diverse signalling and/or regulatory networks. In addition, based on its involvement in polar growth, pathogenicity and stress response, a complete discovery of MCC/eisosome formation will deepen our understanding of these processes.

References

Alvarez FJ, Douglas LM, Konopka JB (2009) The Sur7 protein resides in punctate membrane subdomains and mediates spatial regulation of cell wall synthesis in Candida albicans. Commun Integr Biol 2:76–77

Athanasopoulos A, Boleti H, Scazzocchio C, Sophianopoulou V (2013) Eisosome distribution and localization in the meiotic progeny of Aspergillus nidulans. Fungal Genet Biol 53:84–96. https://doi.org/10.1016/j.fgb.2013.01.002

Athanasopoulos A, Gournas C, Amillis S, Sophianopoulou V (2015) Characterization of AnNce102 and its role in eisosome stability and sphingolipid biosyn-

thesis. Sci Rep 5:15200. https://doi.org/10.1038/srep15200

Audhya A, Loewith R, Parsons AB et al (2004) Genome-wide lethality screen identifies new PI4,5P2 effectors that regulate the actin cytoskeleton. EMBO J 23:3747–3757. https://doi.org/10.1038/sj.emboj.7600384

Bartlett K, Gadila SKG, Tenay B et al (2015) TORC2 and eisosomes are spatially interdependent, requiring optimal level of phosphatidylinositol 4, 5-bisphosphate for their integrity. J Biosci 40:299–311. https://doi.org/10.1007/s12038-015-9526-4

Cowart LA, Gandy JL, Tholanikunnel B, Hannun YA (2010) Sphingolipids mediate formation of mRNA processing bodies during the heat-stress response of Saccharomyces cerevisiae. Biochem J 431:31–38. https://doi.org/10.1042/BJ20100307

Daleke DL (2003) Regulation of transbilayer plasma membrane phospholipid asymmetry. J Lipid Res 44: 233–242. https://doi.org/10.1194/jlr.R200019-JLR200

Deng C, Xiong X, Krutchinsky AN (2009) Unifying fluorescence microscopy and mass spectrometry for studying protein complexes in cells. Mol Cell Proteomics 8:1413–1423. https://doi.org/10.1074/mcp.M800397-MCP200

Douglas LM, Konopka JB (2014) Fungal membrane organization: the eisosome concept. Annu Rev Microbiol 68:377–393. https://doi.org/10.1146/annurev-micro-091313-103507

Douglas LM, Wang HX, Li L, Konopka JB (2011) Membrane compartment occupied by Can1 (MCC) and eisosome subdomains of the fungal plasma membrane. Membranes (Basel) 1:394–411. https://doi.org/10.3390/membranes1040394

Douglas LM, Wang HX, Konopka JB (2013) The MARVEL domain protein Nce102 regulates actin organization and invasive growth of Candida albicans. MBio 4: e00723-13. https://doi.org/10.1128/mBio.00723-13

Fadri M, Daquinag A, Wang S et al (2005) The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2. Mol Biol Cell 16:1883–1900. https://doi.org/10.1091/mbc.E04-07-0564

Fröhlich F, Moreira K, Aguilar PS et al (2009) A genome-wide screen for genes affecting eisosomes reveals Nce102 function in sphingolipid signaling. J Cell Biol 185:1227–1242. https://doi.org/10.1083/jcb.200811081

Grossmann G, Malinsky J, Stahlschmidt W et al (2008) Plasma membrane microdomains regulate turnover of transport proteins in yeast. J Cell Biol 183:1075–1088. https://doi.org/10.1083/jcb.200806035

Grousl T, Opekarová M, Stradalova V et al (2015) Evolutionarily conserved 5′-3′ exoribonuclease Xrn1 accumulates at plasma membrane-associated eisosomes in post-diauxic yeast. PLoS One 10:1–19. https://doi.org/10.1371/journal.pone.0122770

Hagen DC, McCaffrey G, Sprague GF (1986) Evidence the yeast STE3 gene encodes a receptor for the peptide pheromone a factor: gene sequence and implications for the structure of the presumed receptor. Proc Natl Acad Sci U S A 83:1418–1422. https://doi.org/10.1073/pnas.83.5.1418

Kabeche R, Baldissard S, Hammond J et al (2011) The filament-forming protein Pil1 assembles linear eisosomes in fission yeast. Mol Biol Cell 22:4059–4067. https://doi.org/10.1091/mbc.E11-07-0605

Kabeche R, Roguev A, Krogan NJ, Moseley JB (2014) A Pil1-Sle1-Syj1-Tax4 functional pathway links eisosomes with PI(4,5)P2 regulation. J Cell Sci 127:1318–1326. https://doi.org/10.1242/jcs.143545

Kabeche R, Madrid M, Cansado J, Moseley JB (2015) Eisosomes regulate phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) cortical clusters and mitogen-activated protein (MAP) kinase signaling upon osmotic stress. J Biol Chem 290:25960–25973. https://doi.org/10.1074/jbc.M115.674192

Kamble C, Jain S, Murphy E, Kim K (2011) Requirements of Slm proteins for proper eisosome organization, endocytic trafficking and recycling in the yeast Saccharomyces cerevisiae. J Biosci 36:79–96

Khalaj V, Azizi M, Enayati S et al (2012) NCE102 homologue in Aspergillus fumigatus is required for normal sporulation, not hyphal growth or pathogenesis. FEMS Microbiol Lett 329:138–145. https://doi.org/10.1111/j.1574-6968.2012.02513.x

Kollath-Leiß K, Bönniger C, Sardar P, Kempken F (2014) BEM46 shows eisosomal localization and association with tryptophan-derived auxin pathway in Neurospora crassa. Eukaryot Cell 13: 1051–1063. https://doi.org/10.1128/EC.00061-14

Kumar A, Kollath-Leiß K, Kempken F (2013) Characterization of bud emergence 46 (BEM46) protein: sequence, structural, phylogenetic and subcellular localization analyses. Biochem Biophys Res Commun 438:526–532. https://doi.org/10.1016/j.bbrc.2013.07.103

Kusumi A, Fujiwara TK, Chadda R et al (2012) Dynamic organizing principles of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of singer and Nicolson's fluid-mosaic model. Annu Rev Cell Dev Biol 28: 215–250. https://doi.org/10.1146/annurev-cellbio-100809-151736

Lee J-H, Heuser JE, Roth R, Goodenough U (2015) Eisosome ultrastructure and evolution in fungi, microalgae, and lichens. Eukaryot Cell 14: 1017–1042. https://doi.org/10.1128/EC.00106-15

Lemmon MA (2007) Pleckstrin homology (PH) domains and phosphoinositides. Biochem Soc Symp 74:81. https://doi.org/10.1042/BSS0740081

Li L, Naseem S, Sharma S et al (2015) Flavodoxin-like proteins protect Candida albicans from oxidative stress and promote virulence. PLoS Pathog 11:

e1005147. https://doi.org/10.1371/journal.ppat.1005147
Loibl M, Grossmann G, Stradalova V et al (2010) C terminus of Nce102 determines the structure and function of microdomains in the Saccharomyces cerevisiae plasma membrane. Eukaryot Cell 9: 1184–1192. https://doi.org/10.1128/EC.00006-10
Luo G, Gruhler A, Liu Y et al (2008) The sphingolipid long-chain base-Pkh1/2-Ypk1/2 signaling pathway regulates eisosome assembly and turnover. J Biol Chem 283:10433–10444. https://doi.org/10.1074/jbc.M709972200
Malinsky J, Opekarova M (2016) New insight into the roles of membrane microdomains in physiological activities of fungal cells. Int Rev Cell Mol Biol. https://doi.org/10.1016/bs.ircmb.2016.02.005
Mascaraque V, Hernaez ML, Jimenez-Sanchez M et al (2013) Phosphoproteomic analysis of protein kinase C signaling in Saccharomyces cerevisiae reveals Slt2 mitogen-activated protein kinase (MAPK)-dependent phosphorylation of eisosome core components. Mol Cell Proteomics 12: 557–574. https://doi.org/10.1074/mcp.M112.020438
Mercker M, Kollath-Leiß K, Allgaier S et al (2009) The BEM46-like protein appears to be essential for hyphal development upon ascospore germination in Neurospora crassa and is targeted to the endoplasmic reticulum. Curr Genet 55:151–161
Mochizuki S, Harada A, Inada S et al (2005) The Arabidopsis WAVY GROWTH 2 protein modulates root bending in response to environmental stimuli. Plant Cell 17:537–547. https://doi.org/10.1105/tpc.104.028530
Moor H, Mühlethaler K (1963) Fine structure in frozen-etched yeast cells. J Cell Biol 17:609–628
Moreira KE, Walther TC, Aguilar PS, Walter P (2008) Pil1 controls eisosome biogenesis. Mol Biol Cell 20:809–818. https://doi.org/10.1091/mbc.E08-03-0313
Moreira KE, Schuck S, Schrul B et al (2012) Seg1 controls eisosome assembly and shape. J Cell Biol 198: 405–420. https://doi.org/10.1083/jcb.201202097
Murphy ER, Boxberger J, Colvin R et al (2011) Pil1, an eisosome organizer, plays an important role in the recruitment of synaptojanins and amphiphysins to facilitate receptor-mediated endocytosis in yeast. Eur J Cell Biol 90:825–833. https://doi.org/10.1016/j.ejcb.2011.06.006
Nagarajan VK, Jones CI, Newbury SF, Green PJ (2013) XRN 5′→3′ exoribonucleases: structure, mechanisms and functions. Biochim Biophys Acta 1829: 590–603. https://doi.org/10.1016/j.bbagrm.2013.03.005.XRN
Olivera-Couto A, Aguilar PS (2012) Eisosomes and plasma membrane organization. Mol Genet Genomics 287:607–620. https://doi.org/10.1007/s00438-012-0706-8
Olivera-Couto A, Graña M, Harispe L, Aguilar PS (2011) The eisosome core is composed of BAR domain proteins. Mol Biol Cell 22:2360–2372. https://doi.org/10.1091/mbc.E10-12-1021
Olivera-Couto A, Salzman V, Mailhos M et al (2015) Eisosomes are dynamic plasma membrane domains showing pil1-lsp1 heteroligomer binding equilibrium. Biophys J 108:1633–1644. https://doi.org/10.1016/j.bpj.2015.02.011
Opekarová M, Robl I, Tanner W (2002) Phosphatidyl ethanolamine is essential for targeting the arginine transporter Can1p to the plasma membrane of yeast. Biochim Biophys Acta 1564:9–13
Pastor-Flores D, Schulze JO, Bahí A et al (2016) Lipid regulators of Pkh2 in Candida albicans, the protein kinase ortholog of mammalian PDK1. Biochim Biophys Acta 1861:249–259. https://doi.org/10.1016/j.bbalip.2015.12.016
Reijnst P, Walther A, Wendland J (2011) Dual-colour fluorescence microscopy using yEmCherry-/GFP-tagging of eisosome components Pil1 and Lsp1 in Candida albicans. Yeast 28:331–338. https://doi.org/10.1002/yea
Roche CM, Loros JJ, McCluskey K, Glass NL (2014) Neurospora crassa: looking back and looking forward at a model microbe. Am J Bot 101:2022–2035. https://doi.org/10.3732/ajb.1400377
Seger S, Rischatsch R, Philippsen P (2011) Formation and stability of eisosomes in the filamentous fungus Ashbya gossypii. J Cell Sci 124:1629–1634. https://doi.org/10.1242/jcs.082487
Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. Science 175: 720–731
Singer-Krüger B, Nemoto Y, Daniell L et al (1998) Synaptojanin family members are implicated in endocytic membrane traffic in yeast. J Cell Sci 111(Pt 2):3347–3356
Sivadon P, Peypouquet MF, Doignon F et al (1997) Cloning of the multicopy suppressor gene SUR7: evidence for a functional relationship between the yeast actin-binding protein Rvs167 and a putative membranous protein. Yeast 13:747–761. https://doi.org/10.1002/(SICI)1097-0061(19970630)13:8<747::AID-YEA137>3.0.CO;2-L
Spellman PT, Sherlock G, Zhang MQ et al (1998) Comprehensive identification of cell cycle – regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. Mol Biol Cell 9: 3273–3297
Spira F, Mueller NS, Beck G et al (2012) Patchwork organization of the yeast plasma membrane into numerous coexisting domains. Nat Cell Biol 14:640–648. https://doi.org/10.1038/ncb2487
Strádalová V, Stahlschmidt W, Grossmann G et al (2009) Furrow-like invaginations of the yeast plasma membrane correspond to membrane compartment of Can1. J Cell Sci 122:2887–2894. https://doi.org/10.1242/jcs.051227

Truong-Quang B-A, Lenne P-F (2014) Membrane microdomains: from seeing to understanding. Front Plant Sci 5:18. https://doi.org/10.3389/fpls.2014.00018

Valencik M, Pringle J (1995) Schizosaccharomyces pombe bem1/bud5 suppressor (bem46) mRNA. In: EMBL database. Access number U29892

Vangelatos I, Roumelioti K, Gournas C et al (2010) Eisosome organization in the filamentous ascomycete Aspergillus nidulans. Eukaryot Cell 9: 1441–1454. https://doi.org/10.1128/EC.00087-10

Vaskovicova K, Stradalova V, Efenberk A et al (2015) Assembly of fission yeast eisosomes in the plasma membrane of budding yeast: import of foreign membrane microdomains. Eur J Cell Biol 94:1–11. https://doi.org/10.1016/j.ejcb.2014.10.003

Walther TC, Brickner JH, Aguilar PS et al (2006) Eisosomes mark static sites of endocytosis. Nature 439:998–1003. https://doi.org/10.1038/nature04472

Walther TC, Aguilar PS, Fröhlich F et al (2007) Pkh-kinases control eisosome assembly and organization. EMBO J 26:4946–4955. https://doi.org/10.1038/sj.emboj.7601933

Wang HX, Douglas LM, Aimanianda V et al (2011) The Candida albicans Sur7 protein is needed for proper synthesis of the fibrillar component of the cell wall that confers strength. Eukaryot Cell 10: 72–80. https://doi.org/10.1128/EC.00167-10

Wang HX, Douglas LM, Vesela P et al (2016) Eisosomes promote the ability of Sur7 to regulate plasma membrane organization in Candida albicans. Mol Biol Cell 27:1663–1675. https://doi.org/10.1091/mbc.E16-01-0065

Whelan WL, Gocke E, Manney TR (1978) The CAN1 locus of Saccharomyces cerevisiae: fine-structure analysis and forward mutation rates. Genetics 91:35–51

Yadeta KA, Elmore JM, Coaker G (2013) Advancements in the analysis of the Arabidopsis plasma membrane proteome. Front Plant Sci 4:86. https://doi.org/10.3389/fpls.2013.00086

Young ME, Karpova TS, Brügger B et al (2002) The Sur7p family defines novel cortical domains in Saccharomyces cerevisiae, affects sphingolipid metabolism, and is involved in sporulation. Mol Cell Biol 22:927–934. https://doi.org/10.1128/mcb.22.3.927-934.2002

Zhao H, Michelot A, Koskela EV et al (2013) Membrane-sculpting BAR domains generate stable lipid microdomains. Cell Rep 4:1213–1223. https://doi.org/10.1016/j.celrep.2013.08.024

Ziółkowska NE, Karotki L, Rehman M et al (2011) Eisosome-driven plasma membrane organization is mediated by BAR domains. Nat Struct Mol Biol 18:854–856. https://doi.org/10.1038/nsmb.2080

The Genus *Periglandula* and Its Symbiotum with Morning Glory Plants (Convolvulaceae)

E. Leistner[1], U. Steiner[2]

CONTENTS

I. The Ecological Role of Natural Products

Microorganisms and plants have one thing in common: both are frequently equipped with an elaborate biosynthetic machinery responsible for the formation of an almost unlimited variety of natural products. Typically, natural products—which are also called secondary metabolites—are characteristic of a limited amount of microbial or plant taxa, e.g., an order, a family, a species, or even a subspecies only. Many of the natural products exhibit physiological activities which is the basis for their use in medical applications (Clardy and Walsh 2004).

The high physiological activities of many natural products had triggered a now historical dispute about the role of natural products in the producing organism. It was proposed that "the multiplicity of natural products is caused by random processes of mutations, i.e. it reflects the gambling of nature rather than a sophisticated strategy" (Mothes 1981; Mothes et al. 1985).

This hypothesis, however, neglects the possibility that mutations may turn out to be detrimental or advantageous to the mutated organism. In the former case, a mutated organism may be eliminated or in the latter case benefit from an increased fitness and a better chance to survive in a certain ecological setting (Zenk 1967). Today the ecological role of natural products is well accepted in the scientific community (Harborne 2004; Eisner 2003; White Jr et al. 2003).

Natural product research entered a new era when it was discovered that plants and fungi elaborated during evolution, another way to acquire natural products. They may not only be formed in biosynthetic processes by one particular organism itself, but instead, a host organism may harbor a natural product-producing microorganism: A plant may be associated with a bacterium (Piel 2004; Strobel et al. 2004; Gunatilaka 2006) or a fungus (Strobel et al. 2004; Gunatilaka 2006), while a fungus may harbor a bacterium (Partida-Martinez and Hertweck 2005).

[1] Institut für Pharmazeutische Biologie, Rheinische Friedrich Wilhelm-Universität Bonn, Bonn, Germany; e-mail: eleistner@uni-bonn.de
[2] Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES), Rheinische Friedrich Wilhelm-Universität Bonn, Bonn, Germany; e-mail: u-steiner@uni-bonn.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

In such associations both organisms may form a symbiotum in which the associated microorganism benefits by receiving nutrients, protection, reproduction, and dissemination, whereas the host takes advantage of physiologically active compounds which may promote plant growth, herbivore deterrence, and/or increased fitness (Arnold et al. 2003; White Jr et al. 2003; Saikkonen et al. 2004).

Symbiota and their contained natural products play a decisive role in evolution as all species evolve in interactions with other species (Saikkonen et al. 2015). A point in case is the beneficial activity of ergot (syn. ergoline) alkaloids which are products of clavicipitaceous fungi colonizing monocotyledonous plants like Poaceae, Juncaceae, and Cyperaceae (Clay and Schardl 2002; White Jr et al. 2003; Schardl et al. 2006). Ergot alkaloids, however, are also present in higher dicotyledonous plants of the family Convolvulaceae (Hofmann 1961, 2006). This disjointed occurrence of a group of natural products in evolutionarily unrelated taxa (fungi and Convolvulaceae plants) seemed to contradict the generally accepted principle of chemotaxonomy that similar or even identical natural products are present in related taxa. It was therefore assumed that during evolution, a horizontal transfer of genes responsible for ergoline alkaloid biosynthesis might have occurred from fungi to higher plants (Groeger and Floss 1998; Tudzynski et al. 2001; Clay and Schardl 2002). Alternatively, it was discussed that ergoline alkaloid biosynthesis was repeatedly invented during evolution (Mothes et al. 1985). In a recent review in this series, Keller and Tudzinsky (2002) dealt with the pharmacological aspects, biochemistry, genetics, and biotechnology of ergot alkaloids in fungi associated with Poaceae. We show in the present review that neither the horizontal transfer of genes encoding the ergot alkaloid biosynthesis nor the repeated invention of a rather complicated biosynthetic pathway took place during evolution but rather that clavicipitaceous fungi not only live on different grasses but also colonize dicotyledonous plants of the family Convolvulaceae (Kucht et al. 2004; Steiner et al. 2006; Ahimsa-Mueller et al. 2007; Markert et al. 2008; Steiner et al. 2008, 2011; Beaulieu et al. 2015). This indicates that ergot alkaloids are components in a fungus/plant symbiotum characterized by mutual defense and interaction which constitutes a driving force for evolutionary processes (Saikkonen et al. 2015, Schardl et al. 2013).

II. The Symbiosis Between Poaceae and Clavicipitaceous Fungi

A rather well-investigated experimental system consists of clavicipitaceous fungi colonizing Juncaceae, Cyperaceae, and Poaceae plants. In these symbiota ergoline alkaloids play an important role (Keller and Tudzynski 2002). The symbiotic fungi belong either to the tribe Clavicipeae or Balanseae within the family Clavicipitaceae (Bacon and Lyons 2005). The morphological associations of the fungi with grasses occur either epicuticular, epibiotic, or endophytic (Bacon and Lyons 2005). In epiphytic growth the fungal mycelium is concentrated on the surface of young leaves, buds, meristematic regions, and reproductive structures (Clay and Schardl 2002). The association between fungi and their plant hosts is likely to be an example of host-symbiont codivergence (Schardl et al. 2008).

The fungus may be asexual belonging to the group of fungi imperfecti and shows a sexual lifestyle or switch between sexual and asexual propagation. In the sexual lifestyle, fungi parasitize a wide range of grasses where they form infections of single grass florets and replace the seed with individual sclerotia (Clay and Schardl 2002).

The asexual fungi are vertically transmitted through seeds. They have never been known to produce infectious spores and rely entirely on seed transmission. Especially the asexual fungi exhibit high host specificity. Most interesting, sexual and asexual fungi may interact in parasexual processes contributing to a high diversity of fungal asexual endophytes (Tsai et al. 1994).

In general, grasses are poor producers of natural products that assist other plants in their long-term strategy to gain an ecological advantage. Grasses, however, have the ability to compensate for this deficiency by acquiring fungi notorious for their poisonous natural products. In some cases fungi can be considered the livestock of grasses.

Fungi associated with plants may produce different classes of alkaloids among which toxic ergot alkaloids are an important group (Schardl et al. 2004, 2007). The main ecological roles of ergot alkaloids in nature are probably to protect the fungi from consumption by vertebrate and invertebrate animals (Schardl et al. 2006). Ergot alkaloids benefit the fungus by protecting the health and productivity of the host (Schardl et al. 2006). Other benefits include growth of the plant, competitive abilities, and resistance to drought (Malinowski and Belesky 2000), pests, and fungal pathogens (Brem and Leuchtmann 2002; White Jr et al. 2003). In some cases, clavicipitaceous fungi are culturable in vitro (Keller and Tudzynski 2002). This allowed to identify the fungus as the producer of ergot alkaloids and revealed that the host plant is not the site of ergot alkaloid biosynthesis.

It was therefore somewhat unexpected when Hofmann (1961, 2006) found that dicotyledonous plants belonging to the family Convolvulaceae contained ergot alkaloids and that these alkaloids were responsible for the hallucinogenic properties enjoyed by Meso- and South American Indians in religious ceremonies (Schultes and Hofmann 1992).

The idea that a fungus could be responsible for the alkaloid occurrence was discussed, but no evidence for the presence of such a fungus was found (Hofmann 2006). This seemed to be in agreement with the notion that plant tissue cultures which are believed to be germfree, i.e., devoid of any microbes, were reported to produce ergot alkaloids (Dobberstein and Staba 1969), a report which, however, remained unsubstantiated (Kucht et al. 2004; Steiner et al. 2006).

III. Epibiotic Clavicipitaceous Fungi Associated with Convolvulaceae

A. Identification of Genus *Periglandula*

1. Microscopic and Electron Microscopic Characterization

The infestation of the clavicipitaceous fungi on *Ipomoea asarifolia* and *Turbina corymbosa*, members of the family Convolvulaceae, is systemic. Evidence of systemic infection came from demonstrations that the fungi are seed transmitted, that surface-sterilized seeds grown in vitro and under germfree conditions result in plantlets which are colonized exclusively by the respective clavicipitaceous fungi, and that they are transmitted through vegetative propagation (Steiner et al. 2008). It is an unusual type of systemic infection under the aspect that there are no signs of penetration into the host tissue, but the growth on the host plants is superficial. Attempts made to visualize the fungus within the stem and leaf tissue, using methodologies commonly employed to detect endophytes in grasses (Bacon and White Jr 1994), were not successful. Up to now the fungi proved to be non-detectable using these procedures. Among the Clavicipitaceae, *Atkinsonella hypoxylon*, *Balansia cyperi*, *B. pilulaeformis*, and *Myriogenospora atramentosa* are examples of epibiotic species that grow on meristematic tissues of host plants (Leuchtmann and Clay 1988, 1989; Luttrell and Bacon 1977; Rykard et al. 1985; Clay and Frentz 1993). The clavicipitaceous fungi colonizing members of the Convolvulaceae inhabit an epibiotic niche and thus seem most comparable to the epibiotic members of the grass borne Clavicipitaceae. The mutualistic endophyte *Epichloe typhinum* (formerly *Neotyphodium typhinum*) also forms a stable external mycelial net on the leaves of the host plant (Moy et al. 2000). This suggested a possible alternative pathway of fungal dispersal and transmission to hosts, i.e., through epiphyllously produced conidia.

The clavicipitaceous fungi form colonies on the upper surfaces of young unfolded leaves

which are visible to the naked eye as shown for *T. corymbosa* (Fig. 1A) as well as for *Ipomoea asarifolia*. On *T. corymbosa* colony distribution mainly follows the veins of the leaves (Fig. 1A, B), in contrast to the distribution on *I. asarifolia* which is more random. These colonies differ in size and mycelium density, and depending on the developmental stage, the fungi produce synnemata-like structures. No stromata with perithecia and ascospores were detected in the mycelium mats. Maybe the environmental conditions are not suitable for the development of the sexual stage of the fungi, that they lost the ability to reproduce sexually or that the mating type is lacking. On the lower (abaxial) side of the leaves, no mycelium was detected. Visual inspection of leaf buds when opened by manipulation showed that the fungus was well established as dense white mycelial layers on the adaxial leaf surfaces of both plants *I. asarifolia* and *T. corymbosa* at this early stage of leaf development. The mycelium is formed by tightly packed hyphae in the cavity between the leaf halves (Fig. 1C). Sections through colonized tissue revealed that fungal mycelium was entirely superficial. The hyphae, measured approx. 1.5 µm across, were hyaline, thin-walled, and septated. Chlamydospore-like structures and synnemata are produced (Steiner et al. 2011). Indicated by the intense mycelium development, the space between the upper surfaces of folded leaves probably offers a refuge of protection to the fungus. As leaves expand and mature, the hyphae are evident as isolated only microscopically visible clumps, often near or around peltate glandular trichomes, and the ends of the hyphae often appeared broken (Fig. 1D).

The epibiotic fungi of *I. asarifolia* (Steiner and Leistner 2012; Steiner et al. 2015) and *T. corymbosa* (Fig. 1D, E) are closely associated with the secretory glands on the adaxial leaf surface, an anatomic feature which may be essential for the ergot alkaloid biosynthesis in the epibiotic fungus/plant association (Steiner et al. 2006, 2008, 2015). In cell cultures which harbor the fungus, no ergot alkaloids are synthesized and no secretory glands are developed.

Members of the Convolvulaceae like *I. asarifolia* and *T. corymbosa* formed peltate glandular trichomes (Fig. 1E), which consist of one basal cell, one stalk cell, up to eight glandular secretory cells, and a subcuticular oil storage cavity that is derived from the cuticle of the secretory cells. Metabolites are released after rupture of the cuticle. As indicated by staining with the lipophilic dye Nile red and chemical analysis, these specialized structures contain essential oils and palmitic acid (Kucht et al. 2004; Steiner et al. 2015). The secretory glands and their specific metabolites may be the basis of a metabolic dialogue between the fungus and the plant (Steiner et al. 2008, Steiner et al. 2015). The fungi may feed on the volatile oil and derive precursors like terpenes and fatty acids for the ergot alkaloid biosyntheses from the oil. The fungi inhabit the epibiotic niche of glandular cells on the upper surface of leaves. This observation is supported by showing hyphae of the clavicipitaceous fungus on *T. corymbosa* outside of the subcuticular oil storage cavity as well as inside of this compartment embedded in an electron dense matrix (Fig. 1F). The localization of mycelium with glandular cells ensures the close association of the fungus with the host tissues. A continuous maintenance of the symbiotic relationship requires that the fungus derives energy from the host plant. In clavicipitaceous epibiotic fungi, substrate utilization depends on the availability of organic material from the waxy cuticle covering the plant surface and exuded compounds, lipids, amino acids, and vitamins. The main energy-yielding compounds are simple sugars that in the case of endophytic mycelia are derived from the apoplasm through intercellular fungal hyphae (White and Morgan-Jones 1996). In clavicipitaceous fungi present on *I. asarifolia* and *T. corymbosa*, superficial fungal hyphae with tip enlargements tightly adherent to the glandular cells as well as to the cuticle have been observed (Steiner et al. 2015). It seems reasonable to postulate a selective and efficient exchange of metabolites between fungus and plant.

Physiological changes paralleled by morphological adaptations of the host have been described for some endophytic associations (Bacon and White Jr 2000). In *M. atramentosa*, plant host changes in the epidermal cell size and shape suggest the activity of growth regu-

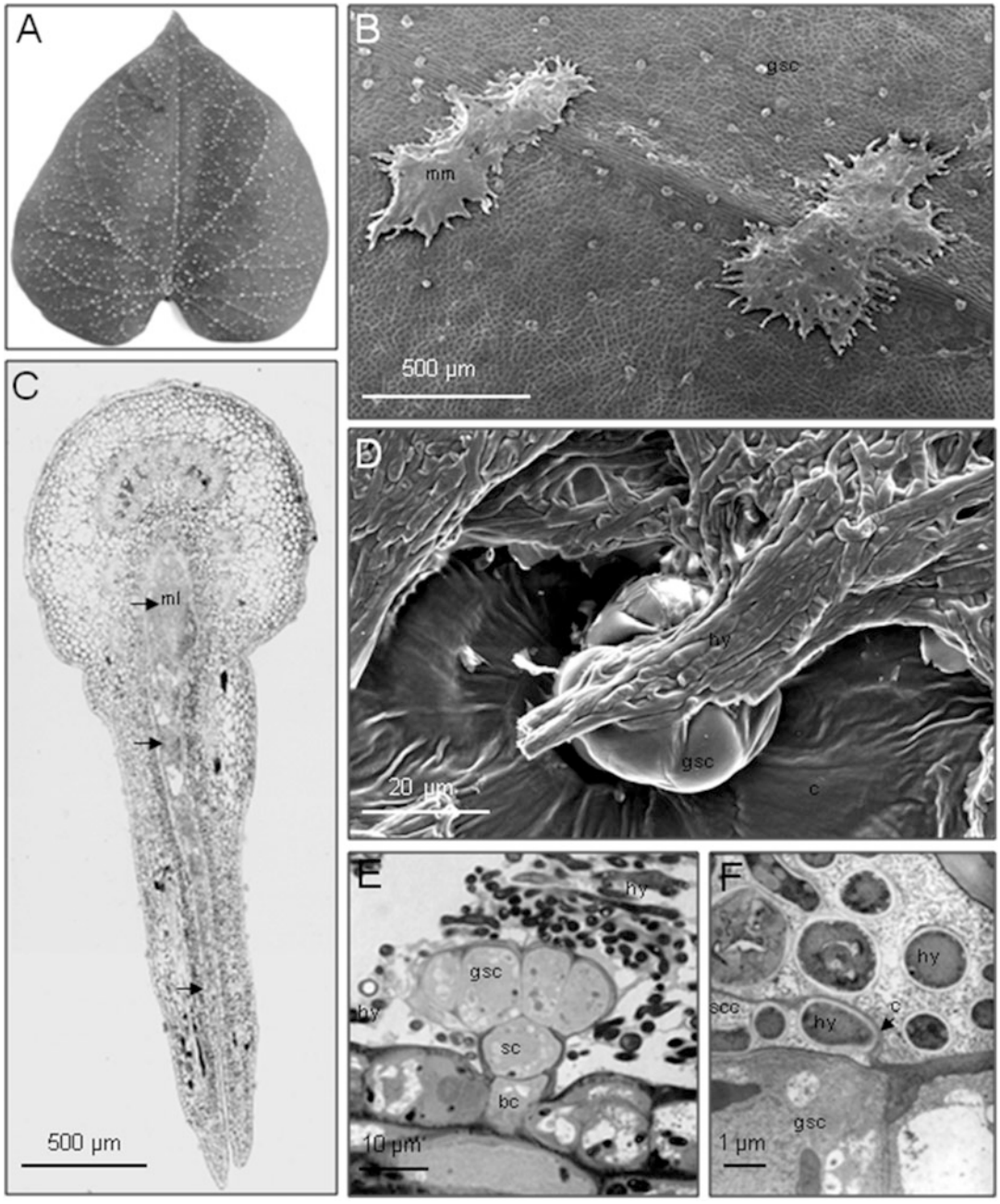


Fig. 1 Colonization of *Turbina corymbosa* with the clavicipitaceous fungus *Periglandula turbinae*: (**A**) Colonies formed by white mycelium on the adaxial surface of a young unfolded leaf. Preferential development on the veins is visible with the naked eye. (**B**) Aggregated hyphae differentiating typical mycelium mats (mm) consisting of several layers which cover leaf areas with peltate glandular trichomes and are adhered to the cuticle. (**C**) Cross-section of a folded leaf bud showing that the fungus is well established on the adaxial leaf surfaces at this early stage of plant development. The mycelium is formed by tightly packed hyphae as a mycelium layer (ml, arrows) in the cavity between the halves of the leaf. (**D**) Close association of secretory cells (gsc) on the adaxial leaf surface with hyphae (hy) which often encircle the peltate glandular trichomes of the plants. (**E**) Cross-section of a peltate glandular trichome composed of

latory substances which are either produced by the fungus or secreted into the host or that are produced by the host in response to the fungal symbiont (Bacon and White Jr 2000). The epiphytic proliferation of hyphae on the cuticle may be additionally enabled through degradation of the cuticular layers of the leaf surfaces. Previous ultrastructural studies of the host-fungus interfaces of the clavicipitaceous fungi on *I. asarifolia* and *T. corymbosa* revealed progressive cuticular disintegration. Substrate utilization studies showed that epiphytic *Atkinsonella hypoxylon* possess the capacity to colonize and degrade paraffin wax droplets (White Jr et al. 1991). *A. hypoxylon* grows superficially on young leaves of grasses as an epiphyte, perhaps degrading wax in the cuticle to obtain nutrients for epiphytic growth (White Jr et al. 1991). Leaves and inflorescence primordia within the stroma never develop a cuticular layer that would impede flow of nutrients and moisture to the fungus. Through these modifications of the host tissues, the endophyte removes barriers to nutrient flow into the mycelium. Very similar to this situation, the cuticle covering the glandular cells of the Convolvulaceae appears thinner and therefore more permeable than the cuticle on epidermal cells (Steiner et al. 2015).

Clavicipitaceous fungi have evolved to survive as saprophytes, degrading organic material, as well as biotrophs of plants, fungi, nematodes, and insects. They are described to have become particularly successful as endophytes and epibionts of grasses. The association between clavicipitaceous fungi and their hosts constitutes unique biotrophic symbioses where the stages of physiological adaptation to the plant host may yield an understanding of how evolution among these fungi and their hosts (Schardl et al. 2008, 2013; Young et al. 2015) has progressed. With the detection of *Periglandula* species on Convolvulaceae able to synthesize ergot alkaloid known to play a role in enhanced resistance to diseases, pests, and tolerance to drought, it is shown that such association has not only in grass hosts but also in dicots evolutionary value. The colonization of a unique plant niche, the clavicipitaceous fungi on Convolvulaceae, represents a novel finding among beneficial plant-fungus symbioses in non-graminaceous plants.

2. Phylogenetic Trees and Taxonomy

Among a broad spectrum of plant-associated fungi isolated from the plant *I. asarifolia*, only the epiphytic leaf-associated fungus belonged to the family Clavicipitaceae within the order Hypocreales (Steiner et al. 2006). Conventional techniques to continuously cultivate the epiphytic fungi from *I. asarifolia* or *T. corymbosa* on synthetic media usually supporting fungal growth turned out to be negative indicating that the leaf material contains factors or structures essential for a prolonged growth of the fungus. On potato dextrose agar, a very limited growth which soon discontinued after inoculation was observed, and some morphological features such as synnemata and chlamydospore-like structures were noted (Steiner et al. 2011). A morphological differentiation of fungi on *I. asarifolia* from those on *T. corymbosa* was not possible. All experiments to characterize these fungi in terms of taxonomy are therefore based on molecular biological techniques (Steiner et al. 2006, 2011).

Removal of fungal mycelium from the leaf surface of convolvulaceous plants was inter alia possible by ultrasonic treatment. This method gave access to extraction and analysis of DNA derived from the externally plant-associated mycelium (Markert et al. 2008). One of the important observations was detection of the whole set of genes necessary for the biosynthesis of ergot alkaloids in the fungus present on *I. asarifolia* which was highly suggestive of the presence of a clavicipitaceous fungus (Markert

Fig. 1 (continued) basal cell (bc), stalk cell (sc), and secretory cells (gsc) showing the epiphytic development of mycelium embedded in a mucilage matrix concentrated on the cuticle over a subcuticular oil storage cavity. (**F**) Electron microscopic view of secretory cells with hyphae outside and inside of the subcuticular oil storage cavity (scc) bordered by the cuticle (c, arrow). No evidence for direct penetration of the plant cells is visible

et al. 2008; Schardl et al. 2013). In-depth investigation of the enzyme (DmaW) initiating the biosynthetic pathway leading to ergot alkaloids provided further evidence for the clavicipitaceous nature of the epibiotic fungus on *I. asarifolia* (Markert et al. 2008).

Construction of phylogenetic trees has been repeatedly and successfully employed in the systematic classification of grass borne clavicipitaceous fungi (Spatafora and Blackwell 1993; Glenn et al. 1996; Kuldau et al. 1997; Reddy et al. 1998; Lewis et al. 2002; Bischoff and White Jr 2005; Sung et al. 2007).

Sequencing of various genes and construction of phylogenetic trees from 18S rDNA, internal transcribed spacer, and 4-[γ,γ-dimethylallyl]tryptophan synthase (DmaW) catalyzing the committed step in ergot alkaloid biosynthesis pointed to a clavicipitaceous origin of the fungus on *I. asarifolia* (Ahimsa-Mueller et al. 2007). Essentially the same results were observed when the fungi associated with *I. asarifolia* (red variety), *T. corymbosa*, and *Ipomoea tricolor* were investigated (Ahimsa-Mueller et al. 2007; Leistner and Steiner 2009) indicating that our observations are not restricted to the white blooming *I. asarifolia* plant and its associated fungus but are of a broader significance. The fungi formed a monophyletic clade within the family Clavicipitaceae in PCR-generated partial sequences of B-tubulin (*tubB*) , RNA polymerase II large subunit (*rpbA*), and mitochondrial ATP synthase subunit 6 (*Atp6*) (Steiner et al. 2011). Based on these observations, they were placed into a new genus which was named *Periglandula* with reference to its close association of fungal hyphae with peltate glandular trichomes (Fig. 2A; Steiner et al. 2006; Steiner and Leistner 2012).

In addition, these experiments showed that the fungi on *T. corymbosa* and *I. asarifolia* could be distinguished by the sequences from *rpbA*, *Atp6*, γ-actin (*atgG*), translation elongation factor 1-alpha (*tefA*), and the 4-[γ,γ-dimethylallyl] tryptophan synthase gene (*dmaW*). Thus, the fungi on *T. corymbosa* and *I. asarifolia* belong to the same family, Clavicipitaceae, and the same genus, *Periglandula*, but are not identical in every aspect. They clearly differ not only in their host specificity and their alkaloid spectra (Ahimsa-Mueller et al. 2007) but also in the *Atp6*, *actG*, *dmaW*, *rpbA*, and *tefA* genes. All sequences obtained from the fungus associated with the red blooming *I. asarifolia* plant were identical to those from the fungus living on the white blooming host variety. Only the *rpbA* sequences of both fungi living on the two plant varieties of *I. asarifolia* differed in one base pair of the *rpbA* gene (Steiner et al. 2011). This, however, cannot be taken as evidence for the presence of two different fungal species.

The fungi on *I. asarifolia* and *T. corymbosa* are considered separate species and named *Periglandula ipomoeae* U. Steiner, E. Leistner et Schardl and *Periglandula turbinae* U. Steiner, E. Leistner et Schardl, respectively. Since the red blooming *Ipomoea asarifolia* plant is associated with a fungus which by molecular techniques cannot be discerned from the one on the white blooming *I. asarifolia* plant, the former was also named *Periglandula ipomoeae* U. Steiner, E. Leistner et Schardl (Steiner et al. 2011).

While *I. asarifolia* and *T. corymbosa* are New World tropical vines, eight Convolvulaceae plants from Asia, Australia, Africa, and North America were recently demonstrated to be ergot alkaloid positive (Beaulieu et al. 2015). The alkaloid content coincided in each case with the presence of a leaf-associated epibiotic fungal *Periglandula* symbiont. A phylogenetic tree calculated from sequences of the translation elongation factor 1-alpha (*tefA*) showed a monophyletic clade with the above-described *Periglandula* fungi *P. ipomoeae* and *P. turbinae* hosted by *I. asarifolia* and *T. corymbosa*, respectively (Fig. 3). The newly described fungi (Beaulieu et al. 2015) fall into four different chemotypes. It may be an intriguing question how this relates to the architecture and evolution of ergot alkaloid biosynthetic gene clusters within the genus *Periglandula*.

B. Seed Transmittance of Epibiotic Fungi Colonizing Convolvulaceae

A freshly harvested and surface-sterilized seed grown under germfree conditions gives a plant

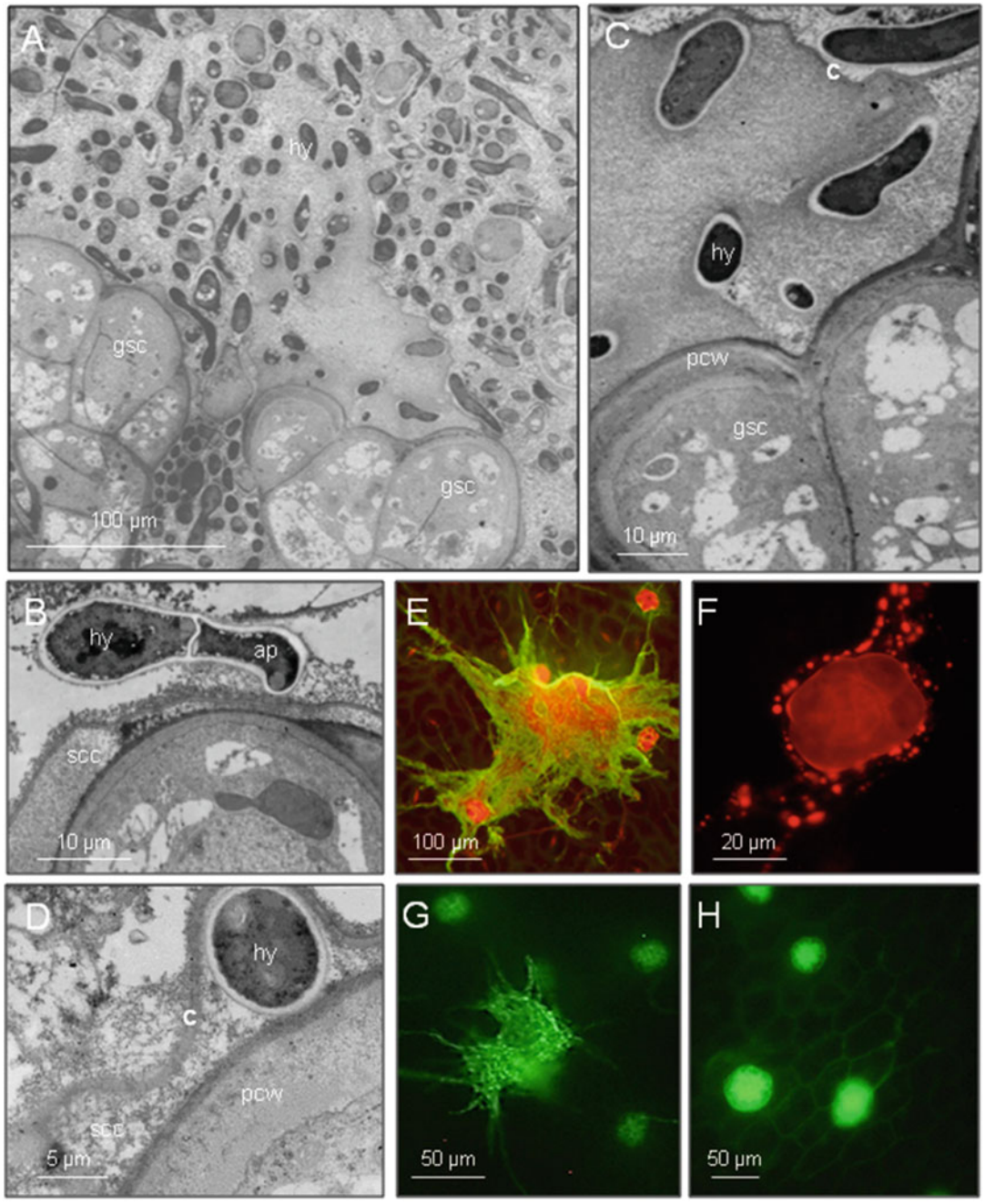


Fig. 2 The glandular trichome as interface of the plant fungus symbiotum *Periglandula/Ipomoea:* (**A**) Mycelium encircle glandular secretory cells (gsc) embedded in a dense matrix. (**B**) Contact site of hyphae (hy) forming an appressorium-like structure (ap) on the cuticle (c) of the secretory cell covering the subcuticular oil storage cavity (scc). (**C**) Hyphae colonizing the outer side of the cuticle and the subcuticular cavity after disruption of the cuticle; (**D**) alteration in the structure of the plant cell wall (pcw) at the contact site with the fungus; accumulation of mitochondria, membrane systems, and ribosomes as indicators of high metabolic activity at the plant/fungus contact site; (**E**) release of lipids (stained with Nile red) by secretory glandular cells encircled by the fungus (stained green with WGA); (**F**) lipid vacuoles (stained with Nile red) in hyphae encircle glandular cell; (**G**) uptake of 6(5)carboxyfluorescein diacetate (green fluo-

colonized by the epibiotic clavicipitaceous fungus. This plant contains ergot alkaloids. The epibiotic fungus is the only fungus that is detectable by SSCP on this particular plant. Such a fungus is detectable in seeds of *Ipomoea asarifolia* and *I. violacea* (Steiner et al. 2006; Ahimsa-Mueller et al. 2007). This shows that the fungus is seed transmitted and points to the host specificity typical of asexual clavicipitaceous fungi (see below).

The viability of the seed-transmitted fungus very likely is limited and depends on age and storage (Schardl 1994) as well as moisture and storage temperature (Welty et al. 1987) of the seed. An *Ipomoea violacea* plant devoid of ergot alkaloids derived from an alkaloid and clavicipitaceous fungus containing seed has recently been described (Ahimsa-Mueller et al. 2007). In this particular case, the viability of the seed exceeds the viability of the inhabiting fungus. This may be a reason for contradictory reports on the occurrence of ergot alkaloids in the seeds of *Ipomoea* species (Eich 2008).

It follows that the presence or absence of ergot alkaloids in a convolvulaceous plant or seed may be an unsuitable character for taxonomic classifications, but instead the ability of a plant taxon to host an ergot alkaloid-producing clavicipitaceous fungus should be the character in question.

C. Plant Growth Under Germfree Conditions

The notion that fungicides eliminate ergot alkaloids from the plant was a clear indication that ergot alkaloids in Convolvulaceae plants are of fungal origin (Kucht et al. 2004). This observation was somewhat unusual because it had been reported that ergot alkaloids are produced by plant cell cultures established from different Convolvulaceae plants (Dobberstein and Staba 1969). Plant cell cultures are usually germfree; they should not contain any microbes and can therefore be used as a test system to probe the biosynthetic capacities of plant cells.

Numerous attempts, however, to reproduce this result (Dobberstein and Staba 1969) and to find a plant cell culture raised from *I. asarifolia*, *T. corymbosa*, and *Ipomoea tricolor* (L) (Convolvulaceae) showing ergot alkaloid production were unsuccessful in our hands (Hussein 2004; Kucht et al. 2004). Indeed, thin-layer chromatography combined with vanUrk's spray reagent were used by Dobberstein and Staba (1969) to detect ergot alkaloids, techniques which are of limited reliability in the identification of natural products (Jenett-Siems et al. 1994, 2004; Kucht et al. 2004).

Again, it was a surprise when we found that the epibiotic fungus lived together with the plant cells in the callus and cell suspension culture. Microscopic examination, single-strand conformation polymorphism (SSCP), and sequencing of the internal transcribed spacer revealed the presence in the cell culture of the epibiotic fungus *P. ipomoeae* previously detected on the leaf surface of *I. asarifolia*. Other endophytic fungi which had been isolated from the intact *I. asarifolia* plants were not detectable by SSCP within the callus and cell suspension culture (Steiner et al. 2006).

When a callus culture was subjected to a new hormone regime (the amount of benzylaminopurine was lowered from 2 mg/l to 0.01 mg/l), a plantlet regenerated from the callus. This plantlet was colonized by the fungus and contained ergot alkaloids (Steiner et al. 2006, 2008).

These observations show also that an intact *I. asarifolia* plant colonized by the fungus *P. ipomoeae* is required for the successful synthesis of ergot alkaloids and gives an idea about the extreme specificity between the epibiotic fungus and the *I. asarifolia* plant (Steiner et al. 2008). It is in line with these conclusions that we were hitherto unable to grow the fungi *P. ipomoeae* or *P. turbinae* in vitro (Steiner et al. 2006). Apparently the plant contains some kind

Fig. 2 (continued) rescence) into the mycelium of the fungus and into glandular trichomes; (**H**) epidermal cells remained unstained (modified from Steiner et al. 2015)

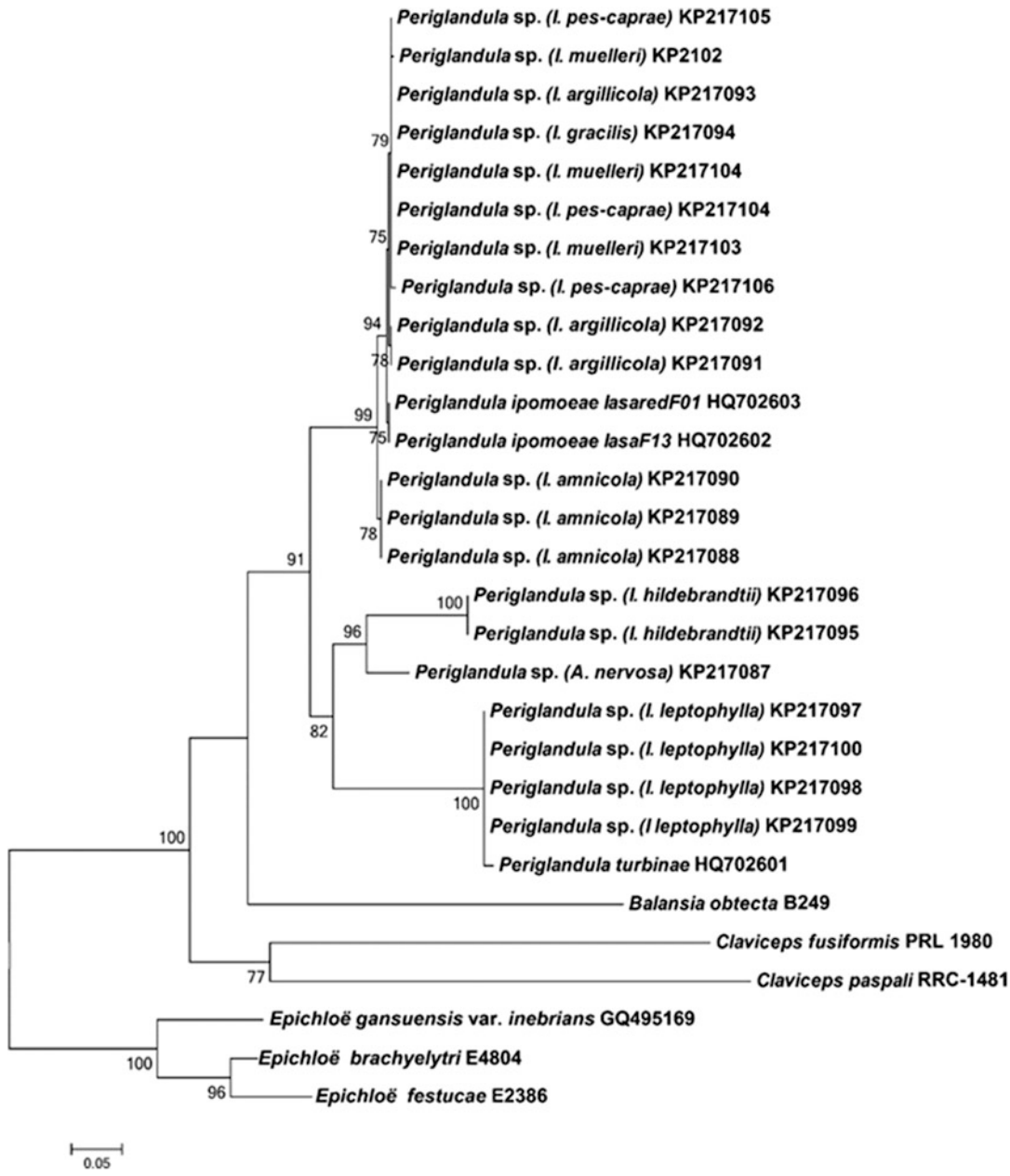


Fig. 3 Phylogenetic diversity of *Periglandula* species (Clavicipitaceae) forming a symbiotum on 11 different morning glory hosts (Convolvulaceae). The plants occur on four different continents and comprise mainly tropical and subtropical vines but also shrub growth forms (e.g., *T. corymbosa*). The fungi form a monophyletic group within the Clavicipitaceae. The maximum likelihood phylogeny was calculated from sequences of the translation factor 1-gene (*tefA* locus). Node confidence values (calculated with the approximate likelihood ratio) greater than 75% are shown. The branch lengths are in the units of number of base substitutions per site. Taxon names are followed by the strain ID and when applicable GenBank accession number. *Periglandula* spp. from new host species in the Convolvulaceae are listed with their host plant's name (Beaulieu et al. 2015; reprinted with permission from Mycologia. ©The Mycological Society of America)

of component essential for fungal growth. The specificity between the plant and its associated fungus is also evident from the fact that different plant taxa within the Convolvulaceae (e.g., *Ipomoea* or *Turbina*) are colonized by related but different clavicipitaceous fungi (Ahimsa-Mueller et al. 2007; Beaulieu et al. 2015).

This raises the question as to how the specificity between the fungus and the host plant is brought about (Steiner et al. 2008). Interesting, the fungus apparently has a very high affinity to the secretory glands on the adaxial leaf surface (Kucht et al. 2004; Steiner and Leistner 2012). This seems to be unusual because essential oils may have an antifungal activity (Chang et al. 2008). It is conceivable that during evolution, clavicipitaceous fungi were able to overcome this barrier and to take advantage of oil components using these compounds as mediators of specificity and even as substrates to feed upon.

The volatile oil of *I. asarifolia* consists of many minor but five major components, the latter of which are sesquiterpenes (Kucht et al. 2004) and palmitic acid (Steiner et al. 2015). Sesquiterpenes play an important role in ecological interactions between plants and insects (Schnee et al. 2006; Gershenzon and Dudareva 2007). Our observations raise the question if this class of terpenoids is also essential for the interaction between different Convolvulaceae species and their associated clavicipitaceous fungi.

D. Biosynthesis and Accumulation of Ergot Alkaloids in the Fungus/Plant Symbiotum

Ergot alkaloids are natural products of high physiological activity. They are described to confer drought resistance, herbivore deterrence, and fitness to the host plant (Malinowski and Belesky 2000; White Jr et al. 2003; Bacon and Lyons 2005; Gershenzon and Dudareva 2007). This raises the question as to how this may be brought about when plant-associated clavicipitaceous fungi are the site of ergot alkaloid biosynthesis. Indeed, Convolvulaceae plants do not seem to have the biosynthetic capacity to produce ergot alkaloids: neither the genes nor the enzymatic machinery were detectable in the shoots. The complete genetic material responsible for ergot alkaloid biosynthesis was clearly found in the associated fungi present on *I. asarifolia* and *T. corymbosa* (Markert et al. 2008; Schardl et al. 2013). The determinant step in ergot alkaloid biosynthesis is the prenylation in 4 position of tryptophan catalyzed by 4-[γ,γ-dimethylallyl] tryptophan synthase (DmaW) (Groeger and Floss 1998; Keller and Tudzinski 2002). The encoding gene—which has different synonyms, i.e., dmaW or cpd1 (Schardl et al. 2006) or fgaPT2 (Unsöld and Li 2005; Gerhards et al. 2014)—is clearly present in the fungus and is part of a cluster (Schardl et al. 2013) in which the ergot alkaloid genes are oriented as is found in *Claviceps* but different from *Aspergillus* and *Epichloe* species (Markert et al. 2008; Gerhards et al. 2014). A reverse genetics experiment showed that the fungus is also the site of transcription of the dmaW gene (Markert et al. 2008). A polyclonal antibody directed against the DmaW enzyme locates the target antigen to the fungal hyphae but not to the glandular trichomes which are devoid of the enzyme. It follows that not only transcription but also translation in ergot alkaloid biosynthesis are the capacity of the fungus (Steiner et al. 2015).

Initial attempts to detect ergot alkaloids in the fungal mycelium present on *I. asarifolia* and *T. corymbosa* failed although two different analytical approaches were used (Markert et al. 2008). When a sample of the mycelium found on *T. corymbosa* was directly placed into the injection port of a GC/MS system, a trace of agroclavine was detectable and clearly identified by comparison with an authentic sample. No alkaloid was detectable when a mycelial sample from *I. asarifolia* was checked in the same way (W. Boland 2008, personal communication). When the leaf material was analyzed for ergot alkaloids after removal by ultrasonic treatment of the mycelium, alkaloids were qualitatively and quantitatively detected in the plant material showing that the plant leaf material contains almost all alkaloids whereas the producing fungus *P. turbinae* contained only a trace of agroclavine (Markert et al. 2008).

Thus, biosynthesis of alkaloids takes place in the mycelium; however, ergot alkaloids accumulate in the host plant. We therefore postulate a transport system that translocates ergot alkaloids from the mycelium into the plant tissue (Steiner et al. 2015). In an experimental system similar to the one discussed here, transport was postulated to occur through the apparently intact cuticle (Smith et al. 1985).

The hyphae of *Periglandula* spp. characteristically showed a close association with the peltate glandular trichomes of the host plants being concentrated on the cuticle above the subcuticular oil storage cavity (Fig. 2A-D) This close symbiotum establishment apparently starts with appressorium-like hyphal tips attaching to the cuticle covering oil cavities (Fig. 2B). Cross sections of glandular trichomes showed hyphae on top and underneath the cuticle after disruption where the fungus is in close contact with the cell walls of the host plant (Fig. 2C,D). These cells show a high density of organelles, mitochondria, and ribosomes indicating their high metabolic activity, while the plant cell wall features a reduced thickness and appears somewhat translucent. The peltate glandular trichomes involved in this process secrete a mixture of volatile and fat oil in which the fungal hyphae are immersed at a later stage of development (Fig. 2C,E). Hyphae encircling the glandular trichomes contained a huge number of globular structures (Fig. 2F). Since these structures stain with "Nile red," they are likely to be functional equivalents of lipid vesicles. The oil secreted by the plant very likely plays a nutritional role for the fungus. Besides their secretion capacity, the glandular trichomes additionally may provide a site of entry into the plant for metabolites like the alkaloids produced by *Periglandula* species. The transport capabilities of the glandular trichomes were tested using 6(5)carboxyfluorescein diacetate. The uptake of this low-molecular-weight compound into glandular trichomes and underlying mesophyll cells as well as into fungal cells was observed (Fig. 2G,H). This indicates that the hydrophobic quality of the cuticle on the epidermal cells restricts uptake of low-molecular-weight compounds, and it may indicate that a secretion of low-molecular compounds like ergot alkaloids from the fungal hyphae and specific uptake into the peltate glandular trichomes is likely. Thus, glandular trichomes may have a dual function for they secrete oil which attracts and sustains the fungus but also absorb ergot alkaloids which play an ecological role in the life of the plant (Steiner et al. 2015). It is remarkable that a two-way transport system in glandular trichomes of *Drosera rotundifolia* leaves has already been postulated by Charles Darwin (1875).

E. *Periglandula* and the Evolution of the Ergot Biosynthetic Pathways

The process of ergot alkaloid biosynthesis may be divided into initial steps leading to a basic core structure like chanoclavine present in all ergot alkaloid-producing fungi and subsequent steps in which the core structure is decorated by a series of metabolic reactions encoded by mid- and late pathway genes and enzymes. Different evolutionary processes such as integration of AT-rich and transposon-derived repeat blocks into ergot alkaloid gene clusters may result in gene losses, gains, or neofunctionalizations (Schardl et al. 2013; Young et al. 2015). Clavicipitaceous strains that produce only chanoclavine are derived by losses of genes as evidenced by remnants and pseudogenes often remaining in the genome (Young et al. 2015). Thus, the biosynthetic process may have come to a halt at chanoclavine due to a vanishing biosynthetic capacity because a once complete biosynthetic pathway was truncated during evolution by multiple frameshifts and stop codons or both (Young et al. 2015). Gene losses are often present in the Clavicipitaceae but are absent from *P. ipomoeae* which is not only equipped with a complete set of genes responsible also for mid-pathway and late steps in alkaloid biosynthesis but produces also natural products like simple amides (Ahimsa-Müller et al. 2007; Eich 2008) and an ergopeptine like

ergobalansine (Eich 2008; Jennett-Siems et al. 1994, 2004). Both types of compounds are end products of the ergot biosynthetic pathway (Young et al. 2015). For this reason the *P. ipomoeae* gene cluster is considered a basal cluster with an ancient character. It is doubtful if this applies also to the newly described *Periglandula* strains (Beaulieu et al. 2015). At least some of them seem to lack end pathway alkaloids like simple amides and ergobalansine. Diversification of ergot biosynthetic pathways may also occur as a result of a relaxed specificity in ergot alkaloid peptide synthases (Robinson and Panaccione 2015).

Plants of the tribus Ipomoeeae, the largest tribe within the Convolvulaceae, are host to the *Periglandula* fungi. Based on whole plastome sequences, the tribe was shown to be monophyletic, and the subtribal classification identifies two major clades: the Astripomoeinae and the Argyreiinae with the former diverging into five and the latter into two subclades. The genus *Ipomoea* is present in each of these subclades demonstrating it is not monophyletic as traditionally recognized. In the phylogenetic description of the Ipomoeeae results based on DNA sequences, morphology, and RFLP analyses are largely congruent (Eserman et al. 2014). The tribe comprises an estimate of 650–900 plant species. Following Eich's (2008) analyses of the occurrence of alkaloids in Convolvulaceae plants, it was assumed that 50% of all plants in this tribe may be ergot alkaloid positive meaning that up to 450 clavicipitaceous *Periglandula* fungi, each one living on its own convolvulaceous host, may exist (Eserman et al. 2014). The association with ergot alkaloid-producing fungi is the ancestral condition in the Ipomoeeae. But ergot alkaloid biosynthesis has been lost four times during evolution from the plant/fungus symbiota within the tribe Ipomoeeae diverging into different clades and subclades (Eserman et al. 2014). Apparently, not only loss of individual alkaloids must have occurred during evolution but also loss of complete clavicipitaceous fungal strains from the symbiota.

IV. Additional Fungus/Plant Symbiota in Dicotyledonous Plants

Convolvulaceae are a family which are not only notorious for the presence of ergot alkaloids but may also contain simple tropane alkaloids such as calystegines and the indolizidine alkaloid swainsonine. The latter compound is remarkable as it occurs also in plants of the Fabaceae and Malvaceae. The alkaloid is toxic and causes a lysosomal storage disease in grazing animals resulting in a staggering walk and lack of muscular coordination. The tropane alkaloids and swainsonine are constituents of *Ipomoea carnea*. This plant is equipped with two types of trichomes on the adaxial leaf surface: simple trichomes and peltate glandular trichomes as seen on the adaxial leaf surface of *I. asarifolia* and *T. corymbosa*. The peltate trichomes are associated with a vertically transmitted fungal symbiont belonging to the Ascomycete order Chaetothyriales which is responsible for the presence of swainsonine in *I. carnea*, while calystegines are products of the host plant. It is striking that distantly related fungi (Hypocreales and Chaetothyriales) that produce different alkaloids (ergot alkaloids and swainsonine) converged upon a similar life history within the same plant family (Convolvulaceae) (Cook et al. 2013).

Another interesting fungus/plant association has been described for locoweed plants belonging to the family Fabaceae. *Astragalus mollissimus*, *Oxytropis lambertii*, and *Oxytropis sericea* are collectively called locoweed and are colonized by endophytes which seem to be closely related to the genus *Embellisia*. Locoism is observed in cattle intoxicated by locoweed plants. The causative agent seems to be again the indolizidine alkaloid swainsonine. This alkaloid is also known to be a product of in vitro grown *Rhizoctonia leguminicola* cultures (Braun et al. 2003).

An interesting association consisting of *Ipomoea batatas (L.) Lam.* (i.e., sweet potato)

(Convolvulaceae) and *Fusarium lateritium* Nees:Fr has also been reported. As described for our clavicipitaceous fungi (Sect. III), *F. lateritium* is primarily located between the halves of young unfolded leaves of the *I. batatas* plant (Hyun and Clark 1998). Yet there is another feature of this fungus/plant association which we also observed in our system (Sect. III): The fungus is associated on the phylloplane with pearl glands and is located around the bases of trichomes (Clark 1992). The fungus apparently produces trichothecenes and protects the host plant against infection by pathogenic *Fusarium oxysporum* f. sp. *batatas* (Wollenw.) W.C. Snyder and H.N. Hans. However, the associated *F. lateritium* may also be the cause for the chlorotic leaf distortion (CLD) disease mediated by trichothecenes (Clark 1994). After light activation of trichothecenes during prolonged exposure of the plant to sunlight, CLD occurs. Plants usually recover when cloudy weather prevails. Thus, the associated fungus may exert a beneficial and a detrimental effect on the host plant, and in both cases trichothecenes are likely to be the causative agent.

Two new clavicipitaceous fungi belonging to a newly established genus (*Hypodermium*) were isolated from an unidentified Asteraceae plant (genus *Bernonia*). The fungi were named *Hypodermium bertonii* (Speg.) J. White, R. Sullivan, G. Bills et N. Hywel-Jones and *Hyperdermium pulvinatum* J. White, R. Sullivan, G. Bills et Hywel-Jones. As with the clavicipitaceous fungi described in Sect. III, the fungi are epibiotic. They belong to the subfamily Cordycipitoideae (Sullivan et al. 2000). An entirely superficial mycelium was observed on a South American Asteraceae plant, *Baccharis coridifolia* DC. The endophyte belongs to the Hypocreales, an order which accommodates also the family Clavicipitaceae. The fungus occurs not only epibiotic but also in meristematic tissue of leaf primordia. No reproductive structures were detectable. The plant is toxic, and it was assumed that the epibiont is a trichothecene producer. Since this fungus and graminaceous Clavicipitaceae (Chap. II) are not closely related, colonizations (that must have occurred during evolution) were assumed to be distinct events (Bertoni et al. 1997).

The same conclusion was drawn for a *Mentha piperita* L. plant colonized by a Pyrenomycete which is also associated with glandular trichomes (Mucciarelli et al. 2002), a striking observation which led to speculations about the possible function of the secretory glands and trichomes in the establishment of a symbiotic association. It is possible,that also in this case the glandular trichomes are entry gates for the fungus in its attempt to establish a molecular dialogue with the host plant (compare Steiner et al. 2008, 2015).

V. Conclusions

The data described in Sect. III solve a historical mystery and explain why ergot alkaloids occur in disjointed taxa, clavicipitaceous fungi, and convolvulaceous plants. They dispute the possibility that during evolution a horizontal transfer of genes responsible for the synthesis of ergot alkaloids occurred from fungi to plants. They also show that there is no necessity to invoke a repeated invention of the ergot alkaloid biosynthetic pathway during evolution. In fact, genes present in *P. ipomoeae*, *P. turbinae*, *Claviceps purpurea*, *Claviceps fusiformis*, *Balansia obtecta*, *Epichloe coenophialum*, as well as *Aspergillus fumigatus* involved in the biosynthesis of ergot alkaloids are orthologs which share a high similarity (Markert et al. 2008; Schardl et al. 2013). The gene cluster responsible for ergot alkaloid biosynthesis in *P. ipomoeae* is likely to have a conserved character which may represent an early point in the evolutionary processes that diversified the gene clusters and molecular structures of ergot alkaloids in symbiota consisting of different higher plant species and their epibiotic clavicipitaceous fungi (Schardl et al. 2013).

It is also clear that the association between fungus and convolvulaceous plant is asymptomatic and that a molecular dialogue occurs between associated fungi and convolvulaceous plants indicating that both are members of a symbiotum in which biosynthesis and accumulation of ergot alkaloids are spatially separated and sequestered in different organisms. The molecular dialogue between both organisms

requires a subcuticular interaction between fungal hyphae and cell walls of peltate glandular trichomes. It is likely that the oil secreted by the trichomes is of nutritional value to the fungus and mediates the contact between both organisms. As shown here, however, secretion of oil is not the only function of the trichomes, but rather they seem to have a second function by absorbing ergot alkaloids. We believe that peltate glandular trichomes are two-way systems in this symbiotum (Steiner et al. 2015).

One of the unsolved questions is if there are also sexual forms of these vertically transmitted *Periglandula* fungi. It is also unknown if and how the plant-associated fungi spread in the symbiotum. Despite repeated attempts to localize hyphae, spores, or propagules within the host plants, structures of the fungi inside the host remained undetected until now.

References

Ahimsa-Mueller MA, Markert A, Hellwig S, Knoop V, Steiner U, Drewke C, Leistner E (2007) Clavicipitaceous fungi associated with ergoline alkaloid-containing Convolvulaceae. J Nat Prod 70:1955–1960

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U S A 100:15649–15654

Bacon CW, Lyons P (2005) Ecological fitness factors for fungi within the Balansiae and Clavicipiteae. In: Dighton J, White JF, Oudemans P (eds) The fungal community, its organisation and role in the ecosystem, 3rd edn. CRC Taylor and Francis, Boca Raton, pp 519–532

Bacon CW, White JF Jr (1994) Stains, media, and procedures for analyzing endophytes. In: Bacon CW, White JF Jr (eds) Biotechnology of endophytic fungi of grasses. CRC, Boca Raton, FL, pp 47–58

Bacon CW, White JF Jr (2000) Microbial endophytes. Marcel-Dekker, New York, pp 341–388

Beaulieu WT, Panaccione DG, Ryan KL, Kaonongbua W, Clay K (2015) Phylogenetic and chemotypic diversity of *Periglandula* species in eight new morning glory hosts (Convolvulaceae). Mycologia 107:667–678

Bertoni MD, Romero N, Reddy PV, White JF Jr (1997) A hypocralean epibiont on meristems of *Baccharis coridifolia*. Mycologia 89:375–382

Bischoff JF, White JF Jr (2005) Evolutionary development of the Clavicipitaceae. In: Dighton J, White JF Jr, Oudemans P (eds) The fungal community – its organisation and role in the ecosystems (3rd ed.), CRC Taylor and Francis, Boca Raton, pp 505-518

Braun U, Romero J, Liddell C, Creamer R (2003) Production of swainsonine by fungal endophytes of locoweed. Mycol Res 107:980–988

Brem D, Leuchtmann A (2002) Intraspecific competition of endophyte infected vs uninfected plants in two woodland grass species. Oikos 96:281–290

Chang H-T, Cheng Y-H, C-L W, Chang S-T, Chang T-T, Su Y-C (2008) Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi. Bioresour Technol 99:6266–6270

Clardy J, Walsh C (2004) Lessons from natural molecules. Nature 432:829–837

Clark CA (1992) Histological evidence that *Fusarium lateritium* is an exopathogen on sweet potato with chlorotic leaf distortion. Phytopathology 82:656–663

Clark CA (1994) The chlorotic leaf distortion pathogen, *Fusarium lateritium*, cross protects sweet potato against Fusarium wilt caused by *Fusarium oxysporum f.sp. batatas*. Biol Contr 4:59–66

Clay K, Frentz IC (1993) *Balansia pilulaeformis*, an epiphytic species. Mycologia 85:527–534

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am Nat 160:S99–S127

Cook D, Beaulieu WT, Mott IW, Riet-Correa F, Gardner DR, Grum D, Pfister JA, Clay K, Marcolongo-Pereira C (2013) Production of the alkaloid Swainsonine by a fungal endosymbiont of the Ascomycete order Chaetothyriales in the host Ipomoea carnea. J Agric Food Chem 61:3797–3803

Darwin C (1875) Insectivorous plants. John Murray, London

Dobberstein RH, Staba EJ (1969) *Ipomoea, Rivea* and *Argyreia* tissue cultures: influence of various chemical factors on indole alkaloid production and growth. Lloydia 32:141–177

Eich E (2008) Solanaceae and Convolvulaceae: secondary metabolites- biosynthesis, chemotaxonomy, biological and economic significance (a handbook). Springer, Berlin Heidelberg

Eisner T (2003) For love of insects. Harvard University Press, Cambridge

Eserman LA, Tiley GP, Jarret RL, Leebens-Mack JL, Miller RE (2014) Phylogenetics and diversification of morning glories (tribe ipomoeeae, convolvulaceae) based on whole plastome sequences. Am J Bot. https://doi.org/10.3732/ajb.1300207

Gerhards N, Neubauer L, Tudzynski P, Li SM (2014) Biosynthetic pathways of ergot alkaloids. Toxins 6:3281–3295

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414

Glenn AE, Bacon CW, Price R, Hanlin RT (1996) Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88:369–383

Groeger D, Floss HG (1998) Biochemistry of ergot alkaloids-achievements and challenges. In: Cordell GA (ed) The alkaloids: chemistry and biology, vol 50. Academic, New York, pp 171–218

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69:509–526

Harborne JB (2004) Introduction to ecological biochemistry, 4th edn. Academic, London

Hofmann A (1961) Die Wirkstoffe der mexikanischen Zauberdroge "Ololuiqui". Planta Med 9:354–367

Hofmann A (2006) LSD-mein Sorgenkind, die Entdeckung einer Wunderdroge. Deutscher Taschenbuchverlag, Munich

Hussein YHA (2004) Biochemical analysis of Convolvulaceae plant tissue cultures for the presence of ergoline alkaloids. PhD thesis, Botany Department, Faculty of Science, Zagazig University, Zagazig, Egypt

Hyun J-W, Clark CA (1998) Analysis of *Fusarium lateritium* using RAPD and rDNA RFLP techniques. Mycol Res 102:1259–1264

Jenett-Siems K, Kaloga M, Eich E (1994) Ergobalansine/ergobalansinine, a proline-free peptide type alkaloid of the fungal genus *Balansia* is a constituent of *Ipomoea piurensis*. J Nat Prod 57:1304–1306

Jennett-Siems K, Kaloga M, Eich E (2004) Ergobalansine/ergobalansinine, a proline-free peptide-type alkaloid of the fungal genus Balansia is a constituent of Ipomoea piurensis. J Nat Prod 67:2160

Keller U, Tudzynski P (2002) Ergot alkaloids. In: Osiewacz HD (ed) The Mycota, industrial applications, vol X. Springer, Berlin, pp 157–181

Kucht S, Gross J, Hussein Y, Grothe T, Keller U, Basar S, Koenig WA, Steiner U, Leistner E (2004) Elimination of ergoline alkaloids following treatment of *Ipomoea asarifolia* (Convolvulaceae) with fungicides. Planta 219:619–625

Kuldau GA, Liu JS, White JF Jr, Siegel MR, Schardl CL (1997) Molecular systematics of Clavicipitaceae supporting monophyly of genus Epichloë and form genus *Ephelis*. Mycologia 89:431–441

Leistner E, Steiner U (2009) Fungal origin of ergoline alkaloids present in Dicotyledonous plants (Convolvulaceae). In: Anke T, Weber D (eds) The Mycota. Physiology and Genetics, vol XV. Springer, Berlin, pp 197–208

Leuchtmann A, Clay K (1988) *Atkinsonella hypoxylon* and *Balansia cyperi*, epiphytic members of the Balansiae. Mycologia 80:192–199

Leuchtmann A, Clay K (1989) Morphological, cultural and mating studies on *Atkinsonella*, including *A. texensis*. Mycologia 81:692–701

Lewis EA, Bills GF, Heredia G, Reyes M, Arias RM, White JF Jr (2002) A new species of endophytic balansia from Veracruz, Mexico. Mycologia 94:1066–1070

Luttrell ES, Bacon CW (1977) Classification of *Myriogenospora* in the Clavicipitaceae. Can J Bot 55:2090–2097

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses. Crop Sci 40:923–940

Markert A, Steffan N, Ploss K, Hellwig S, Steiner U, Drewke C, Li S-M, Boland W, Leistner E (2008) Biosynthesis and accumulation of ergoline alkaloids in a mutualistic association between *Ipomoea asarifolia* (Convolvulaceae) and a clavicipitalean fungus. Plant Physiol 147:296–305

Mothes K (1981) The problem of chemical convergence in secondary metabolism. In: Kageyama M, Nakamura K, Oshima T, Uchida T (eds) Science and scientists. Japan Scientific Societies Press, Tokyo, pp 323–326

Mothes K, Schütte HR, Luckner M (1985) Biochemistry of alkaloids. VEB Deutscher Verlag der Wissenschaften, Berlin

Moy M, Belanger F, Duncan R, Freehoff A, Leary C, Meyer W, Sullivan R, White JF Jr (2000) Identification of epiphyllous mycelial nets on leaves of grasses infected by clavicipitaceous endophytes. Symbiosis 28(4):291–302

Mucciarelli M, Scannerini S, Bertea CM, Maffei M (2002) An ascomycetous endophyte isolated from *Mentha piperita* L.: biological features and molecular studies. Mycologia 94:28–39

Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884–888

Piel J (2004) Metabolites from symbiotic bacteria. Nat Prod Rep 21:519–538

Reddy PV, Bergen MS, Patel R, White JF Jr (1998) An examination of molecular phylogeny and morphology of the grass endophyte *Balansia claviceps* and similar species. Mycologia 90:108–117

Robinson SL, Panaccione DG (2015) Diversification of ergot alkaloids in natural and modified fungi. Toxins 7:201–218

Rykard DM, Bacon CW, Luttrell ES (1985) Host relations of *Myriogenospora atramentosa* and *Balansia epichloë* (Clavicipitaceae). Phytopathology 75:950–956

Saikkonen K, Wäli P, Helander M, Feath SH (2004) Evolution of endophyte-plant symbiosis. Trends Plant Sci 9:275–280

Saikkonen K, Young CA, Helander M, Schardl CL (2015) Endophytic Epichloe species and their grass hosts: from evolution to applications. Plant Mol Biol. https://doi.org/10.1007//s11103-015-0399-6

Schardl CL (1994) Molecular and genetic methodologies and transformation of grass endophytes. In: Bacon CW, White JF Jr (eds) Biotechnology of endophytic fungi of grasses. CRC, Taylor and Francis, Boca Raton, pp 151-166

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbiosis of grasses with seedborn fungal endophytes. Annu Rev Plant Biol 55:315–340

Schardl CL, Panaccione DG, Tudzynski P (2006) Ergot alkaloids-biology and molecular biology. In: Cordell GA (ed) The alkaloids: chemistry and biology, vol 63. Academic, New York, pp 45–86

Schardl CL, Grossman RB, Nagabhyru P, Faulkner JR, Mallik UP (2007) Loline alkaloids: currencies of mutualism. Phytochemistry 68:980–996

Schardl CL, Craven KD, Speakman S, Stromberg A, Lindstrom A, Yoshida R (2008) A novel test for host-symbiotum codivergence indicates ancient origin of fungal endophytes in grasses. Syst Biol 57:483–498

Schardl CL, Young CA, Hesse U, Amyotte SG, Andreeva K et al (2013) Plant-symbiotic fungi as chemical engineers: multi-genome analysis of the Clavicipitaceae reveals dynamics of alkaloid loci. PLoS Genet 9(2):e1003323. https://doi.org/10.1371/journal.pgen.1003323

Schnee C, Köllner TG, Held M, Turlings TCJ, Gershenzon J, Degenhardt J (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc Natl Acad Sci U S A 103:1129–1134

Schultes RE, Hofmann A (1992) Plants of the gods, their sacred, healing and hallucinogenic powers. Healing Arts, Vermont

Smith KT, Bacon CW, Luttrell ES (1985) Reciprocal translocation of carbohydrates between host and fungus in Bahiagrass infected with *Myriogenospora atramentosa*. Phytopathology 75:407–411

Spatafora JW, Blackwell M (1993) Molecular systematics of unitunicate perithecial ascomycetes: the Clavicipitales-Hypocreales connection. Mycologia 85:912–922

Steiner U, Leistner E (2012) Ergoline alkaloids in convolvulaceous host plants originate from epibiotic clavicipitaceous fungi of the genus *Periglandula*. Fungal Ecol 5:316–321

Steiner U, Ahimsa-Mueller MA, Markert A, Kucht S, Gross J, Kauf N, Kuzma M, Zych M, Lamshoeft M, Furmanowa M, Knoop V, Drewke C, Leistner E (2006) Molecular characterisation of a seed transmitted clavicipitaceous fungus occurring on dicotyledonous plants (Convolvulaceae). Planta 224:533–544

Steiner U, Hellwig S, Leistner E (2008) Specificity in the interaction between an epibiotic clavicipitalean fungus and its convolvulaceous host in a fungus/plant symbiotum. Plant Signal Behav 3:704–706

Steiner U, Leibner S, Schardl CL, Leuchtmann A, Leistner E (2011) *Periglandula* a new fungal genus within the Clavicipitaceae and its association with Convolvulaceae. Mycologia 103:1133–1145

Steiner U, Hellwig S, Ahimsa-Müller MA, Grundmann N, Li SM, Drewke C, Leistner E (2015) The key role of peltate glandular trichomes in symbiota comprising Clavicipitaceous fungi of the genus *Periglandula* and their host plants. Toxins 7:1355–1373

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Sullivan RF, Bills GF, Hywel-Jones NL, White JF Jr (2000) Hypodermium: a new clavicipitalean genus for some tropical epibionts of dicotyledonous plants. Mycologia 92:908–918

Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Mol Phylogenet Evol 44:1204–1223

Tsai H-F, Liu J-S, Staben C, Christensen MJ, Latch CM, Siegel MR, Schardl CL (1994) Evolutionary diversification of fungal endophytes of tall fescue grass by hybridisation with *Epichloë* species. Proc Natl Acad Sci U S A 91:2542–2546

Tudzynski P, Correia T, Keller U (2001) Biotechnology and genetics of ergot alkaloids. Appl Microbiol Biotechnol 57:593–605

Unsöld IA, Li S-M (2005) Overproduction, purification and characterization of FgaPT2, a dimethylallyltryptophan synthase from *Aspergillus fumigatus*. Microbiology 151:1499–1505

Welty RE, Azevedo MD, Cooper TM (1987) Influence of moisture content, temperature, and length of storage on seed germination and survival of endophytic fungi in seeds of tall fescue and perennial ryegrass. Phytopathology 77:893–900

White JF Jr, Morgan-Jones G (1996) Morphological and physiological adaptations of Balansieae and trends in the evolution of grass endophytes. In: Redlin SC, Carris LM (eds) Endophytic Fungi in Grasses and Woody Plants. APS Press, St. Paul, pp 133–154

White JF Jr, Bacon CW, Hinton DM (1991) Substrate utilization in selected *Acremonium*, *Atkinosella*, and *Balansia* species. Mycologia 83:601–610

White JF Jr, Bacon CW, Hywel-Jones NL, Spatafora JW (2003) In: Bennet JW (ed) Clavicipitalean fungi, evolutionary biology, chemistry, biocontrol, and cultural impacts, Mycology series, vol 19. Marcel Dekker, New York

Young CA, Schardl CL, Panaccione DG, Florea S, Takach JE, Charlton ND, Moore N, Webb JS, Jaromczyk J (2015) Genetics, genomics and evolution of ergot alkaloid diversity. Toxins 7:1273–1302

Zenk MH (1967) Biochemie und Physiologie sekundärer Pflanzenstoffe. Ber Dt Bot Ges 80:573–591

Volatiles in Communication of *Agaricomycetes*

Ursula Kües[1], Weeradej Khonsuntia[1], Shanta Subba[1], Bastian Dörnte[1]

CONTENTS

[1] Molecular Wood Biotechnology and Technical Mycology, University of Goettingen, Goettingen, Germany; e-mail: ukuees@gwdg.de; wkhonsu@gwdg.de; ssubba@gwdg.de; bdoernt@gwdg.de

I. Introduction

Any environment offers multiple kinds of biotic and abiotic signals to its inhabitants. These possibly send out themselves signals into their environment, nondirectionally as **cues** such as due to their pure presence and their general organismal properties and habits and others evolved and directed for an intended purpose as **signals** in the closer biological sense. **Signaling** thereby has always two **directional components**, i.e., **sending** of the signal and **sensing** of the signal. Signaling can thus be a mean of **communication** (**information transfer**) between a **sender** and a **receiver**. Such biological signaling can be **bidirectional** (communication and counter-communication, talk and crosstalk) if both communicating partners are senders and receivers. Receptive organisms of signals can be the receivers as the intended addressees, other fortuitous **recipients**, and also **eavesdroppers** taking advantage of signals proffered for an unrelated function. Reception of a signal might elicit **decisions** and **reactions**. A fully covering definition of biological communication includes also the **responses** provoked by the transfer of signal information (see, e.g., the definitions presented in Scott-Phillips 2008). Signals send out to a receiver can be suitably **honest** and reliable if both sender and receiver share common interests and have benefits. Signals can however also have degrees of **misinformation** potential and might be used for **deception** and **manipulation** of recipients. **Signal parasitism** is the most categorial form of deception in which senders for their benefits mimic existing signals (**signal**

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

mimicry) to take advantages from cheated receivers (Carazo and Font 2013; Mokkonen and Lindstedt 2016; Weldon 2016).

Organisms communicate between their own cells; with individuals of the same species; with individuals of other species within a common taxonomic rank, e.g., within a genus, a family, an order, a class, a phylum, and a kingdom; and also and possibly even more with individuals from above the kingdom level. As for any other type of organisms, this is also true for the fungi (Kües and Navarro-Gonzaléz 2009; Li et al. 2016a).

Fungi communicate within an individual with **autoregulatory** signals, for example, for hyphal branching (Ugalde and Rodriguez-Urra 2014, 2016), for nutrient translocation (Moore 1998; Kües and Navarro-Gonzaléz 2009; Boswell 2012; Straatsma et al. 2013), for controlling morphology and asexual sporulation (Chen and Fink 2006; Tsitsigiannis and Keller 2006; Ugalde and Rodriguez-Urra 2014, 2016), for sexual reproduction, and for establishing in coordinated manner multicellular structures with distinct cellular functions such as the fruiting bodies of higher fungi with their specific tissues, cells, and body plans (Kües and Navarro-Gonzaléz 2009, 2015; Cottier and Mühlschlegel 2012; Martin et al. 2013; Ugalde and Rodriguez-Urra 2014, 2016). Within a species, communication may target at sexual reproduction such as between different strains via mating-type-specific pheromones (Kües et al. 2011; Pöggeler 2011; Kües 2015; Wöstemeyer et al. 2016) or by competition for space and food resources, at germination, fungal growth, morphogenesis, and development through more general signals and through specific **quorum sensing** mechanisms (Kües and Navarro-Gonzaléz 2009, 2015; Albuquerque and Casadevall 2012; Amare and Keller 2014; Ugalde and Rodriguez-Urra 2014, 2016; Avbelj et al. 2016; Wongsuk et al. 2016; Polke et al. 2017). Similar general mechanisms may act when different fungal species compete for space and food (Boddy 2000; Avbelj et al. 2016).

Inter-kingdom quorum sensing interactions have been established between fungi and prokaryotes (Kües and Navarro-Gonzaléz 2009; Dixon and Hall 2015), distinct fungal signals positively and negatively influence bacterial motility and can trigger specific changes in bacterial morphology (Schmidt et al. 2016; Jones and Elliott 2017; Jones et al. 2017), and bidirectional fungal-bacterial communication can determine metabolism and differentiation of both (Spraker et al. 2014). Cross-kingdom communication between eukaryotes via fungal quorum sensing molecules and other signals has been verified between fungi and plants and between fungi and animals (Fischer and Keller 2016; van't Padje et al. 2016). Intense cross-kingdom signaling occurs between fungi and plants, for example, in precontact communication between symbiotic and pathogenic fungi and their hosts preceding tropic fungal growth toward a plant structure (Kües and Navarro-Gonzaléz 2009; Oldroyd 2013; Raudaskoski and Kothe 2015; Zeilinger et al. 2016a). Moreover, fungi may take influence by interspecific interactions on behavior of other fungi with their hosts (Cale et al. 2016). Sending own signals and sensing host signals can be crucial particularly for fungi growing in other organisms, whether in a plant or in an animal (Kües and Navarro-Gonzaléz 2009; Delaunois et al. 2014; Yarden 2014; Braunsdorf et al. 2016). Fungi for various reasons can add to or alter also their hosts' own signals (Kües and Martin 2011; Farre-Armengol et al. 2016; Werner et al. 2016; Peris et al. 2017). Sessile fungi may send out inter-kingdom signals to attract, e.g., mobile animals to use them as vectors for spore dispersal (Policha et al. 2016) or as a food source (Hsueh et al. 2017). Moreover, signals of repellent nature might be sent out to predators, likely without a preliminary contact, to elicit avoidance for fungal protection (Thakeow et al. 2007; Holighaus and Rohlfs 2016), or others to plant roots as phytotoxins to suppress non-favorable plant growth (Splivallo et al. 2007a; Tarkka and Piechulla 2007; Streiblová et al. 2012). On the other hand, other organisms' signals might block fungi in their growth (Garbeva et al. 2014; Cespedes et al. 2015; Weisskopf et al. 2016).

In summary, "**food, growth and differentiation, development**, and **sex**" in combination

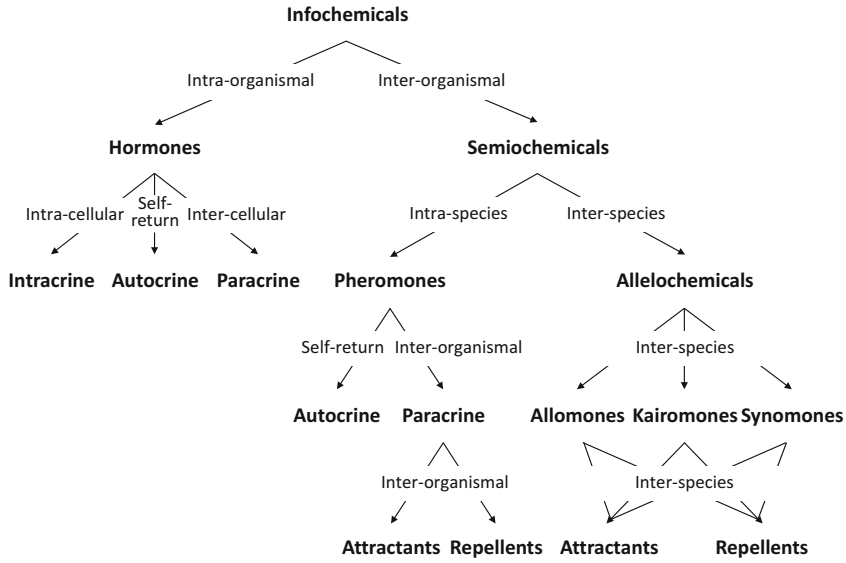


Fig. 1 Hierarchy in terminology of message-bearing biochemical compounds defined by place of production and place of reception and reaction (see text for further explanations)

with **defense** might thus be elemental drivers of communication between different biological partners. However, ecosystems are highly complex with many different players interacting and communicating with each other at the same time in the same environment with a multitude of different signals (Wenke et al. 2010; Bitas et al. 2013; Hung et al. 2015; Werner et al. 2016; Leach et al. 2017). Multiple simultaneous sending and receiving of signals by the various organisms in a given environment and the ever changing spatiotemporal dynamics could stress the inhabitants to filter out what is specifically relevant for them and their functional guilds in the **noisy environment**. Specialized molecular sensors and receptors, upstream of intracellular signaling pathways, are used in signal perception to differentiate in the warfare of signal sensing (Braunsdorf et al. 2016; Kou and Naqvi 2016; Zeilinger et al. 2016a).

II. Chemical Communication: Infochemicals

One way to communicate is to employ **biochemical compounds** as signals. Message-bearing **biochemicals** used individually or as mixtures in biological communication are overlappingly defined as **infochemicals** (Dicke and Sabelis 1988; Wheatley 2002; Fig. 1) or, as the more common and potentially more specific term for communication between different organisms, as **semiochemicals**, named so by the Greek word semeion for signal (Beck et al. 2017; Fig. 1).

Infochemicals and semiochemicals can be further differentiated into:

1. **Hormones**, signaling molecules transported to places of action within the body of the producing organism for **intraorganismal** message transmission. Hormones can be further differentiated into **intracrine** when operating within a producing cell, **autocrine** when returning back to the producing cell, and **paracrine** when acting on other cells than the producing cell (Fig. 1).
2. **Pheromones**, signaling molecules freely secreted from organisms in order to act in **intraspecies** message transmission. Pheromones act **autocrine** when targeting at the producing individual, **paracrine** for communication between different individuals, and as **sex and mating-type pheromones** when signaling between partners of

distinct sex or mating type specifically in communication for sexual reproduction (Fig. 1).

3. **Allelochemicals**, signaling molecules which act in **interspecies** message transmission. Allelochemicals further subdivide into **allomones** when benefitting the emitter, **kairomones** when benefitting the receiver, and **synomones** when benefitting both sender and receiver (Fig. 1).

Notable, a pheromone of one species might simultaneously serve as an allelochemical for other species. Moreover, by the downstream reactions triggered by a received signal, pheromones and allelochemicals might function either as **attractants** when the signal turns the receiver toward the sender or as **repellents** when the signal results in an avoidance reaction of the receiver (Ruther et al. 2002; Sbarbati and Osculati 2006; Beck et al. 2017; Fig. 1). The terminology can be even more distinctive in classification when considering distinct reactions of the receiver. **Primer** and **releaser** distinguish **physiological** and **behavioral** responses, while the specific purpose and function of reactions can be addressed in further terms for behaviors like, as specifically appointed for animal kairomones, foraging, enemy avoidance, sexual, and aggregation (Ruther et al. 2002).

Specific examples in fungal communications for most of these distinct general situations and the definitions as summarized in Fig. 1 can be found in the literature cited above in the introduction and in the text of the following sections.

III. Volatile Organic Compounds in Fungi

Semiochemicals are often **volatile organic compounds (VOCs)**, carbon-based, and often lipophilic chemicals with low molecular weight (<300–400 Da) and high vapor pressure (at 25 °C >100 kPa) with which organisms can communicate over shorter and also longer distances. From their place of production, VOCs can easily travel through different media, first of all through the atmosphere but also through liquids and porous soils (Bitas et al. 2013; Peñuelas et al. 2014; Li et al. 2016a). Senders can thus reach receivers through the elements air, water, and soil.

Fungi produce and employ a broad range of VOCs for short- and long-distance-communication, such as molecules derived from fatty acids (e.g., hydrocarbons, aliphatic alcohols, ketones; **oxylipins** = oxidized fatty acids and metabolites derived there from; Brodhun and Feussner 2011), polyketides, aromates, terpenes, sulfur compounds, nitrogen compounds, and halogenated compounds [for a specific compilation of fungal volatiles, see the recent extensive review by Dickschat (2017), for volatile sesquiterpenes (C15 terpenoids) in addition the review by Kramer and Abraham (2012)]. Understanding the fungal **volatilomes**, **volatomes**, or **volatolomes** (terms interchangeably used for the blends of all VOCs emitted directly and indirectly by the metabolism of an organism) and their biological and ecological functions has recently been moved more into focus of research (Hung et al. 2015; Spiteller 2015; Li et al. 2016a; Werner et al. 2016).

Fungal VOCs are also of broad interest for human applications by their pharmacological and further biotechnological potentials. VOCs from edible and medicinal mushrooms, for example, may confer for the consumer an appetizing odor and flavor to the fruiting bodies and derived food products. Other VOCs can indicate as **off-odors** and **off-flavors** food spoilage, while other **malodors** possibly signal inedible and toxic characters of mushrooms (Fons et al. 2003; Chiron and Michelot 2005; Sherratt et al. 2005; Combet et al. 2006; Cho et al. 2008; Verginer et al. 2010; Splivallo et al. 2011; Fuijoka et al. 2013; Ashmore et al. 2014; Nosaka and Miyazawa 2014; Usami et al. 2014, 2015; Vita et al. 2015; Vadatzadeh et al. 2015; see Sect. VI.D). Moreover, VOCs of edible and medicinal mushrooms may confer also good health-promoting and pharmaceutical effects (Wasser and Weis 1999; Baby et al. 2015; Pennerman et al. 2015; Kües and Badalyan 2017). Fungal VOCs give various other fermented food sources (bread, cheeses, soy products, etc.) and alcoholic and nonalcoholic beverages (wine, beer, coffee, etc.) their good tastes and smells or contribute to their overall chemical blends of VOCs (Gkatzionis et al. 2014; Zhang et al. 2014; Pires et al. 2014; Carrau et al. 2015; Li et al. 2015; Zhao et al. 2015; Hirst and Richter 2016; García-Estrada and Martín 2016; Lee et al. 2016; Varela 2016; Belda et al. 2017; Rahayu et al. 2017). Not surprisingly,

therefore, there is an increasing demand on specific fungal VOCs in flavor, cosmetic, detergent, and pharmaceutical industry (Fraatz et al. 2009a; Carlquist et al. 2015; Forti et al. 2015; Heitmann et al. 2017; Vespermann et al. 2017).

Microbial volatiles can protect plants from pathogens. In addition to plant-promoting bacteria, specific fungi find also more and more application in agriculture in eco-friendly plant protection and growth promotion (Kanchiswamy et al. 2015a, b). Protective effects of fungal VOCs against postharvest decays and molding for application in crop storage are under study (e.g., Lee et al. 2009; Corcuff et al. 2011; Di Francesco et al. 2015; Liarzi et al. 2016; Arrarte et al. 2017; Parafati et al. 2017; Toffano et al. 2017; Mari et al. 2016). Particularly interesting for fungal biocontrol are **mycoparasitic** species of the *Trichoderma* genus which use VOCs among other antagonistic measures in attacking their fungal targets (Susi et al. 2011; Zeilinger et al. 2016b). Fungal VOCs can be appointed in highly specific insect and nematode pest management strategies (Schalchli et al. 2014; Holighaus and Rohlfs 2016; Beck and Vannette 2017). Cocktails of VOCs particularly rich in compounds with **antifungal** and **antibacterial** properties produced by certain strongly **antagonistic** fungi of mostly **endophytic** origin are suggested to be used in applications of "mycofumigation" (biofumigation using fungi and fungal products) to replace chemical biocides for protecting, e.g., crops, agricultural products, and building materials from undesired fungal or bacterial growth or from arthropod infestation and for decontamination of animal waste (Strobel 2006, 2011; Alpha et al. 2015; Hutchings et al. 2017; Suwannarach et al. 2013, 2017), although importantly, not without safety warnings for potential health risks to humans by the recognized and unrecognized mechanisms of cellular actions of respective antimicrobial fungal VOCs (Bennett and Inamdar 2015; Hung et al. 2015) such as, for example, the damage of DNA caused by the DNA-methylating volatile fungal agent *N*-methyl-*N*-nitrosoisobutyramide (Alpha et al. 2015; Hutchings et al. 2017).

Fungi produce alcoholic VOCs of different C chain lengths, from ethanol to decanol, and moreover various terpenes which are discussed as potential sources for green chemicals and as surrogates for gasoline, jet fuel, and diesel fuel in a form of "mycodiesel." To overcome natural low yields, fungal genes for VOC production could be identified and used for pathway engineering in suitable hosts for recombinant optimized production (Strobel 2014; Spakowicz and Strobel 2015).

Finally, blends of emitted fungal VOCs might be used in chemotaxonomy and fungal identification (Boustie et al. 2005; Malheiro et al. 2013; Müller et al. 2013; Oliveira et al. 2015) and for detection of unwanted fungal species in specific environments such as urban outdoor environments (Garcia-Alcega et al. 2017), buildings and building materials (Parkinson et al. 2009; Schuchardt and Kruse 2009; Polizzi et al. 2012; Betancourt et al. 2013; Konuma et al. 2015; Micheluz et al. 2016; Pantoja et al. 2016), and food (Magan and Evans 2000; Nieminen et al. 2008; Salvador et al. 2013; Pan et al. 2014; Wang et al. 2016; Ji et al. 2017) and, because of the nondestructive methodical character, also for detection of fungi on historical parchments and other valuable objects (Sawoszczuk et al. 2015, 2017a, b).

Hundreds of different fungal VOCs have chemically been described, several of which are emitted (not only) by many fungal taxa and probably many more which are more specific to smaller fungal groups or even individual species. While not necessarily restricted to a fungal origin and possibly biased by the type of fungi preferentially analyzed in research, many of the so far detected and defined VOCs have been found emitted from yeasts and filamentous species of the *Ascomycetes* or from selected species of the *Agaricomycetes* and some other *Basidiomycetes*. Biological functions are defined or proposed for only smaller subgroups of named fungal VOCs (Chiron and Michelot 2005; Korpi et al. 2009; Effmert et al. 2012; Davis et al. 2013; Lemfack et al. 2014; Halbwachs et al. 2016; Dickschat 2017). Usually, cocktails of a broad range of VOCs are released. Truffles, for example, can emit especially rich fragrances with up to 200 different compounds (see, e.g., Fiecchi et al. 1967; Talou et al. 1987, 1989a, b; Gioacchini et al. 2005, 2008; March et al. 2006; Splivallo et al. 2007b, 2011; Culleré et al. 2010, 2013; Liu et al. 2015; Zhang et al. 2016b; Kamle et al. 2017; Liu and Li 2017), although not all are produced by the fungi themselves but originate from bacteria (Splivallo and Ebeler 2015; Splivallo et al. 2015; Vadatzadeh et al. 2015; Splivallo and Culleré 2016) living in the fungal fruiting bodies in possible symbiosis (Benucci and Bonito 2016; Le Roux et al. 2016). Furthermore, other fungi associated with the tubers (Buzzini et al. 2005) and even the host trees might also have some kind of influence on the complex truffle scent compositions (Culleré et al. 2017).

The body of knowledge on appearance of VOCs and possible VOC functions is much larger in *Ascomycetes* than in *Basidiomycetes* as evidenced in many of the references cited above and in Sect. I and in the previous chapter 5 "Communication of Fungi on Individual, Species, Kingdom and above Kingdom Levels"

in the first edition of Mycota XV (Kües and Navarro-Gonzaléz 2009). Biosynthetic pathways of production are however clear only for some VOCs, such as for certain compounds arising from amino acids and for some others arising from fatty acids (Korpi et al. 2009; Bennett and Inamdar 2015) and, promoted by fungal whole genome sequencing, also for a first of the many different fungal sesquiterpenes (Wawrzyn et al. 2012; Quin et al. 2013; Kües and Badalyan 2017). However, VOCs detected in the presence of fungi can present also volatile degradation products of fungal substrates owing to the extracellular mode of fungal nutrition (Jonsell et al. 2003; Bennett and Inamdar 2015), or they may arise through spontaneous non-specific chemical reactions of (volatile) compounds of fungal origin (Bennett and Inamdar 2015), or they might be added by other organisms as documented, e.g., for truffles (see above).

In this chapter, we further focus on fungal VOCs with recognized functions in communication by *Agaricomycetes*. Among the *Agaricomycetes* are species which produce particularly rich cocktails of VOCs of many different kinds while technical improvements made it possible to identify many of them in their chemical nature (Chiron and Michelot 2005; Dickschat 2017). Nevertheless, their biological functions including possible roles in communication in their ecosystems remain understudied (Halbwachs et al. 2016). As will be seen in the following, our general knowledge with few exceptions on chemically defined VOCs in *Agaricomycetes* is still very restricted while ecological ideas on organismal interactions are rising.

Generally, it is not as easy to identify volatile compounds in odors which are often blends of multiple VOCs of very different chemical natures, properties, and concentrations, while individual VOCs may contribute in minute amounts significantly to an overall outcome of a scent. Techniques for separation, concentration, identification, and quantification applied in research in determination of VOCs and their sensitivities and specificities in individual VOC detection are therefore important to notice since they can very much influence the outcome of the VOCs which are detected (see, e.g., Splivallo et al. 2007b; Thakeow et al. 2007; Costa et al. 2013; Culleré et al. 2013; Taşkin et al. 2013; Liu et al. 2015; Zhang et al. 2016b; Zhang and Li 2010; Hung et al. 2015). For example, VOCs emitted by a (non-destructed) specimen might be headspace captured from the object at rational temperatures over a period of time using, e.g., different fibers in SPME (solid-phase microextraction), stir bar sorptive extraction (SBSE), or graphic traps as collectors (see, e.g., Zhang and Pawliszyn 1993; Zhang et al. 1994; Fäldt et al. 2000; Jeleń 2003; Zeppa et al. 2004; Gioacchini et al. 2005; Splivallo et al. 2007b; Diaz et al. 2009; Combet et al. 2009; Fuijoka et al. 2013; Müller et al. 2013), or fungi might be ground to powder in liquid nitrogen and VOCs be extracted in suitable organic solvents (see Chen and Wu 1984; Mau et al. 1992; Cruz et al. 1997; Grosshauser and Schieberle 2013). Different variants of one- and two-dimensional high-resolution gas chromatography (GC) with distinct capillary columns for separation by specific compound characters can be applied in combination with distinct mass spectrometry (MS) techniques and suitable ever-growing spectra libraries for organic compounds to identify and possibly quantify different chemical types of VOCs (see, e.g., Aprea et al. 2007; Splivallo et al. 2007b; Grosshauser and Schieberle 2013; Taşkin et al. 2013; Dickschat 2014; Radványi et al. 2015; Liu and Li 2017). Olfactory tests with trained human individuals might be further sensitive measures to identify fungal scents and orthonasal odor thresholds (Grosshauser and Schieberle 2013). Effective electronic noses have been developed as devices to mimic human olfactory in VOC mixture perception and identification (Baietto et al. 2010; Abdullah et al. 2011; Fuijoka et al. 2013; Zhou et al. 2015; Zhang et al. 2016b). They use non-specific tuned sensor arrays in a non-separative mechanism where odors are perceived as global fingerprints and are compared with known odors digitized in a digital pattern recognition system (Thakeow et al. 2007). Biosensors based on antennae of insects specialized on fungi and fungal-infested substrate can be particularly useful in detection of specific fungal VOCs because of their high selectivity and sensitivity. Antennae from different insects respond differentially to specific microbial VOCs, in line with insect behavioral responses. However, reactions to linear and branched eight-carbon compounds as indicators of microbial activities are common to antennae of insects regardless of ecological preferences (Thakeow et al. 2007, 2008; Drilling and Dettner 2009; Abraham et al. 2014; Holighaus et al. 2014; Balakrishnan et al. 2017).

IV. The Mushroom Odor

Many fruiting bodies of *Agaricomycetes* have a special fungi-typical smell, commonly known as mushroom odor. The basic compounds of mushroom odor are eight-carbon volatiles of

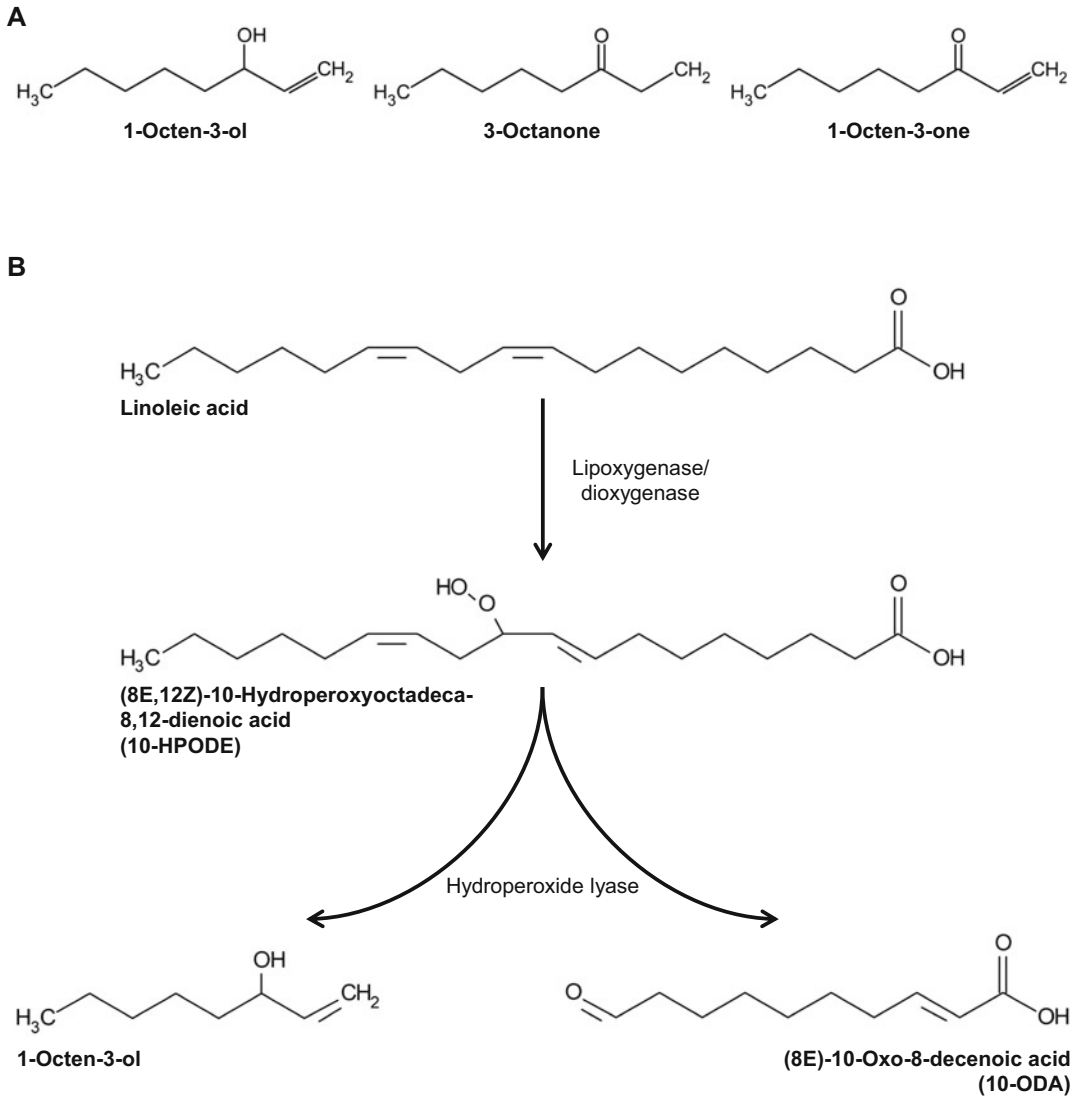


Fig. 2 (**a**) Linear eight-carbon compounds found in typical mushroom odors. (**b**) Proposed pathway of 1-octen-3-ol production in *Agaricus bisporus* (after Wurzenberger and Grosch 1982, 1984c and Combet et al. 2006)

which the linear oxylipins 1-octen-3-ol (the "mushroom alcohol" or "matsutake alcohol" as the principle compound contributing to the mushroom odor), 3-octanone, and 1-octen-3-one (Fig. 2a) present main compounds as oxidation products of fatty acids (Combet et al. 2006). The edible Champignon de Paris, *Agaricus bisporus* (button mushroom as common name), for example, emits significant amounts of these compounds as key odorants, together with other minor oxylipins (Maga 1981; Tressl et al. 1982; Mau et al. 1992; Cruz et al. 1997; Venkateshwarlu et al. 1999; Zawirska-Wojtasiak et al. 2007; Combet et al. 2009; Fuijoka et al. 2013). 44.3–97.6% of total volatile fractions are reported, while calculated values depend much on the methods used for extraction (Combet et al. 2009).

1-Octen-3-ol dominates in solvent extracts from disrupted whole-cell samples and may be produced in part due to cellular damage. Using intact sporophores and SPME for VOC detection,

3-octanone is however the main compound detected in the mushroom headspace (Combet et al. 2009). Further kinds of volatiles add to the mushroom scent (Wąsowicz 1974; Sulkowska and Kaminski 1974; Dijkstra and Wikén 1976; Çağlarirmak 2008; Grosshauser and Schieberle 2013; Ashmore et al. 2014) and may distinguish (as taxonomy keys) in composition and olfactorial perception between different *Agaricus* species (Wood et al. 1990, 1998; Wood and Largent 1999; Rapior et al. 2002; Callac and Guinberteau 2005; Chen et al. 2015).

Wurzenberger and Grosch (1982, 1984a, b, c) concluded for *A. bisporus* that 1-octen-3-ol and (8E)-10-oxo-trans-8-decenoic acid (10-ODA) are enzymatic breakdown products of the 18-carbon-long linoleic acid as the major fatty acid of the fungus (Fig. 2b). A lipoxygenase, respectively, a dioxygenase, and a hydroperoxide lyase are proposed to be responsible for the oxidative cleavage of linoleic acid (Mau et al. 1993; Husson et al. 2001; Morawicki et al. 2005; Combet et al. 2009).

So far, a lipoxygenase with high specificity toward linoleic acid but with 13(*S*)-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HPODE; Fig. 2b) as main oxidative product was purified and characterized from the oyster mushroom *Pleurotus ostreatus* (Kuribayashi et al. 2002). Lipoxygenase LOX(Psa)1 from the related *Pleurotus sapidus* (Fraatz et al. 2009b; Leonhardt et al. 2013; Plagemann et al. 2013, 2014), more specifically defined as dioxygenase with lipoxygenase-type oxidations (Krügener et al. 2010; Plagemann et al. 2013), also uses linoleic acid as substrate to yield 13-HPODE and, as an additional unusual substrate, the sesquiterpene (+)-valencene which is converted by the enzyme into the grapefruit aroma (+)-nootkatone. Further work in *Pleurotus pulmonarius* suggests the presence of two distinct lipoxygenase-type enzymes for the production of 13-HPODE and 1-octen-3-ol (Assaf et al. 1995, 1997). A confirmed intermediate product in 1-octen-3-ol formation in *A. bisporus* is 10-hydroperoxyocta-decadienoic acid (10-HPODE; Wurzenberger and Grosch 1982, 1984a, b, c) which in three further reaction steps can lead to 1-octen-3-ol (Brodhun and Feussner 2011). Analogously to enzymatic production of 1-octen-3-ol and 10-ODA from linoleic acid (Fig. 2b), 10-ODA in *A. bisporus* can alternatively derive from linolenic acid (from the hydroxyperoxid isomer 10-hydroxy-*E*8,*Z*-12,*Z*-15-octadecatrienoic acid, 10-HPOT), together with the two octadienols 1,*Z*-5-octadien-3-ol and *Z*-2, *Z*-5-octadien-1-ol (Tressl et al. 1982; Wurzenberger and Grosch 1986). Further, reductase activities in *A. bisporus* lead from 1-octen-3-one to 1-octen-3-ol and 3-octanone (Chen and Wu 1984).

The amounts of emitted eight-carbon volatiles vary much over the lifetime of a fruiting body of *A. bisporus* (Mau et al. 1993; Cruz et al. 1997; Combet et al. 2009), an observation which serves in food business as quality measure of freshness and maturity of the mushroom crop (Eastwood and Burton 2002). Eight-carbon compounds rise in abundance in early fruiting steps and 3-octanone further during sporulation (Mau et al. 1993; Cruz et al. 1997; Combet et al. 2009). Biologically, emission of eight-carbon volatiles serves both, intra-colony communication and communication with organisms of other types.

Fruiting body appearance and the fungal smell are two factors which are used by human consumers to assess the quality of button mushrooms, while preferences for young closed mushrooms (such as typically preferred in Germany) over matured open mushrooms with fuller odors (portobello mushrooms as common in supermarkets in Britain) may mislead consumers in terms of richness and quality of full aroma and taste (Eastwood and Burton 2002). Storage conditions, food processing, and microbial infections can alter the mushroom scents by reducing strengths of VOCs or by addition of other VOCs including also off-odors (Mau et al. 1993; Soler-Rivas et al. 1999; Combet et al. 2009; Grosshauser and Schieberle 2013; Pei et al. 2016; Radványi et al. 2015, 2016; Djekic et al. 2017).

A. 1-Octen-3-ol as Autoregulator of Fruiting Body Development

1-Octen-3-ol (Fig. 2a) in *A. bisporus* is an effective **autoregulator** for regulation of initiation of fruiting body development (Noble et al. 2009; Eastwood et al. 2013). As a first step toward fruiting, *A. bisporus* hyphae aggregate into mycelial cords. Primary hyphal knots (PKs) arise on these by intense localized branching. Further development turns them into circular secondary hyphal knots (SKs) which are 1–2 mm in diameter. Subsequent tissue differentiation leads to primordia (pinheads), but only some of them will differentiate into mature mushrooms (Umar and van Griensven 1997; Morin et al. 2012; Eastwood et al. 2013; Straatsma et al. 2013; Kües and Navarro-Gonzaléz 2015; Fig. 3a). 1-Octen-3-ol and temperature signals are crucial but independent

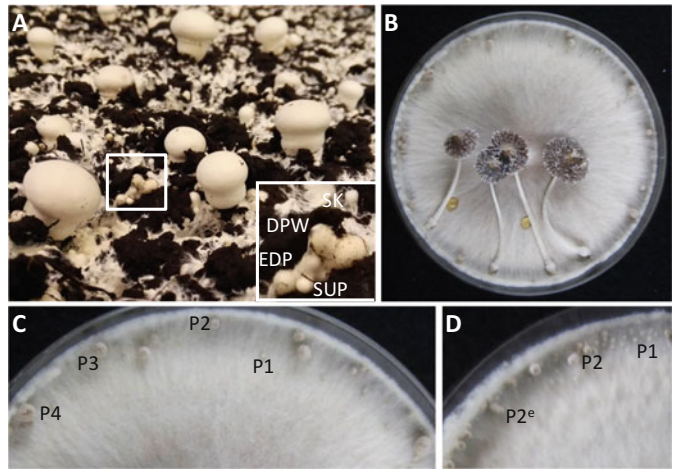


Fig. 3 Fruiting body development of *Agaricus bisporus* and *Coprinopsis cinerea*. (**a**) *A. bisporus* culture on a casing layer with early structures in fruiting body development (pinheads) and young fruiting bodies (buttons). The inset shows an enlarged view on secondary hyphal knots (SK) and arrested pinhead structures, further differentiated into smooth undifferentiated primordia (SUP), elongated differentiated primordia (EDP), and differentiated primordia with waist (DWP), after Eastwood et al. (2013). (**b**) and (**c**) Culture of *C. cinerea* with matured fruiting bodies and primordia of stages P1–P4 (shown enlarged in the sector of the culture presented in **c**) which, after synchronized secondary hyphal knot formation, successively on consecutive days aborted development in favor of the finally only four remaining that reached stage P5 and matured into fruiting bodies (Kües and Navarro-Gonzaléz 2015; Kües et al. 2016b). (**d**) Lack of aeration leads to block in primordia development. Primordia which were still active at the day of Petri dish closure with Parafilm (here after the P1 stage had been reached) can show some unusual elongated stipe growth ($P2^e$) while the caps arrest in development (Subba and Kües, unpublished)

master regulators of the early events in fruiting, while they act at distinct steps in development. The decision of primary hyphal knot development to occur on the vegetative mycelium is suppressed in the presence of 1-octen-3-ol at a level of 350 ppm (2 mg/l air). In the further development, temperature reduction (from 25 to 18 °C) controls the entry from early smooth undifferentiated primordia (SUP) into primordial tissue differentiation, while an increase in CO_2 concentration leads to arrest in development of elongated differentiated primordia (EDP) and differentiated primordia with waist (DWP) at the pinhead stage and affects the absolute number of mature fruiting bodies (Flegg 1979; Noble et al. 2009; Berendsen et al. 2013; Eastwood et al. 2013; Fig. 3a). Sufficient ventilation can relieve from the repression of initiation of fruiting body development by 1-octen-3-ol and also from the block in progressing of primordia development by CO_2 (Noble et al. 2009).

1-Octen-3-ol is produced by *A. bisporus* in the vegetative mycelium and during fruiting (Pfeil and Mumma 1992; Mau et al. 1997; Cruz et al. 1997; Combet et al. 2009; Noble et al. 2009), especially at the young developmental stages (stage 1 = pin, 4–5 mm; stage 2 = button, 20–30 mm in diameter with closed caps and non-stretched veil; stage 3 = closed caps, medium stage, 35–50 mm in diameter with still closed veil; Hammond and Nichols 1975; Hayes 1978; Kües and Navarro-Gonzaléz 2015). In commercial button mushroom cultivation, casing (i.e., addition of a nutrient-poor peat- or compost-based top layer onto the growth substrate when fully colonized by the vegetative mycelium) is required as essential step for efficient fruiting body induction (Flegg and Wood 1985; Rühl and Kües 2007). 1-Octen-3-ol as negative autoregulator of fruiting is selectively absorbed by the casing layer but not the also produced 3-octanone (Pfeil and Mumma 1992; Noble et al. 2009).

Timings and places of 1-octen-3-ol production as of CO_2 emission during *A. bisporus* cultivation make an autoregulatory function

well conceivable, in order to define and synchronize in the growing mycelium the ideal start of fruiting and in order to save, relocate, and use valuable resources in support of the only 5–10% structures selected to complete the fruiting body development for spore production (Mau et al. 1993; Cruz et al. 1997; Noble et al. 2017; Combet et al. 2009), as response to avoid competition for specific nutrition between too many and too irregularly outgrowing primordia (Straatsma et al. 2013). A number of genes are induced in cultures under high 1-octen-3-ol concentration (among genes for a Frt1-type repressor of dikaryotic genes, various metabolic enzymes, glutathione transferase, and two specific hydrophobins), and some others (including genes for isocitrate lyase and two specific hydrophobins) are repressed (Eastwood et al. 2013). The significance of regulation of these specific genes by 1-octen-3-ol is however yet unclear. Continuous exposure of cultures with 1-octen-3-ol negatively affects the radial mycelial growth of *A. bisporus* colonies (Berendsen et al. 2013).

Emitted 1-octen-3-ol has further jobs in controlling development of *A. bisporus* fruiting bodies. 1-Octen-3-ol stimulates growth of supportive *Pseudomonas* species which in turn degrade the fruiting-inhibiting volatile 1-octen-3-ol and also other inhibitory VOCs (e.g., 2-ethyl-1-hexanol released from growth substrate), thereby promoting the frequency of initiation of fruiting of the fungus (Noble et al. 2009; Berendsen et al. 2013). On the contrary, 1-octen-3-ol acts as inhibitor of germination of conidiospores of the ascomycetous mycopathogen *Lecanicillium fungicola* which causes dry bubble disease by infection of fruiting bodies. *L. fungicola* is however unable to germinate in mycelial cultures of *A. bisporus* and to attack these, possibly through a hindrance by 1-octen-3-ol emission. A block in fruiting of *A. bisporus* by 1-octen-3-ol will additionally protect the fungal colony from the mycopathogen by not opening a portal for entry for *L. fungicola* (Calonje et al. 2000; Bernardo et al. 2004; Berendsen et al. 2012, 2013).

Other ascomycetous mycopathogens of *A. bisporus* (*Mycogone perniciosa*, *Trichoderma aggressivum*) are similarly sensitive to 1-octen-3-ol (Berendsen et al. 2013). Damaged mushrooms produce even more 1-octen-3-ol (Combet et al. 2009), likely as wound-activated chemical protection against entry of harmful molds and bacteria (Beltran-Garcia et al. 1997; Okull et al. 2003; Spiteller 2008). Although 3-octanone (Fig. 2a) does not affect fruiting in *A. bisporus* (Noble et al. 2009), the compound can also inhibit *L. fungicola* (Berendsen et al. 2013) and other *Ascomycetes* (Nidiry 2001). 10-ODA produced by *A. bisporus* together with 1-octen-3-ol has also antifungal activities (Okull et al. 2003; Spiteller 2008). This oxylipin has autoregulatory functions and stimulates mycelial growth and stipe elongation of *A. bisporus* and apparently also the initiation of fruiting body development (Mau et al. 1992; Mau and Beelman 1996; Champavier et al. 2000).

Chen et al. (2013) reported ethylene production by *A. bisporus* as another autoregulatory VOC with inhibitory effects on mycelial growth and on primordium initiation. In co-culture with *Pseudomonas putida*, the bacteria attach to fungal hyphae, consume produced ethylene, and in turn stimulate hyphal growth and primordium formation (Chen et al. 2013; Meng et al. 2014; Zhang et al. 2016a).

Our group recently observed that blocks in aeration hinder initiation of fruiting body development and halt primordia development in the ink cap *Coprinopsis cinerea* (Subba and Kües unpublished; Fig. 3d). It is yet unclear whether these blocks are due to an accumulation of emitted volatile compounds in the headspace of undisturbed fungal cultures or due to accumulation of CO_2 known in various *Agaricales* including *A. bisporus* to block fruiting body development (Niederpruem 1963; Flegg and Wood 1985; Kamra and Zadražil 1986; Kües and Liu 2000; Oei 2003). Like the button mushroom, *C. cinerea* initiates fruiting at multiple places on its substrates, but many of the initial structures will be given up every day during the phases of tissue differentiation in primordia development (successive primordial stages P1–P4; Fig. 3c) in favor of a few that reach the final but still closed primordial stage P5 in which all tissues and cells are established, karyogamy in basidia happened, and meiosis for further progress toward basidiospore production

is initiated and which will finally mature into fully developed fruiting bodies with elongated stipes and opened caps with dark-brown-stained basidiospores on their gill surfaces (Madelin 1956; Navarro-Gonzaléz 2008; Kües and Navarro-Gonzaléz 2015; Kües et al. 2016b; Fig. 3b and c). Similar as in the button mushroom, autoregulatory VOCs could have also a role in the control of progress of development in *C. cinerea*. 1-Octen-3-ol emission increases upon initiation of fruiting body development in *C. cinerea* with highest concentrations being emitted during the days of tissue formation in the primordia (up to primordial stage P4). 3-Octanone emissions at the same time go down (Chaisaena 2008; Thakeow 2008).

B. 1-Octen-3-ol and Other Eight-Carbon Volatiles as Allelochemicals in Communication with Invertebrates

1-Octen-3-ol (Fig. 2a) and other eight-carbon compounds are very potent in attracting insects, mites, and other invertebrates to mycelia and fungal fruiting bodies, both **fungivores** and **fungivore predators** following direct and indirect signaling of potential food resources (Pierce et al. 1991; Fäldt et al. 1999; Thakeow et al. 2007, 2008; Chaisaena 2008; Thakeow 2008). With and without a further aspect of food, sexual reproduction is another reason to be attracted by fungal odors. Fruiting bodies or fungal-infested substrates can be ideal places for **oviposition** (laying eggs) by invertebrates. They can provide an ideal material consistency for oviposition, can shield eggs and the brood by good insulation for keeping an ideal temperature, and will provide good aeration and protect against potential water soaking by porous mycelial tissues and hyphal surface repellency, respectively, and, of course, they can also serve as food sources for the brood (Jonsell and Nordlander 2004; Thakeow et al. 2007; Halbwachs et al. 2015; Holighaus and Rohlfs 2016; Kobayashi et al. 2017). Further, finding fruiting bodies of wood-decaying fungi can lead the animals to wooden substrate (Thakeow et al. 2007).

The 1-octen-3-ol as part of the typical mushroom odor serves to specifically attract, e.g., insects to mushrooms, such as the mycophagous beetle *Cis boleti* to the fruiting bodies of *Trametes gibbosa* (Thakeow 2008; Thakeow et al. 2008), the beetles *Sulcacis affinis* and *Tritoma bipustulata* living in *Trametes versicolor* (Drilling and Dettner 2009), and various other beetles of a saproxylic (= dependent on dead or decaying wood or on other wood-inhabiting taxa) lifestyle and the predator on fungus-insects *Lordithon lunulatus* to fruiting bodies of wood-decaying polypores (Fäldt et al. 1999). Among other eight-carbon compounds from mushrooms (*cis*-octa-1,5-dien-3-ol, *trans*-octa-1,5-dien-3-ol, 3-octanone; Vanhealen et al. 1980; Chaisaena 2008), 1-octen-3-ol is also an attractant for various mites, for instance, the mushroom mite *Tyrophagus putrescentiae* (also known as house, dust, and storage mite; Fig. 4b). Mushroom mites enter emitting fungal cultures for consumption of mycelium (Fig. 4). Grazing damages the mycelial layer (Fig. 4b–e), which in turn causes wound-induced increased 1-octen-3-ol production and leads to aggregation of the mites for grazing at the wounded places (Chaisaena 2008; Navarro-Gonzaléz 2008; Singhadaung et al. 2016; Fig. 4c).

An interesting case in parallel of mushroom signaling to invertebrates is the visual and olfactory mushroom mimicry of *Dracula* orchids (Kaiser 1993, 2006; Policha et al. 2016). At least 11 different species of mushroom-visiting drosophilid flies specifically visit flowers of *Dracula lafleurii*. In order to attract the mycophilous flies for pollination, the flowers use both as traits, their mushroom look and their mushroom-like odor (Endara et al. 2010; Policha et al. 2016). The *R*-(−) enantiomer of 1-octen-3-ol is the dominant compound in the flower's scent (Policha et al. 2016) as it is in mushroom odors (Zawirska-Wojtasiak 2004; Thakeow et al. 2008; Policha et al. 2016). Emission of 1-octen-3-ol by the orchid is restricted to the faked gilled labellum of the flowers (Policha et al. 2016).

Another example of simultaneous visual and chemical mushroom mimicry is the South American tropical understory tree *Duguetia cadaverica* with foul-smelling flowers resembling in fragrance compositions (1-octen-3-ol, 3-octanone, (*E*)-2-octenol, 4-methylpentanoic acid, indole, and sulfides) the putrid-smelling stinkhorns such as *Phallus impudicus* (Fig. 5) and *Clathrus archeri* (Borg-Karlson et al. 1994; Johnson and Jürgens 2010; Kaiser 2006; Teichert et al. 2012; Sect.

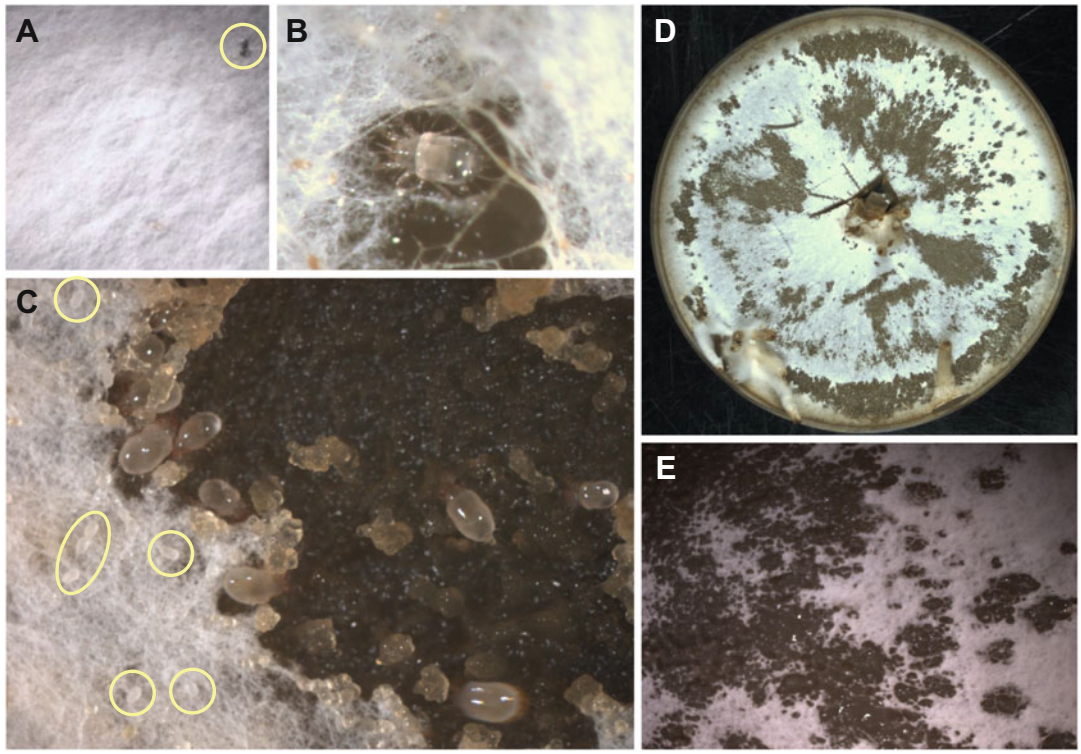


Fig. 4 Mushroom (dust) mites of the species *Tyrophagus putrescentiae* are easily attracted to fungal cultures in order to consume the obviously tasty mycelium of the fungus *Coprinopsis cinerea*. (**a**) A tiny hole (encircled) in the mycelium of a vegetative culture indicates first infestation with a mite. (**b**) A mite feeds on hyphae in such small mycelial perforation. (**c**) Aggregates of grazing mites in a mycelial perforation of increasing diameter. Note the eggs of the mites (encircled) on the mycelial surface and the clumps of unstained feces on the grazed agar surface in between the mites. (**d**) Overview and (**e**) enlarged sector of a *C. cinerea* culture infected by mites: Perforations in the mycelial carpet indicate loss of mycelium by grazing mites. From Kües and Navarro-Gonzaléz (2009), with alterations

VII). The strongly scented pollination chambers of the flowers of the tree with spongy stinkhorn-like tissue at the entry are visited in mornings by a beetle species of the genus *Pycnocnemus* serving as putative pollinator (Teichert et al. 2012). Beetles of this genus belong to the *Oxycnemus* complex which grow and develop in large fruiting bodies of *Agaricomycetes*. Many of them are specialists on stinkhorns (Leschen 1999; Teichert et al. 2012; see Sect. VII).

Female and male sexes might differentially react on VOCs. 1-Octen-3-ol acts on its own as deterrent to both sexes of the mushroom pest fly *Megaselia halteria*, while in combination with other eight-carbon compounds (3-octanone, 1-octen-3-one), it may attract specifically females to volatile-producing *A. bisporus* mushroom culturing beds for oviposition (Grove and Blight 1983; Pfeil and Mumma 1993; Tibbles et al. 2005). In accordance with observed mycelial VOC production patterns (see Sect. IV.A), *M. halteria* in high numbers is attracted to mushroom compost with fully grown mycelium while casing cuts down on *M. halteria* infestations (Scheepmaker et al. 1996).

M. halteria is one possible attracted pest which is feared in mushroom cultivation. However, it is not always 1-octen-3-ol that leads the way of pests to the mushrooms. The sciarid mushroom fly *Lycoriella ingenua* as one most severe pest in mushroom cultivation is not attracted by *A. bisporus* mycelium themselves. Instead, VOCs produced by microbes in the mushroom composts and, if present, VOCs provided by the mycoparasitic green mold *T. aggressivum* and other

Fig. 5 *Phallus impudicus*. Dried specimen from the historical mushroom collection of the Faculty of Forest Sciences and Forest Ecology in the Department of Molecular Wood Biotechnology and Technical Mycology of the University of Goettingen

ascomycetous contaminants in mushroom compost lead the female fungus gnats to the *A. bisporus* mycelium (Scheepmaker et al. 1996; Cloonan et al. 2016a, b).

For the fungivorous *C. boleti* and *S. affinis* and for various saproxylic generalist species living in rotten wood (*Malthodes fuscus*, *Anaspis marginicollis*, and *Anaspis rufilabris*), also preferentially female beetles are attracted by 1-octen-3-ol (Fäldt et al. 1999; Thakeow et al. 2008). Interestingly in the case of *C. boleti*, it is specifically the (*S*)-(+)-1-octen-3-ol enantiomer as minor VOC (7% of total 1-octen-3-ol) emitted from the *T. gibbosa* brackets which attracts the females (Thakeow et al. 2008). Mate finding by males of such species is then mediated through insect sexual pheromones, while in other saproxylic beetle species, both sexes are attracted by the host odor (Jonsson et al. 2003). Fäldt et al. (1999) found that attraction by 1-octen-3-ol in combination with octane-3-one was sex-independent for the predator *L. lunulatus* visiting polypore mushrooms for insect prey hunting. Further, both sexes of the leaf miner moth *Epinotia tedella* (larvae are specialized on spruce needles) react on 1-octen-3-ol (Fäldt et al. 1999).

Concentration influences the behavior of the invertebrates. A same compound might act as attractant and as repellent for a same animal. While being an attractant, 1-octen-3-ol at higher concentrations can thus repel the mushroom phorid fly *M. halteria* (Pfeil and Mumma 1993), fungus gnats like *Bradysia* sp. nr. *coprophila* (Cloyd et al. 2011), fungivorous beetles as exemplified for *Bolitophagus reticulatus* (Holighaus et al. 2014), mycophagous *Collembola* as *Proisotoma minuta* (Sawahata et al. 2008), and also fungivorous slugs (Wood et al. 2001). However, experiments for repellency tend to use unnaturally high concentrations of the VOCs. Their actual relevance for processes in nature is unclear (Thakeow et al. 2007; Kües and Navarro-Gonzaléz 2009; Holighaus and Rohlfs

2016). Contrariwise however in nature, actual concentrations of eight-carbon volatiles emitted by a fungus might communicate the right time of arrival to an animal or might be used to differentiate host from nonhost fungal species. In addition, more than one VOC might be used in additive manner for repellency, or VOCs with contrasting behavioral responses could counterbalance each other.

As coacting repellents and antifeedants, 1-octen-3-ol together with clitolactone, 5-(chloromethyl)-3-methyl-2 (5H)-furanone can protect different *Clitocybe* mushrooms from the mycophagous banana slug *Ariolimax columbianus* (Wood et al. 2001, 2004). 1-Octen-3-ol and methyl cinnamate were shown in combination to repel the mycophagous collembolan *P. minuta* from *Tricholoma matsutake* fruiting bodies (Sawahata et al. 2008). In case of *B. reticulatus*, 1-octen-3-ol is a repellent and 3-octanone an attractant (Holighaus et al. 2014). Ethanol is also an attractant to beetles of this species (Jonsell et al. 2003). Mushroom odors in synergy with ethanol emitted from decaying wooden substrate (Gara et al. 1993; Jonsell et al. 2003) convey the actual status of mushrooms to the beetles. *B. reticulatus* is not much attracted to fresh and young fruiting bodies which release higher amounts of the repellent 1-octen-3-ol in contrast to partially dead brackets which release highest amounts of 3-octanone (Jonsell et al. 2003; Holighaus et al. 2014).

V. Other Mushroom Volatiles, Odor Bouquets, and Invertebrates

Main volatile compounds emitted from fresh mature mushrooms are usually 1-octen-3-ol and 3-octanone (Fig. 2a) in varied concentrations (Mau et al. 1997, 1998; Venkateshwarlu et al. 1999; Fons et al. 2003; Wu et al. 2005a, b; Chaisaena 2008; Thakeow 2008; Zhang et al. 2008; Piovano et al. 2009; Szumny et al. 2010; Holighaus et al. 2014; Bozok et al. 2015, 2017; see above Sect. IV), while the absolute emissions and relative amounts of the compounds can also be different between caps and their gills and stipes (Noël-Suberville et al. 1996; Cho et al. 2006, 2008). Many other additional VOCs of different chemical kinds variably specify the scents of individual mushroom species (see, e.g., Pyysalo 1976; Fons et al. 2003; Rapior et al. 1997, 1998, 2000, 2002, 2003; Boustie et al. 2005; De Pinho et al. 2008; Ouzouni et al. 2009; Malheiro et al. 2013; Kleofas et al. 2015; Zhou et al. 2015), although geographical origins, genetic differences, and nutrient compositions in growth substrates can have influences on the individual scents (Wu et al. 2005a; Omarini et al. 2010; Lin et al. 2011; Ding and Hou 2012; Li et al. 2016b). Odor compositions change from mycelium to sporocarps, during the different stages in the process of fruiting body development and during aging, as, e.g., reported for *A. bisporus* (see above Sect. IV. A), *C. cinerea* (Chaisaena 2008; Thakeow 2008), *Polyporus sulphureus* (Wu et al. 2005a), *Taiwanofungus camphoratus* (Lin et al. 2011, 2017), *T. matsutake* (Wood and Lefreve 2007; Li et al. 2016b), and *Volvariella volvacea* (Mau et al. 1997).

A. Feeding and Breeding

Different invertebrates are attracted over the process of development and the lifetime of mushrooms to a fungus, **specialists** focusing on one or a few fungal species and **generalists** found on many different species (Figs. 6 and 7a, d, and e) and probably also many indifferent visitors which drop in by chance. Generalists such as the slug *Arion* sp. (Figs. 6, 7a) in need to find their food including mushrooms by a fine sense for faint gaseous smells (Buller 1909, 1922) may more likely (although not exclusively) react on general odors such as the eight-carbon compounds of the typical mushroom scent and visit detected mushrooms as a valuable source for food. Among fungivorous insects, there is a tendency that the strength of host specificities (**monophagy** or **oligophagy** feeding on one or a few related species) links to longer life spans of fruiting bodies (e.g., annual fruiting bodies and perennial conks of polypores compared to ephemeral agaric mushrooms) with monophagous species being early colonizers, while **polyphagy** (feeding on fruiting bodies of more than one host family/order; Põldmaa et al. 2016) is typical for consumption of short-lived mushrooms (such as ephemeral fleshy agarics) by insects and for phases in development of mushroom decay, with the exception of the short-lived *Boletes*- which host more dipteran specialists than

Fig. 6 The slug *Arion* sp. is attracted by an aging *Coprinopsis lagopus* mushroom, heads straight toward the mushroom, and consumes much of the spore-bearing cap and the utmost upper parts of the stipe onto which loads of the black basidiospores were fallen, to after the meal turn away from the remains of the fruiting body without further considering the main part of the stipe or any other *C. lagopus* mushrooms from the same colony carrying only few nutrient-rich spores. Times of the series of observations (at a sunny, mild late August morning) are indicated. Insets show the mushroom cap prior and after grazing by the slug

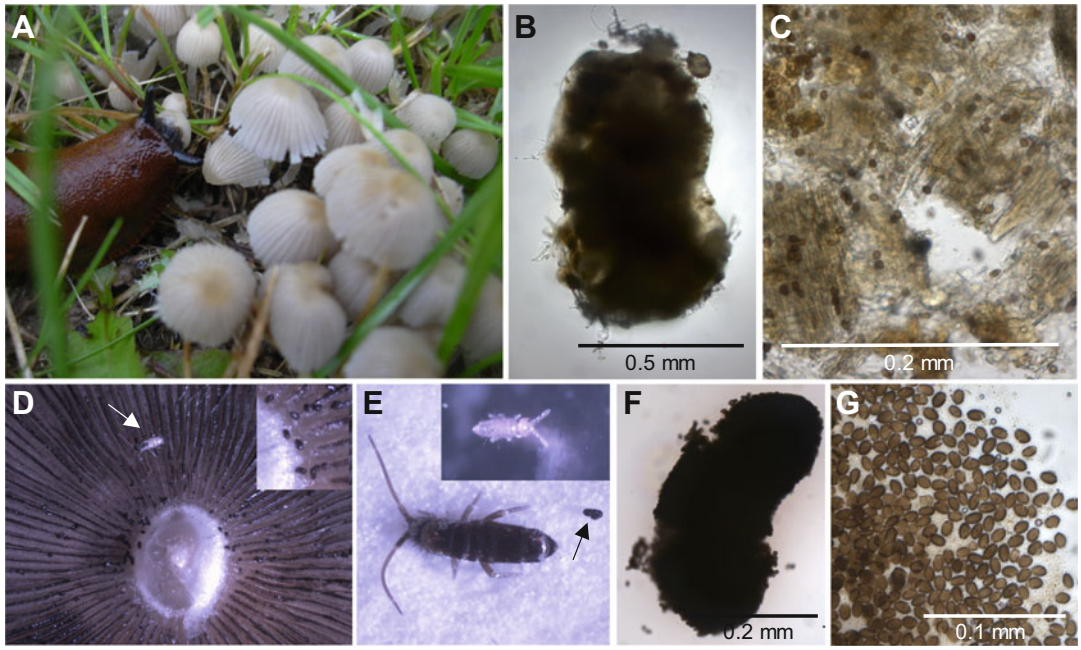


Fig. 7 (**a**) The slug *Arion* sp. grazing on tufts of fruiting bodies of *Coprinellus disseminatus* grown on a meadow. (**b**–**f**) An experiment for baiting animals to fresh fungal *Coprinopsis cinerea* cultures with mushrooms: Petri dish cultures of *C. cinerea* containing fully developed primordia (progressing P5 stage) ready to undergo overnight fruiting body maturation (see Fig. 3b) were moved either with covering lids or without lids at an evening in the later summer (in August) into a meadow underneath some bushes. Overnight, the fruiting bodies matured and night-active slugs and springtails were apparently attracted to the cultures for consuming the fungus. Slugs appeared to have consumed the complete culture with mycelium and fruiting bodies inclusive of the basidiospores as well as the agar. Fecal pellets (**b**) left in the open Petri dishes contained hyphal fragments as well as masses of dark brown-pigmented basidiospores (**c**), suggesting that the fruiting bodies were fully developed before they were consumed. While the lids of closed Petri dishes (with vents) hindered larger animals reaching the fungal cultures, larvae and adult stages of collembolans were found in the cultures either at the underside of the opened mushroom cap (**d** see tip of arrow; inset: enlarged view on the transition between fruiting body gills and the centered stipe reveals many black-stained fecal pellets laid onto the mushroom tissues) or within the still present vegetative mycelium on the agar (see examples in **e**; the arrow in the figure points at a fecal pellet laid by the adult animal). Also the collembolans consumed the basidiospores, and loads of intact spores are seen in the squeezed fecal pellets (**f**, **g**). Reproduced from Kües and Navarro-Gonzaléz (2009)

other taxa of mushrooms (Hackman and Meinander 1979; Lacy 1984; Bruns 1984; Jonsell and Nordlander 2004; Yamashita and Hijii 2007b; Jakolev 2012; Leather et al. 2014; Thorn et al. 2015; Jonsell et al. 2016; Põldmaa et al. 2016).

Fungivory is expected to usually have rather negative to in the best case neutral impacts on the fungus when eaten mushrooms are immature and still without spores. In case of mature fruiting bodies, an impact of animal feeding might be negative or positive on the fungal fitness, depending on whether spores are digested or whether spores survive and are (also) distributed for germination onto new substrate and whether germination rates are even boosted up. Finally, fruiting body consumption by animals after spore shedding should be neutral for the fungus (Tuno et al. 2009). The type of feeding by animals, either on tissue parts of fruiting bodies, unselectively on all parts of the whole mushroom, or specifically on hymenial tissues with basidia and the basidiospores, plays a role for the degree of impact of feeding on young and mature mushrooms (Guevara et al. 2000c; Krivosheina 2008; Tuno et al. 2009; Kadowaki 2010a). Both generalists and specialists may variably have an influence on enhancing spore germination rates. Spores of many reluctantly germinating basidiomycetous species, especially of Russulae and Lactarii, germinate after passing the digestive tracts of certain slugs as generalist mushroom consumers, and that can even happen after toads have eaten the slugs (Buller 1909).

Fungivorous insects may feed as adults or in young developmental life stages on fruiting bodies. Chemical cues reporting the mushroom status and probably active chemical defenses in younger mushrooms will influence the arrivals of the fungivorous insects (Jonsell and Nordlander 2004). Further, specialists which seek mushrooms of specific fungal species for, e.g., **breeding** (and following larval feeding on the fungi) may react differentially on very specific odor compounds and odor profiles (Epps and Arnold 2010; Kadowaki 2010a; Jonsell et al. 2016). Not surprising therefore, attraction of invertebrates to fresh mushrooms tends to be different and more specific than to decaying fruiting bodies, i.e., for **early arrivers** and for **late arrivers** (Guevara et al. 2000b; Epps and Arnold 2010; Jonsell and Nordlander 2004; Orledge and Reynolds 2005; Kadowaki 2010a; Hågvar and Steen 2013; Hågvar et al. 2014), and beetle and fly diversity increases with sporophore ages (Krivosheina 2008; Epps and Arnold 2010; Thunes and Willassen 1997; Thunes et al. 2000), while mushroom durability, size, and moisture and microclimate, distances, altitudes, and habitat configuration also have influences (Thunes and Willassen 1997; Jonsell et al. 1999, 2001, 2016; Thunes et al. 2000; Jonsell and Nordlander 2002; Komonen and Kouki 2005; Epps and Arnold 2010; Maraun et al. 2014; Kadowaki and Inouye 2015; Thorn et al. 2015). In addition, **parasitoids** (ovipositing, e.g., into host eggs or host larvae) can arrive on fruiting bodies as a third ecological insect group on mushrooms, to exist parallel to the fungivores feeding on mushrooms as adults and to the breeders with larval feeding. Nature and frequencies of parasitoid communities can be influenced by the fungal host species and by the dominant fungivorous insects (*Coleoptera*, *Lepidoptera* or also *Hymenoptera*) living on the respective fungal host (Drissen et al. 1990; Jonsell et al. 1999, 2001, 2016). However, distinct volatiles of importance selected in evolution for specific and diverse invertebrate attractions and for discrimination of mushroom species, ages of fruiting bodies, and other host's conditions including actual states of populations of invertebrates (neutral in coexistence or possible competitors or possible prey) and their dynamics (Kadowaki et al. 2011) are yet mostly unknown.

B. Beetles

Living sporocarps of the wood-decaying species *Fomitopsis pinicola*, for example, are visited by multiple host and nonhost saproxylic beetles, for instance, in search for feeding on the mushrooms or by using the fungal odor as kairomone to be directed to decaying wood required for oviposition. The undersides of living conks are especially populated by specialized spore-eating beetles. When dying or dead, specialized decomposer communities of certain fungivorous *Cis* beetles and a very diverse mite fauna with tunneling activities, among fungivorous *Carabodes* mites, are prevalent (Hågvar 1999; Thunes et al. 2000; Jonsell et al. 2001; Komonen et al. 2004; Hågvar and Steen 2013). Decaying mushrooms have less striking VOC differences, and scents become more similar to each other, thus attracting more generalist invertebrates (Thunes et al. 2000; Jonsell and Nordlander 2004; Thakeow et al. 2007). Of the multiple mite species found in *F. pinicola* fruiting bodies, trophically diverse oribatid mites dominate, while few seem to feed on the mushrooms in adult state. What attracts the mites to the brackets is unknown although some of them (*Cepheus cepheiformes*, *Haffenrefferia gilvipes*, *Siculobata leontonycha*) appear in part to feed on animal prey (Maraun et al. 2014).

Beetles of the subfamily of *Cisidae* are specialized for feeding and breeding to temporal persistent conks of polypores (Robertson et al. 2004). *Octotemnus glabriculus* and *C. boleti* are ciid tree-fungus beetles with distinct breeding behavior on fruiting bodies of *T. versicolor*. *O. glabriculus* is found in primordia, larvae appear in young expanding fruiting bodies, the adults predominate in young fresh brackets grazing on hymenia, and, later in the year, *C. boleti* beetles as secondary invaders replace *O. glabriculus* in fully developed mushrooms. The two beetle species react on age-related fungal VOC emissions and apparently distinguish in their volatile attractants (Guevara et al. 2000a, b, c). Concerning seasons of year and ages of mushrooms, amounts of 1-octen-3-ol (Fig. 2a) emitted by polypore fruiting bodies can differ by factors of >10 and >100 (Fäldt et al. 1999; Guevara et al. 2000a;

Wu et al. 2005a; Thakeow et al. 2008). For *C. boleti*, it is the emitted 1-octen-3-ol as one compound which attracts the beetles to the brackets (Thakeow et al. 2008). This attraction is probably better promoted by 1-octen-3-ol emissions from wounded mushroom tissue inflicted by the prior feeding activities of the primary invader *O. glabriculus* (Guevara et al. 2000a, c). However, the universal mushroom alcohol 1-octen-3-ol is unlikely the sole VOC of influence. The oligophagous *C. boleti* and *O. glabriculus* beetles have a narrow host range and are attracted to *T. versicolor* and other *Trametes* as preferred hosts, whereas *Ganoderma applanatum* conks are neglected (Guevara et al. 2000b; Thakeow et al. 2008). The specialist beetles *Cis glabratus* and *Cis quadridens* can discriminate between chopped basidiocarps of *F. pinicola* and *F. fomentarius* (Jonsell and Nordlander 1995). The monophagous *Cis nitidus* on the other hand is specifically attracted to *G. applanatum* fruiting bodies, while there are also other ciids, e.g., the polyphagous *Cis bilamellatus*, which show no preference for specific bracket fungi (Guevara et al. 2000b). Unique candidates in the *T. versicolor* bouquet of VOCs for attraction of the specialized beetles are the sesquiterpenes arcurcumene and α-cedrene (Guevara et al. 2000a, b; Fig. 8). In contrast, Thakeow et al. (2008) identified β-bisabolene (Fig. 8) as possible signaling sesquiterpene in the headspace of *T. gibbosa* brackets. In conclusion, two main VOC fractions are considered important in finding a fungal host by ciid beetles, eight-carbon compounds, and terpenoids (Fäldt et al. 1999; Guevara et al. 2000a, b; Thakeow et al. 2008; Drilling and Dettner 2009). Insects with narrower substrate requirements tend to not be much attracted by the common fungal eight-carbon compounds but rather by individual scents of their hosts. Individual terpenoids in the mushroom fragrances can offer such possibilities (Jonsell and Nordlander 1995; Fäldt et al. 1999; Drilling and Dettner 2009). Fäldt et al. (1999) and Holighaus et al. (2014) found the sesquiterpene protoillud-6-ene (Fig. 8) in the scent of *F. fomentarius* conks as potential invertebrate attractant. *T. versicolor* brackets emit a collection of different sesquiterpenes to which antennae of fungivorous *Coleoptera* from different beetle families react (Drilling and Dettner 2009).

Arriving of beetles on polypore conks can differ by the species, by fungal developmental stages, between the early growing stage, mature fruiting bodies, dying sporophores, and decaying conks (Setsuda 1995; Olberg and Andersen 2000; Kadowaki 2010a). Adaptation to fungal development is likely, in particular for mycophagous specialists and for predators of fungivorous insects. The predator beetle *Trogossita japonica* is thus specifically attracted to the annual sporophores of *Cryptoporus volvatus* at the time of sporulation. They parallel overwintering and new generation adults of nitidulid and tenebroid specialists (*Aphenolia pseudosoronia*, *Parabolitophagus felix*, *Ischnodactylus loripes*) which reproduce seasonally on the species (Setsuda 1995; Kadowaki 2010a, b). *C. volvatus* basidiocarps have a special morphology with an extended volva covering the hymenium with the basidiospores so that spores are not freely dispersed but accumulate on the inner surface of the sheath (Park et al. 2014). Synchronization of beetles with sporulation of their host suggests a function in spore dispersal, in accordance with the findings of high spore numbers on bodies of all beetle species visiting the mushrooms (Castello et al. 1976; Setsuda 1995; Park et al. 2014; Sect. V.D), and this further requires for efficiency the development of particular mechanisms of attraction to the fungus. Crude *n*-hexane extracts of *C. volvatus* basidiocarps with a complex mixture of mono- and sesquiterpenes, aliphatic alcohols, and ketones attracted fungivorous *I. loripes* beetles. A mixture of three compounds (*trans*-pinocarveol, isopinocamphone, Fig. 8; (*E*,*Z*)-1,2,5-undecatriene; Fig. 9) in synergy in natural fungal concentrations was found to be sufficient for beetle attraction (Hayashi et al. 1996).

C. Flies and Mites

Mushrooms might increase volatile production at times of sporulation as an adaptation to signal insects and other invertebrates feeding on spores to come (Jonsell and Nordlander

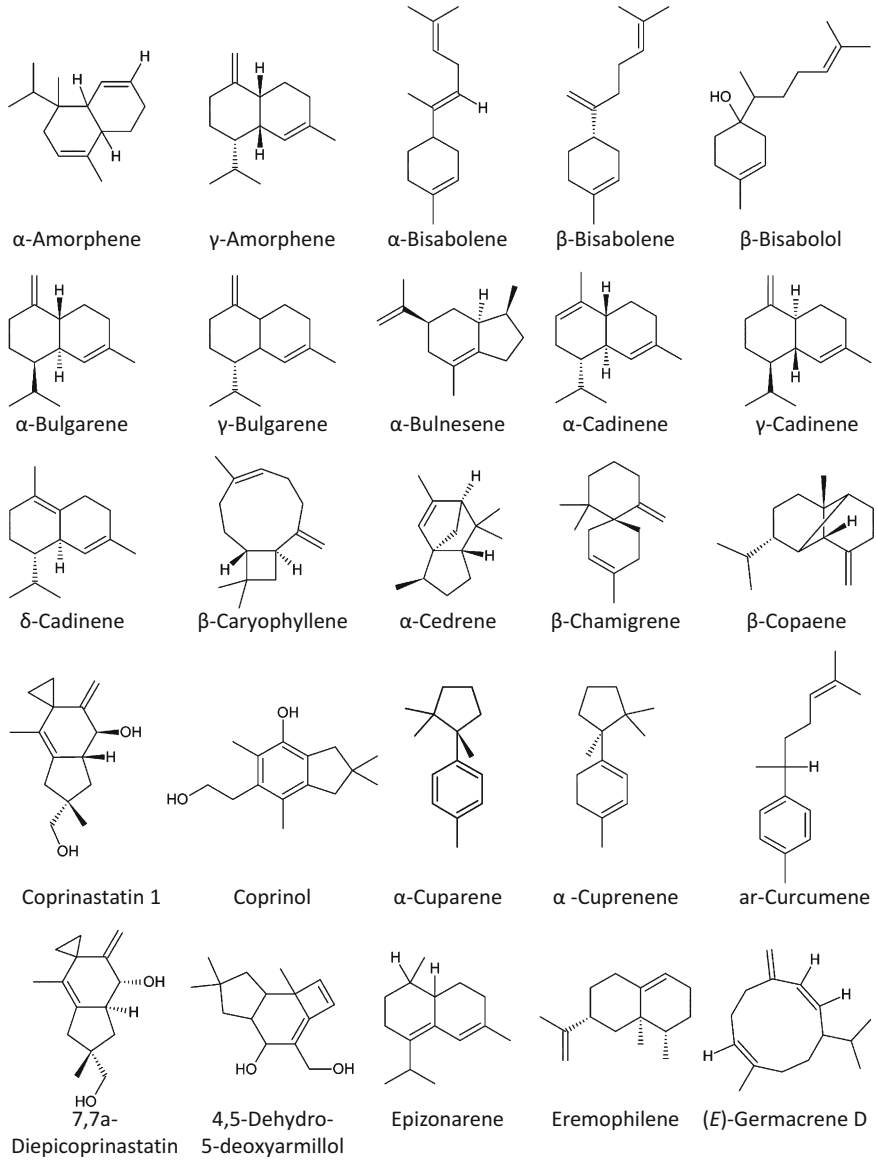


Fig. 8 Selection of sesquiterpenes and sesquiterpene alcohols from *Agaricomycetes* with possible signaling and antimicrobial functions

1995; Fäldt et al. 1999; Figs. 6 and 7a, d, e). Spore dispersal can be a good reason for sessile fungi to signal to motile invertebrates to come and serve as vectors for the spores. For distribution, spores may attach to the outer surface of invertebrate bodies, be collected for transfers in specific host organs (specific sacs on insect bodies for transfer of symbiotic fungi called mycangia), or be eaten and, if not digested, deposited in intact state with feces onto new substrate for germination (Tuno 1999; Lilleskov and Bruns 2005; Kües and Navarro-Gonzaléz 2009; Kobayashi et al. 2017; Fig. 7b, c, f, g). Parts of basidiospores of the polyporous *G. applanatum*, for example, survived passage through the digestive tract of mycophagous drosophilid flies and retained germination ability. Somewhat lower numbers of spores were in

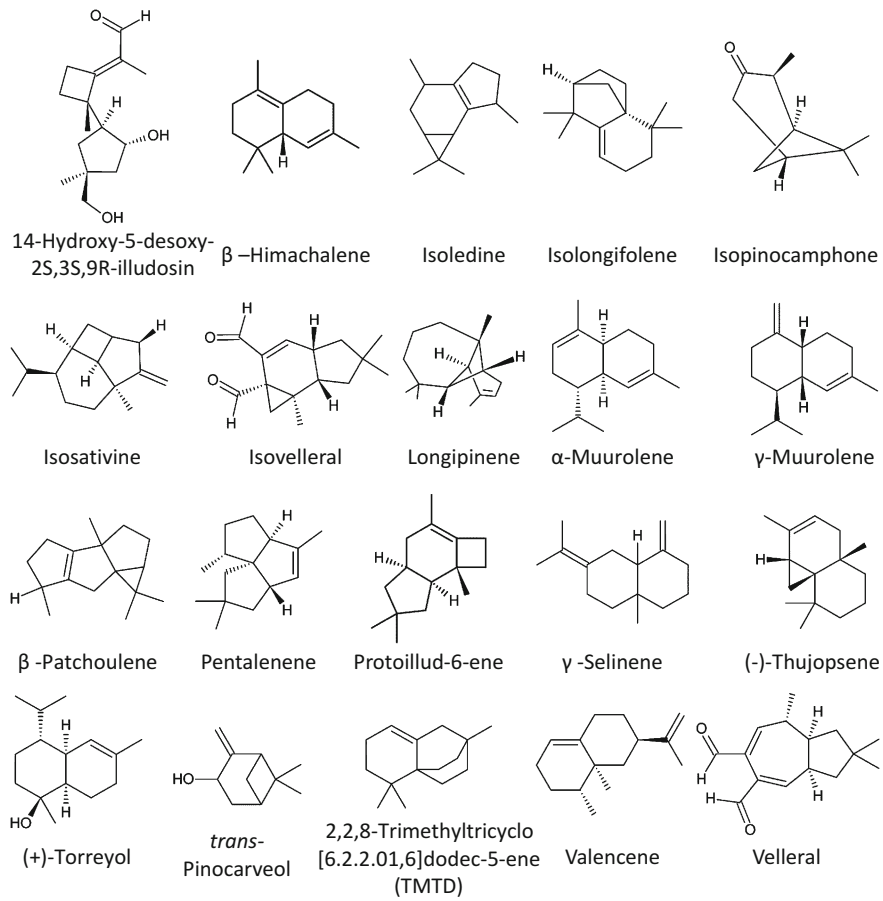


Fig. 8 (continued)

addition recorded on the exoskleleton of the flies (Tuno 1999). Survival and germination of basidiospores consumed by flies are species-dependent with regard to the consumed fungus (e.g., *Agaricus* sp., *Boletellus floriformis*, *Coprinellus micaceus*, *Coprinopsis atramentaria*, *Cortinarius purpurascens*, *Hypholoma lateritium*, *Naematoloma sublaterium*, *Pleurotus djamor*, and *Suillus granulatus* as potential survivors versus *Amanita vaginata*, *Entoloma kujuense*, *Hygrocybe conica*, *Inocybe* sp., and *Russula flavida* as fly-digested species) and to the individual mycophagous fly (e.g., *Drosophila angularis*, *Drosophila brachynephros*). Not mandatory for all species, but ingested colorless spores are more likely be digested by the flies (Kobayashi et al. 2017).

Thick cell walls (Tuno 1999; Garnica et al. 2007; Halbwachs et al. 2015), coloration such as melanin incorporation (Bloomfield and Alexander 1967; Halbwachs et al. 2015; Kobayashi et al. 2017; Fig. 7c, g), and ornamentation on spore cell walls (Garnica et al. 2007; Piattoni et al. 2014; Halbwachs et al. 2015; Beenken et al. 2016; Zambonelli et al. 2017) can be fungal spore adaptations to protect them against digestion by invertebrates. Melanized basidiospores of the dung fungus *C. cinerea* therefore easily survive consumption by diverse animal species and are found back intact in feces of various invertebrates (Buller 1931; Navarro-Gonzaléz 2008; Fig. 7b, c, f, g). Spores of the species which passed the intestine of the mite *T. putrescentiae* retained full germination ability (Navarro-Gonzaléz 2008). Signaling of spore maturity to possible fungivores may make use of more specific signals. In case of *C. cinerea*, it appears to be a sesquiterpene (proposed were

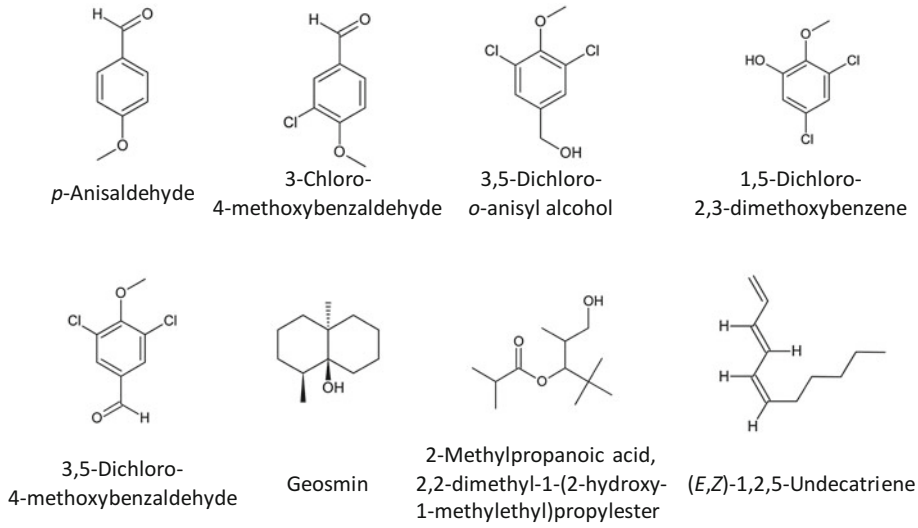


Fig. 9 Volatile semiochemicals and antimicrobial compounds from *Agaricomycetes* other than terpenes

β-himachalene and cuparene, Fig. 8) produced during basidiospore production and mushroom opening which attracts *T. putrescentiae* to leave their grazing places in the vegetative mycelium and to climb up the stipe of the quickly developing fruiting bodies to then ingest the mature melanized spores. Females lay their eggs close to feces with the spores and provide the possibility that hatching larvae might feed on newly germinated mycelium (Chaisaena 2008; Navarro-Gonzaléz 2008; Thakeow 2008).

C. cinerea is one of the few *Agaricomycetes* of which sesquiterpene synthase genes have been cloned and heterologously been expressed for enzymatic characterization (see the recent review by Kües and Badalyan 2017). Of the six different sesquiterpene synthases in *C. cinerea*, heterologously expressed enzyme Cop1 synthesizes β-elemene (as heat-induced Cope rearrangement product from germacrene A), α-muurolene (Fig. 8), δ-cadinene (Fig. 8), and germacrene D (Fig. 8); enzyme Cop2 forms β-elemene (rearrangement product), α-muurolene, δ-cadinene, and the alcohols α-cadinol and germacrene D-4-ol; enzyme Cop3 gives rise to α-muurolene (major product), β-elemene (rearrangement product), α-muurolene, germacrene D, and δ-cadinene; enzyme Cop4 synthesizes δ-cadinene (major product), β-cubebene, sativene, β-copaene (Fig. 8), cubeol, α-acoradine (major product), β-bisabolene (Fig. 8), and others; and enzyme Cop6 produces α-cuprenene (Fig. 8) as the only product, while enzyme Cop5 appears to be nonfunctional. The product range of Cop6 is however enhanced by the two cytochrome P450 monooxygenases Cox1 and Cox2 to α-cuparene (Fig. 8), α-cuparophenol, and further hydroxyl or ketone derivatives. Pentalenene, α-muurolene, α-cuprenene, and δ-cadinene (Fig. 8) are shown to be produced in vivo in the fungal mycelium (Agger et al. 2009; Lopez-Gallego et al. 2010a, b; Lauchli et al. 2014) and, in other studies, also β-caryophyllene (Wihlborg et al. 2008; Fig. 8), coprinastatin 1, 7,7a-diepicoprinastatin 1, 14-hydroxy-5-desoxy-2*S*,3*S*,9*R*-illudosin, 4,5-dehydro-5-deoxyarmillol, coprinol, and more (Pettit et al. 2010a, b; Fig. 8).

D. Springtails

While spore cell walls of certain fungal species are apparently resistant against digestion, spores may nevertheless not always survive. In tests with the collembolan *Ceratophysella* (*Hypogastrura*) *denisana*, basidiospores of 22 different tested *Agaricomycetes* species were all damaged, in best cases only 98% of the spores and in most cases 100% of the spores. Spore cell walls of, e.g., *Aureoboletus thibetanus* were found in feces, but they were cracked leaving the spores empty (Nakamori and Suzuki 2010). Species of the springtail genus *Hypogastrura* differ in the degrees of spore cell wall breaking (Nakamori and Suzuki

2005). On the other hand, *Morulina alata* as a springtail missing molar plates feeds in nature on mushrooms of *Inocybe fastigiata* and *Mycena pura*, and ingested fungal spores remain intact (Nakano et al. 2017). Spore dispersal by springtails through their guts (**endozoochory**) is thus in some cases conceivable (see also Fig. 7d–g). The species *C. denisana* consumes hymenial layers of many different agarics. Animal density defines the degree of hymenium consumption; 50% or more of the hymenial areas were found to be consumed. *C. denisana* leaves its feces with the spores attached to the gill surfaces, blocking spore dispersal by wind (Nakamori and Suzuki 2005; Sawahata 2006; compare also Fig. 7d). Terrestrial slugs are coprophagus (South 1992) and may secondarily consume the spores within the feces when grazing on the mushroom caps.

Another recent study showed that springtails equally often passively transfer fungal spores as appendages on their bodies (**epizoochory** or **ectozoochory**), providing another potential means of spore dispersal (Anslan et al. 2016). Also mushroom-visiting beetles of different species have been reported to carry spores on their bodies for dispersal to new growth substrate, in numbers as high as 10^4 to $>10^5$ (Castello et al. 1976; Park et al. 2014; Jacobsen et al. 2017). Indeed, in some fungal species, for instance, *Tomentella sublilacina*, surface properties of spores are adapted in ornaments (spines, nodules) and hydrophobicity to provide better physical adhesion to exoskeletons of invertebrates (Lilleskov and Bruns 2005; Jacobsen et al. 2017). Springtails might be consumed as prey by *Rhynchodemidae* flatworms. Spores attached to the *Collembola* were found intact back in the gut of the worms (Nakamori and Suzuki 2012). Situations of **mutualism** by endo- or epizoochory, i.e., spore dispersal in return of food, might have then been established between a specific fungus and its fungivore(s) and even possibly further extended in tri-trophic systems to the fungivore predators. Accordingly, a volatile signal emitted by a fungus to attract respective fungivores for the reason of spore dispersal or to attract fungivore predators in order to consume harmful mushroom-grazing animals and also animals with spores attached to the body for endozooic spore dispersal would then be a synomone.

E. *Ganoderma* Conks

From observations of consumption of the thick-walled but non-stained *Ganoderma lucidum* basidiospores by the obligate spore-feeder beetle *Zearagytodes maculifer*, Kadowaki et al. (2011) expect a severe negative impact for this fungal species by beetle foraging. Spore consumption by the beetle causes cracking of spore cell walls and complete breaking of spores and leads to drastically reduced spore survival with no germination. Mutualism in spore dispersal through the gut of the beetles is thus not given. A not yet analyzed and thus not excluded possibility for living spore dispersal could still be provided through endozoochory via the bodies of these beetles. Success of the related *G. applanatum* in dead wood colonization in early succession correlated in another study however positively with abundance of two fungivorous beetles, i.e., the sap beetle *Glischrochilus quadripunctatus* (European bark beetle predator) and the round fungus beetle *Agathidium nigripenne* which suggests that these two beetles disperse the fungus to new substrate (Jacobsen et al. 2015). Tuno (1999) reported that *G. applanatum* spores retain their germination ability after passage through the digestive tract of *Mycodrosophila* flies and linked modification of the outer spore wall coating (Tuno et al. 2009; see below Sect. VII.B). Coated basidiospores of *Ganoderma* species tend to poorly germinate (Nuss 1982; Kadowaki et al. 2011). Coated basidiospores of the species *Ganoderma philippii* appear to even need the passage through the digestive tract of tipulid fly larvae for germination to be activated through cell wall modification in order to after larval defecation be then possibly distributed by adult flies (Lim 1977). *Ganoderma* species can form two distinct types of basidiospores, some early proterospores in developing fruiting bodies which germinate readily and, as the majority in mature conks, coated basidiospores which hesitate to

germinate and may depend for germination on passage through insects (Nuss 1982). Morphological features of potential feeders' mouth parts (suctorial or mandibulate) will determine as one factor the further fate of ingested spores (Tuno et al. 2009; Nakamori and Suzuki 2005, 2010; Nakano et al. 2017) but not only or necessarily, as the differential results on spore-breaking capabilities by different mandibulate species of the genus *Hypogastrura* show (Nakamori and Suzuki 2005).

F. Spore Transport and Dispersal

Thus, a **conflict** is likely to arise when fruiting bodies signal spore maturity to the intended group of invertebrates which could serve for them as vectors for spore dispersal. Unless a very specific signal has been selected in coevolution between a fungus and an animal to only attract the desired useful vector, sending out a signal will likely be caught also by other unwanted organisms which may do harm to the fungus, such as by grazing on the fruiting body hymenia and by consuming and digesting the basidiospores (Guevara et al. 2000c).

Evolution may provide a fungus with other solutions to the problem. *Russula bella* and *Strobilurus ohshimae* thus produce in the hymenium in between the basidia-hair-like deadly cystidia for spore protection against springtails. When, for instance, individuals of the springtail species *C. denisana* get in physical contact with these cystidia, they will be killed. *Mitchellania horrida* springtails on the other hand feed on *S. ohshimae* and can survive when physical contacts with the toxic cystidia are only short (Nakamori and Suzuki 2007, 2008).

Depending on the kind of animal, whether of crawling nature or able to fly or perhaps using tactics for specialized passive transportation such as hijacking flying insects or adopting particular wind dispersal strategies (Clotuche et al. 2013; Perez-Leanos et al. 2017), invertebrate spore vectors will distribute spores only over short distances, less than 1 m per day in case of crawling animals and possibly some 10–100 m in case of flying insects. Spore dispersal by vertebrates with longer movements can instead overcome several meters to several kilometers per day, in correlation to the respective animal lifestyles and the dimensions of their occupied territories (Lilleskov and Bruns 2005; Halbwachs and Bässler 2015).

VI. Odors of Mushrooms and Vertebrates

Mushrooms can be part of larger animal diets. Mushroom-consuming vertebrates encompass some birds, lizards, turtles, and many kinds of smaller and larger mammals including humans (Fogel and Trappe 1978; Johnson 1996; Simpson 2000; Ashkannejhad and Horton 2006; Cooper and Vernes 2011; Schickmann et al. 2012; Wallis et al. 2012; Trierveiler-Pereira et al. 2016; Nuske et al. 2017a, b; Zambonelli et al. 2017). Mushroom consumers are categorized into 1. obligate, 2. preferential, 3. casual or opportunistic, and 4. accidental mycophagists. Digestive strategies of animals to cope with fungi influence the frequencies of mushroom consumption (Wallis et al. 2012). Basidiospores often survive consumption by vertebrates and germinate after defecation (e.g., Cork and Kenagy 1989; Claridge et al. 1992; Colgan and Claridge 2002; Ashkannejhad and Horton 2006; Frank et al. 2009; Castillo-Guevara et al. 2011). Notably, specialists feeding more reliant on fungal diets contribute disproportionally more to fungal dispersal via feces than generalists feeding on mushrooms as supplementary food source (Nuske et al. 2017a).

A. Smaller Mammals

Many inventories are available on consumption of fungal fruiting bodies and on spores in feces for smaller mammals: squirrels, lagomorphs, mice, voles, chipmunks, shrews, murids, rat kangaroos, wallabies, possums, and others. Among them are obligate, preferential, opportunistic, and also accidental mycophagists, with individual preferences for the kinds of mushrooms to be consumed (e.g., see Maser et al. 1986; Blaschke and Bäumler 1989; Johnson

1996; Tory et al. 1997; Carey et al. 2002; Wheatley 2007; Kataržyte and Kutorga 2011; Schickmann et al. 2012; Vernes et al. 2015; Nuske et al. 2017a, b; Meyer et al. 2015; Trierveiler-Pereira et al. 2016; Urban 2016). Many of the consumed fungal species are **hypogeous** (fruiting underground) or **sequestrate** (fruiting bodies are closed and spores are not forcibly discharged) that is why **mycophagy** is pivotal for the dispersal and survival of these species in nature. Spores of hypogeous fungi are significantly more often found in feces of small mammals, e.g., in feces of squirrels (e.g., *Sciurus vulgaris*; Bertolino et al. 2004), flying squirrels (*Glaucomys sabrinus*; Maser et al. 1985; Lehmkuhl et al. 2004; Wheatley 2007), and dormouse (*Glis glis*; Schickmann et al. 2012). Moreover, many of the consumed fungal species are **mycorrhizal**, whether fruiting hypogeous or **epigeous** (fruiting above ground). Endozoochory of mushroom spores by small mammals has thus a very important and not to ignore ecological role for individual fungal species and also for the general forest ecosystems. Consumption by small mobile mammals supports species distribution when spores retained germination ability after defecation. Furthermore, in some situations spore consumption can further enhance spore germination rates (e.g., Buller 1922; Cork and Kenagy 1989; Claridge et al. 1992, 1993; Colgan and Claridge 2002; Bertolino et al. 2004; Caldwell et al. 2005; Frank et al. 2009; Pérez et al. 2012). Studies on the deer mice *Peromyscus alstoni* and *Peromyscus maniculatus* and the epigeous fruiters *Laccaria trichodermophora* and *Suillus tomentosus* showed that mammals by mushroom consumption can selectively and differentially take positive or negative influence on spore germination rates and mycorrhiza formation of distinct fungal species (Castillo-Guevara et al. 2011; Pérez et al. 2012). Selective host-specific mycorrhization of North American pine seedlings in New Zealand by co-invaded *Rhizopogon* and *Suillus* genera was conferred by the brushtail possum *Trichosurus vulpecula* through feces with respective fungal spores (Wood et al. 2015).

Deer mice use olfactory cues for food sensing (Howard et al. 1968; Drickamer 1972). Particularly successful in mushroom collection are squirrels as habitual mycophagist using mushrooms as additive and seasonal food resources and for accumulation of winter stocks. By their hoarding behaviors, finding their food can be twofold for the animals, firstly as new food and secondly as buried food (Buller 1922; Urban 2016; Zambonelli et al. 2017). Volatiles lead them to detect mushrooms in the ground (Buller 1922; Fogel and Trappe 1978; Talou et al. 1990; Johnson 1996; Trappe and Claridge 2010; Splivallo et al. 2011). Visual cues and memory more than olfactory cues can later on direct the squirrels to relocate the own caches with their stored food (McQuade et al. 1986) while detection of foreign caches might again be olfactory-based (Vander Wall 2000). More mycophagous small mammals are shown to rely on odors to detect food sources in the ground (e.g., Tasmanian bettong, *Bettongia gaimardi*; flying squirrel, *G. sabrinus*; swamp wallaby, *Wallabia bicolor*) and, furthermore, to judge the quality of potential food (brushtail possum, *T. vulpecula*) (Maser et al. 1978; Maser and Maser 1988; Donaldson and Stoddart 1994; Pyare and Longland 2001; Bedoya-Pérez et al. 2014). Both VOCs emitted by the fungi and VOCs emitted from other matter usually associated with the fungi (e.g., wood) might be used for detection (Pyare and Longland 2001). Food quality might be judged by emission of specific (in some instances toxic) volatiles recorded as signal for "inedible" (Bedoya-Pérez et al. 2014). Learning to discriminate smells by small mammals must thereby not be underestimated (Rokni et al. 2014; Urban 2016).

As in the case of the invertebrates discussed in the Sect. IV.B above, it is important for the ecological success of a fungal species that ingested spores will survive the passage through the gut system of vertebrates which consume mushrooms. Spores of ectomycorrhizal species and spores of herbivore dung fungi as two important ecological groups of *Agaricomycetes* acquired particularly often protective adaptations for their cell walls against animal digestion (Halbwachs and Bässler 2015).

Coprophilous *Agaricomycetes* are often found on dung of **mycophobic** herbivores such as horses (Buller 1909,

Fig. 10 Fruiting bodies of Coprini in autolysis: (**a**–**c**) *Coprinus comatus* (Agaricaceae). (**d**) *Coprinopsis picacea* (Psathyrellaceae). (**e**) *Coprinopsis atramentaria* (Psathyrellaceae). Note the black-painted grass and herbal leaves beneath *C. comatus* in **b** and **c** and beneath *C. picacea* in **d** stained through dripping down of droplets with blackish fungal spores

1931; Pointelli et al. 1981; Richardson 2002), cattle (Richardson 2001), and elephants (Manimohan et al. 2007), nourishing on type of grasslands. In spite of the animals' **mycophobia**, the melanized thick-walled and sometimes ornamental fungal spores are adapted to passing the herbivores' guts and to germinate on their dung. It is thought that the spores are unwittingly ingested by the herbivores together with plant material consumed as diet. Fruiting bodies of dung fungi of the genera *Coprinopsis* and *Coprinellus* are short-lived and quickly autolyze their caps (Buller 1909, 1922, 1931; Kües 2000; Nagy et al. 2011, 2012; Figs. 3, 5, 6 and 8). Only minor parts of spores of mushrooms might be ejected from the cap prior to autolysis and may possibly enter into the air (Kües 2000; Lakkireddy and Kües 2017). Indeed, spores of Coprini (an artificial morphological fungal form group of cap-autolysing species which is equivalent to the dismissed former family of *Coprinaceae*; in the following cited studies not further differentiated into individual species) are commonly detected in air (see, e.g., the reports by Das and Gupta-Bhattacharya 2012; Almaguer et al. 2014; Martínez Blanco et al. 2016; Sánchez Reyes et al. 2016; Vélez-Pereira et al. 2016). Nevertheless, basidiospores of Coprini in the great majority fall directly down in liquid droplets from the caps onto the ground and substrate beneath (Buller 1909; Kües 2000; Fig. 10). From there, they might be further distributed into the closer surroundings. Spreading spores within the same grassland area by grazing fungivorous invertebrates through epi- and endozoochory from mushrooms and dung patches beneath onto freshly growing plant material in the animals' reach appears well plausible, for the herbivores to take then up deposited spores during grazing on the plants. Lilleskov and Bruns (2005) demonstrated before for the ectomycorrhizal resupinate species *T. sublilacina* that various invertebrates (mites, millipedes, centipedes, beetles) will graze on spore banks of the fungus as compacted spots of spores to then by endo- and ectozoochory locally diffuse the spores from the place into a broader area. Transferring this concept into the Coprini, invertebrate spore dispersal can thus attain an ecological role in near-distance distribution of coprophilous fungi, as an addition to the well-accepted-long-distance spore dispersal by wind (Buller 1909).

B. Larger Mammals

Several larger herbivores avoid eating mushrooms or mushroom-infested plant material and repel from stronger mushroom odors (Överås et al. 1979; Frazier et al. 2000; Kües et al. 2003; Hüttermann and Majcherczyk 2007). However, sheep have been reported to take in mushrooms and particularly young ones even to actively search for these (Warren and Mysterud 1991; Mehli and Skuterud 1998), as have also

species of deers, caribou (Launchbaugh and Urness 1992; Ashkannejhad and Horton 2006; Halbwachs and Bässler 2015), and reindeer (Inga 2007). Deers frequently contain spores of various ectomycorrhizal species in their feces, for example, of the ectomycorrhizal genera *Rhizopogon* and *Suillus*. Defecated spores can mediate mycorrhization of seedlings of their *Pinus* and *Pseudotsuga* symbionts and dropped feces can thus be an entry of tree species into new habitats. *Rhizopogon* species produce hypogeous truffle-like sporocarps and have lost in evolution the typical spore discharge mechanism of *Basidiomycetes*. They rely for dispersal on that animals will find them in the ground by emitted fungal odor (Ashkannejhad and Horton 2006; Nuñez et al. 2013; Wood et al. 2015).

Similar ecological relationships can exist also with primarily carnivorous animals. Grizzly bears (*Ursus arctos horribilis*) excavate and consume mushrooms of ectomycorrhizal species (*Suillus*, *Rhizopogon*, *Russula*, and *Lactarius* species) on occasions, e.g., in shortage of seeds of pine trees. It is interesting to note that mushroom feeding by grizzlies in a study in the Yellowstone region showed a strong correlation specifically to stands of lodgepole pines (*Pinus contorta*), suggesting a contribution of grizzlies to the dispersal of this particular tree species (Mattson et al. 2002).

The omnivorous wild boar (*Sus scrofa*) consumes epigeous and hypogeous mushrooms of a wide range of fungal species, hypogeous fruiting bodies thereby more often than epigeous mushrooms (Piattoni et al. 2012, 2016; Soteras et al. 2017). Wild boars are opportunistic mycophagists. Mushrooms can present up to 7% of the total amount of the animal's diet (Ballari and Barrios-García 2013) and, influenced by the general food availability, mushrooms are taken in by wild boars in 3–20% of the feeding time (Genov 1981; Baubet et al. 2004), with spores found in some instances in nearly all feces (Soteras et al. 2017). As shown for ornamented pigmented thick-walled spores from ascomycetous truffles, cell walls may in part erode during passage through the animal while the spores still germinate (Piattoni et al. 2014; Zambonelli et al. 2017). Feces of wild boars as that from deers can transfer hypogeous ectomycorrhizal asco- and basidiomycetes (reported are, e.g., ascomycetous *Tuber* species on the one hand, basidiomycetous *Rhizopogon* on the other hand) into new habitats (Piattoni et al. 2012, 2014; Nuñes et al. 2013; Livene-Luzon et al. 2017). By the large distances of 1 to >15 km wild boars cover daily (Dardaillon 1986; Sodeikat and Pohlmeyer 2002; Halbwachs and Bässler 2015; Piattoni et al. 2016), paving the way for the wild boars to the sporulating mushrooms through scents of VOCs is then a rewarding investment for a fungus for long-distance distribution. Distribution by migrating wild boars can bring an ectomycorrhizal fungus newly into areas undergoing reforestation, and it can help in mixing the genetic population of a species, especially also when fungal patches are spatially separated (Genard et al. 1988). Wild boars can further assist trees in invasion into new habitats by providing with their feces the inocula for required mycorrhizal partners for the growth of trees (Nuñez et al. 2013; Soteras et al. 2017).

C. Humans

Mushrooms might be edible and tasty to humans or nonedible by too-hard structure and bad taste, or they can be toxic and must be avoided in diets (Sherratt et al. 2005; Kües and Badalyan 2017). Sight and smell can lead a potential consumer to distinguish edible from nonedible and toxic species (Chiron and Michelot 2005; Sherratt et al. 2005).

Discrimination between valuable and hazardous mushrooms in humans is traditionally mediated by learning from the elders in countries with established societal cultures of mushroom hunting and from traditional ethnic tribes with deep ethnomycological local knowledge (Kües et al. 2003; Boa 2004; Łuczaj and Nieroda 2011; Garibay-Orijel et al. 2012; Łuczaj et al. 2015) although physiologically unwarranted human avoidance of mushrooms and mycophobia can be transmitted through negative historical experiences (such as the medieval witch-hunts), superstition, and spread of tales of mycophobic content (Benjamin 1995; Ruan-Soto et al. 2013). Experienced mushroom hunters however commonly use distinct characteristic odors among other factors in decision-making between edible and poisonous

species. Long lists with bloomy odor descriptions for many species are therefore available (Spoerke 1994). However, not all dangerous species may confer an informative odor to humans who typically perform poor at analyzing components of VOC mixtures (Jinks and Laing 1999, 2001; Laing and Francis 1989; Rokni et al. 2014), nor is the smell always bad and abrasive. The highly toxic death cap *Amanita phalloides*, for example, in young age is described to be quite odorless and adopts only later a honey-like sweet to sickly sweetish scent (Bresinsky and Besl 1990). Some bad-smelling species in contrast are eaten by humans, such as the stalk and the down-hangingnet-like skirt (indusium) of the foulish-smelling crinoline stinkhorn *Phallus indusiatus* as fungal delicacy (Mau et al. 2001; Halpern 2007) and the inner parts of *P. impudicus* (Fig. 5) at the young comparably odorless unopened egg stage (Arora 1986; Stijve 1998; Pudil et al. 2014).

D. Toxicity

Mushrooms serve fungal spore production and dispersal (Halbwachs and Bässler 2015; Kües and Navarro-Gonzaléz 2015). Spores of most *Agaricomycetes* are ejected from the basidia into the air (Money 1998; Pringle et al. 2005; Lakkireddy and Kües 2017), often synchronized in large clouds of spores which helps by convectively created winds to move them higher up into the air, above the mushroom cap for longer-distance flights (Dressaire et al. 2015, 2016; Halbwachs and Bässler 2015). Nevertheless, most spores (>95%) seem to fall down in close neighborhood of a fruiting body (<1 m distance), while only very small percentages of spores might then be transported through the air over longer distances (Li 2005; Horton et al. 2013; Galante et al. 2011; Peay et al. 2012; Pringle et al. 2015). Most of the large amounts of spores produced within a single fruiting body might thus remain unproductive in establishing new colonies and for occupying new biotopes. Mammalian consumption of mushrooms and thereby digesting their spores might be considered as further factor to be adverse for spore dispersal and their contribution to establish a new generation (Hanski 1989; Courtney et al. 1990; Sherratt et al. 2005). Evolving toxic compounds as an **anti-predatoradaption** could defend against consumption and loss of spores by larger animals (Sherratt et al. 2005). Toxic mushrooms may further signal their unprofitability for consumers in forms of **aposematism** (anti-predator adaptation where a warning signal confers unprofitability as food to a potential consumer), by warning looks such as through color (Ramsbottom 1953; Sherratt et al. 2005), by luminous light emissions (Sivinski 1981; Spooner and Roberts 2005; Sherratt et al. 2005), and by unpleasant odor (olfactory aposematism; Camazine 1983, 1985; Sherratt et al. 2005). Of these, only the adverse odors appear to significantly correlate to signaling poisonous characters of mushrooms (Sherratt et al. 2005).

Toxicity of mushrooms has not a monophyletic origin. Toxic characters have been acquired multiple times in evolution and also been lost multiple times across the *Agaricomycetes* (Sherratt et al. 2005). Toxicity goes back to a variety of different chemical principles (Berger and Guss 2005a, b; Gonmori et al. 2011; Kües and Badalyan 2017) which easily explains the observation of multiple evolutionary events which are encountered across the *Agaricomycetes* species ranges. Toxins can defend against **predation**. In concept, however, toxins might have been evolved for selecting predators rather than avoiding predators (Tuno et al. 2009). Signaling of toxicity by color or smell can have been superimposed subsequently in evolution. According to Sherratt et al. (2005), poisonous mushrooms more likely smell strongly and more likely unpleasant to humans than edible ones. Bad smell may correlate with bad taste because smell and overall taste tend to interrelate by both addressing the olfactory receptors in the nose. A toxic compound when volatile (aka a **volatoxin**, following the definition by Bennett and Inamdar 2015) could be itself the cue for the bad smell (i.e., odor depends on toxin; Sherratt et al. 2005). A phenol, for example, is made responsible for the characteristic pungent carbolic smell, the bitter taste, and the mild toxicity (symptoms upon consumption: vomiting, diarrhea, headache) of the inedible *Agaricus xanthodermus* (Wood et al. 1998; Gonmori et al. 2011; Figs. 11, 12). However, for other toxins such direct

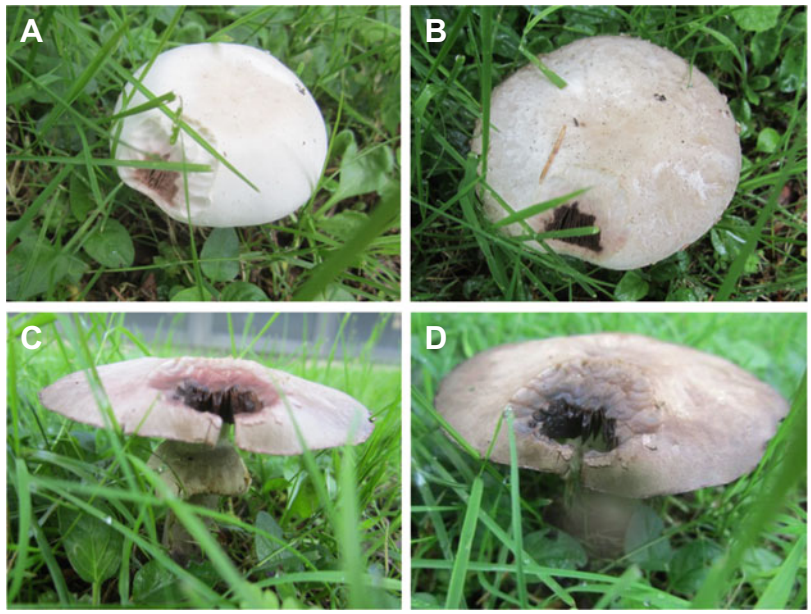


Fig. 11 Development of the *Agaricus xanthodermus* fruiting body over 4 days (in sunny late August). Note on the cap the incisor tooth marks of a squirrel which feeds on mushrooms noxious to humans. (**a**) Fresh bites on the still closed mushroom revealing the young pinkish gills. (**b**) Gills adopt a brown color in the young mushroom. (**c, d**) Aging mushroom

correlation with odor is likely not true, especially when bioactive compounds are nonvolatile such as toxins of proteinaceous nature (see Kües and Badalyan 2017 for examples). Adverse odors must then come from biochemical pathways unrelated to the toxin and have therefore adopted a clear signal character (Sherratt et al. 2005; Halbwachs et al. 2016).

Mycophagy is well distributed under primates, while mushrooms usually present an irregular diet at low rate (Hanson et al. 2003, 2006; Sawada 2014; Sawada et al. 2014; Trierveiler-Pereira et al. 2016). Japanese macaques (*Macaca discata yakui*), for example, eat a large variety of mushrooms (67 possible species in 31 genera were documented). The animals may examine toxic mushrooms by sniffing, nibbling, and careful handling but not significantly more than mushrooms of palatable species. Behavioral studies suggest that the macaques do not perceive warning odors. However, when animals eat a fruiting body unexamined, it is unlikely to be a poisonous one which suggests that they do have acquired some knowledge on edible mushroom species (Sawada et al. 2014). How do other mycophagous species deal with toxic mushrooms? Learning by an animal has been reported once for an opossum (*Didelphis virginiana*) that developed a strong aversion against *Amanita muscaria* after having been ill after mushroom consumption (Camazine 1983). Opossums like many other larger fungivores forage nocturnally at poor sight. Opossums thus use olfactory cues in their strong aversion to toxic mushrooms (Camazine 1983, 1985). Red squirrels (*Tamiasciurus hudsonicus*) on the other hand consume certain mushroom species (e.g., *Lactarius piperatus*, *Russula emetica*) with impunity, irrespective of potential toxins (Buller 1909, 1922; Fogel and Trappe 1978). Scars from squirrel feeding can regularly be observed on species which are unedible for humans by noxious and bitter-taste compounds (see *A. xanthodermus* as an example in Fig. 11). Toxicity of a compound directed toward some but not all animals might rely on respective animals' gut systems and the

Fig. 12 Flies populate *Agaricus xanthodermus* mushrooms (in sunny late August after days of heavy rainfall). (**a**–**g**) Adults of different *Diptera* caught on cap surfaces and gills of young closed or just opened mushrooms. (**h**) Tunneling larvae appear on the surface of a mature mushroom. (**i**) A mature mushroom with smooth non-injured cap surface, 2 days before (**j**–**l**) the mushrooms fully collapsed through decay by massive maggot development. (**m**) A mature mushroom with scars of squirrel feeding, (**n**) 1 day later starting to decay with softened destaining tissue patches in the cap, and (**o**–**q**) 2 days later collapsing through attack by masses of maggots

associated microbes and their enzymes (Bedoya-Pérez et al. 2014; Halbwachs et al. 2016).

Intoxication by mushrooms of domesticated animals like carnivorous cats and dogs has frequently been reported. Incidences often concern unexperienced puppies and kittens prior to learning, while older animals tend to be more cautious (Kües et al. 2003; Cope 2007; Puschner and Wegenast 2012; Seljetun and von Krogh 2017). Dogs with their fine noses can be trained as truffle hounds for mushroom hunting and tracing fruiting bodies in the ground, as a nonconsuming alternative to the omnivorous truffle hogs, domesticated pigs being skillful in olfactorial finding of hypogeous specimens but also interested in eating these (Hall et al. 2007; Kües and Martin 2011).

VII. Stinkhorns

Stinkhorns comprise the family of *Phallaceae* in the order *Phallales*. As their name says, these fungi have adopted strong stinky, often foulish, fecal- or carrion-like smells emitted from the gelatinous gleba containing the spores at the

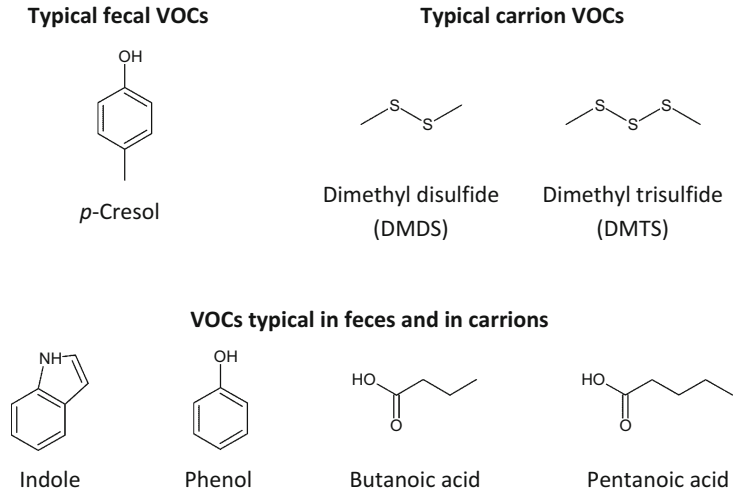


Fig. 13 Major unpleasant chemical compounds found in the scents of mature stinkhorns

end of the receptable, the spongy stalk of the mature sporophores (Arora 1986; Hosaka et al. 2006; Trierveiler-Pereira et al. 2014; Fig. 5). Stinkhorns are among those *Agaricomycetes* which have lost the active basidiospore discharge mechanism. Since the spores collect in the gelatinous gleba at the mushroom cap, they are also not wind-dispersed. Stinkhorns therefore utterly rely on animals for spore dispersal. However, stinkhorn spores are not found in mammalian feces. Mycophagous arthropods, in particular dipteran flies, distribute the species (Ingold 1953; Hosaka and Uno 2012; Tuno 1998).

Mature stinkhorns attract carrion and dung flies and also scarab beetles through optical and olfactorial mimicry of their food and brood sites for spore dispersal and thus behave comparably to sapromyophilous foul-smelling plants pollinated by flies which normally visit dead animals or dung (Stijve 1998; Tuno 1998; Johnson and Jürgens 2010; Teichert et al. 2012). In accordance, mushrooms of the best known stinkhorn species *P. impudicus* (Fig. 5) were found in Ireland in striking association with badger (*Meles meles*) places, likely because of blowflies of the genera *Calliphora* and *Lucilia* which are specialized on carcasses. These flies are dominantly attracted to and rear from badger cadavers. The female flies also feed on the spore-laden mucilage of the *P. impudicus* gleba (Sleeman et al. 1997). Possibly as an adaptation to optimal endozooic spore survival and dispersal and possibly mediated by a laxative effect of gleba fluid, defecation of loads of spores by flies occurs rapidly when the insects are feeding on the fungus, much faster than defecation after consumption of other fly substrates (Ingold 1953; Schremmer 1963; Sleeman et al. 1997). Feeding on gleba and spores might thus be of good benefit for the stinkhorns. This is not necessarily true for the consuming flies because females can fail by low nutrient quantity in the gleba substrate (especially low in protein) to produce fully developed eggs (Stoffolano et al. 1989, 1990). Other likely minor potential epizooic measures of stinkhorn spore dispersal are via sticking spores with the slimy gleba onto the animal bodies (Schremmer 1963; Tuno 1998) and through active collection of spores into the corbiculae (pollen baskets) on the hind legs of carrion-specialized *Hymenoptera* (stingless *Trigona* sp. bees; Burr et al. 1996; Shaw and Roberts 2002).

A. Stinkhorn Odors

The composition of the dung-like odor of the fetid lantern stinkhorn *Lysurus mokusin* consists largely of butanoic acid (20.2%), *p*-cresol (16.7%), phenol (16.4%), pentanoic acid

(10.2%), indole (7.5%), and further aliphatic acids (7.2%) (Chen et al. 2014; Fig. 13). In comparison, Johnson and Jürgens (2010) measured 8.6% butanoic acid, 4.4% *p*-cresol, 2.3% phenol, 0.2% pentanoic acid, 3.0% indole, and 15.2% other aliphatic acids and 21.0% dimethyl disulfide (DMDS) and 40.5% dimethyl trisulfide (DMTS) as headspace volatiles in the scent of the octopus stinkhorn *C. archeri*. Further, 2–20% DMDS and >20% DMTS were detected in headspace analyses of the ripe fruiting bodies of the common stinkhorn *P. impudicus* (Borg-Karlson et al. 1994) and among other volatiles also indole (Pudil et al. 2014). DMDS, DMTS, and indole are however absent in the less complex odor bouquets of the immature stinkhorn egg stage (defined mainly by 1,4-dimethoxybenzene with a sweet floral scent and 3-octanone; Fig. 2a) and also in the very complex VOC mixtures (main volatiles of in total 41 different VOCs found are acetic acid, 2,3-butanediol, 2-phenylethanol, and 4-methybenzaldehyde) of overripe fruiting bodies which lack the gleba through insect feeding (Pudil et al. 2014). Mature stinkhorns mimic in their scents both carrion and fecal odors (Cardona et al. 2005; Johnson and Jürgens 2010; Paczkowski and Schütz 2011; Fig. 13). In varying combinations, *p*-cresol, aliphatic acids, phenol, indole, DMDS, and DMTS (Fig. 13) are typical volatiles in putrid odors of feces, urine, decaying materials mimicking feces, and carrions which are potent attractors to saprophagous, sacrophagous, and coprophagous flies and other insects (Lane and Fraser 1999; Johnson and Jürgens 2010; Jürgens et al. 2006, 2013; Paczkowski et al. 2012, 2015; Chen et al. 2014; Forbes and Perrault 2014; Stavert et al. 2014; Tomberlin et al. 2017). Individual reactions of various carrion insects (*Diptera*, *Coleoptera*, and *Hymenoptera* species) to specific decomposition VOCs (attractants, oviposition stimulants) have in the meanwhile been recorded in experimental test setups in the laboratory (Verheggen et al. 2017). Stinkhorns might use these volatile signals in their own favor as allomones to attract insects for spore dispersal.

B. Mycophagous Generalists

Generalist mycophagous insects are attracted by fetid *L. mokusin* fruiting bodies. The nocturnal earwig *Anisolabis maritima* consumes the gleba with the spores of *L. mokusin* and disperses the spores within feces. Outer coatings of spores are removed, and mucilage from the fungal gleba is digested by the mycophagous earwigs, while spore germination rates are significantly enhanced. *A. maritima* can excrete 1.18×10^7 fungal spores per day and >10 times more per day than fly visitors of the species *Lucilia sericata* (Chen et al. 2014). The putrid scent of *C. archeri* attracts diverse generalist flies and mimics brood and feed places of the flies. *C. archeri* is variably visited by calliphorid flies which lay their eggs on carrions and feed on feces, by sarcophagids which associate with carrions, and by muscids which breed and feed on decaying organic matter including feces (Johnson and Jürgens 2010). Similar observations on generalist flies (often of the genera *Calliphora*, *Lucilia*, and *Sarcophaga*) and carrion-attracted beetles are reported for ripe *P. impudicus* stinkhorns (Schremmer 1963; Smith 1956; Love 1976; Sleeman et al. 1997). Mycophagous specialists (obligate **mycobionts**) are apparently rare on stinkhorns (Krivosheina 2008). Flies of twelve different families were recorded to feed specifically on gleba with spores of crinoline stinkhorns, with eight opportunistic secondary mycophagous and thirteen non-mycophagous drosophilids and only one mycophagous specialist (*Mycodrosophila gratiosa*) which were encountered as the dominant visitor groups attracted to the gleba by the peculiar crinoline stinkhorn smell of decayed fallen fruits. Fed spores were not decayed by the different types of flies and showed high germination rates of

80–90%. Other generalist decomposers on crinoline stinkhorns were mycophagous *Coleoptera* of five different families feeding variably on the gleba, the stem, or the whole mushroom body and mycophagous *Hymenoptera* and *Collembola* feeding on the gleba (Tuno 1998).

C. Breeders and Parasitoids

A vast diversity of insects (*Coleoptera*, *Diptera*, and others) are obligatory or opportunistic breeders in fruiting bodies of *Agaricomycetes*, however, often not species-specific in terms of the respective fungal hosts (Leschen 1999; Krivosheina 2008; Kadowaki 2010a; Hosaka and Uno 2012; Schigel 2012; Roháček and Ševčík 2013; Jonsell et al. 2001, 2016; Kinzner et al. 2016; Fig. 12). In line, various woodland *Drosophila* species, being food generalists, facultative fungal breeders or also some mycophagous specialists, are attracted to *P. impudicus* for oviposition and breeding (Shorrocks and Charlesworth 1982; Drissen et al. 1990; Burla et al. 1991). Female *Drosophila* flies may already oviposit on young stinkhorns at the egg state (Basden 1952; Smith 1956; Shorrocks and Charlesworth 1982), prior to the very fast development of the stinky odors by the gleba at the cap occurring at mushroom maturation [for a time course of stinkhorn egg ripening, stipe elongation, cap and gleba presentation, and odor production in stinkhorn development, see the descriptions by Schremmer (1963), Shorrocks and Charlesworth (1982), and Stijve (1998)]. Depending on the fly species, their eggs are found within the peridial layers left as reminders from the young stinkhorn egg stage as cover on the base of the ripe mushroom, or the insect eggs are also distributed inside the caps and possibly the upper stipes (Smith 1956; Shorrocks and Charlesworth 1982), or they are laid onto the stinkhorns (Basden 1952). Hatching of larvae from drosophilid eggs can be delayed until the mushrooms are ripe or decaying (Basden 1952; Smith 1956). Hatched fly larvae may graze on yeasts using for growth the carbohydrate-rich mucilage of the peridial layers, or the larvae may feed on different tissues of the mushrooms from stipes and caps, in dependence of the preferential oviposition and rearing places of a respective fly species (Kearney and Shorrocks 1981; Shorrocks and Charlesworth 1982; Spencer and Spencer 1997).

The facultative fungal breeder *Drosophila phalerata* oviposits into and propagates very often within the peridial layers of *P. impudicus* (Shorrocks and Charlesworth 1982; Drissen et al. 1990; Burla et al. 1991). This can lead to attract different parasitoid eucoilid and braconid wasps, such as the polyphagous larval parasitoid *Leptopilina clavipes* as an antagonist of *D. phalerata* (Drissen et al. 1990; Janssen et al. 1995a, b; Tuno 1998). *L. clavipes* reacts for attraction on decaying mushroom odors to find and parasitize drosophilid larvae (Vet 1982; Pannebakker et al. 2008).

Leptopilina heterotoma is another closely related general parasitoid of *Drosophila* maggots. *L. heterotoma* is attracted to uninfested stinkhorns but avoids mushrooms which are already populated by its fly host alone and by the dominant competitor *L. claviceps* for the host flies. Avoidance is determined on the solely base of some repellentodor changes by the previously established fungus-insect communities (Janssen et al. 1995a, b). *L. heterotoma* uses the female monoterpenoid sex pheromone (−)-iridomyrmecin as cue in intraspecific competition avoidance and across wasp species borders (*L. clavipes*, *Leptopilina boulardi*; Weiss et al. 2013). As eavesdropper on fly communication, the solitary wasp *L. heterotoma* reacts for attraction toward flies density-dependent on the aggregation pheromones (*cis*-vaccenyl acetate) emitted by the adult drosophilids (Wiskerke et al. 1993; Hedlund et al. 1996; Wertheim et al. 2003, 2006). The wasp may however also appoint odors from host substrates as microhabitats of its prey, the prey food (such as yeasts), and further prey cues (larval excrements, adult traces other than aggregation pheromones) when searching for potential fly hosts (Vet 1982; Dicke et al. 1984; Papaj and Vet 1990; Vet et al. 1998; Wertheim et al. 2003). Ethanol is part in the VOC blends of host habitats which attract *L. heterotoma* (Dicke et al. 1984). Consuming ethanol-containing food in contrast can however protect drosophilids from *L. heterotoma*

attack (Lynd et al. 2017). Food yeasts of *D. phalerata* growing on the carbohydrates in the peridial layers of the stinkhorns (Kearney and Shorrocks 1981; Shorrocks and Charlesworth 1982; Spencer and Spencer 1997) may provide such protective ethanol-containing nutrition. Mutualism driven by a complex chemical communication exists between yeasts and attracted drosophilids as their vectors (Günther et al. 2015). Are there also communication and mutualism between yeasts and drosophilids in the ecological environment of a stinkhorn peridium? Levels of nutritional interactions become then quite complex—e.g., tetra-trophic (*P. impudicus*, yeasts, drosophilid, wasp), quinta-trophic (with two different drosophilids or two wasp species), or more multi-trophic—and require dynamic and flexible decision-makings of the participants under consideration of various types of communications.

D. Phallalophagy

Mycophagous insect communities in individual basidiomycetous mushrooms can generally be very complex in absolute animal numbers and in species numbers (Thunes et al. 2000; Wertheim and van Alphen 2001; Kadowaki 2010a; Yamashita et al. 2015; see as an example the larval and adult fly communities on *A. xanthodermus* in Fig. 12). There can thus be further competition between different insect feeding and breeding in *Agaricomycetes* mushrooms for limited space and fungal food resources, with different situation-dependent outcomes variably for early and for subsequent colonizers (Grimaldi and Jeanike 1984; Shorrocks and Bingley 1994; Rouquette and Davis 2003; Yamashita and Hijii 2007a; Krivosheina 2008; Thorn et al. 2015). Leschen (1999) reviewed the phylogenetic origin of **phallalophagy** ("stinkhorn fungus eating") of the *Oxycnemus* beetle complex which is specialized for breeding in *Phallales*. Proposed by the author, respective nitidulid beetles may arrive very early on the young stinkhorn; prior to that the exposed gleba on the caps becomes active. The beetles will probably react on the mild and faint odors of the young structure (Leschen 1999; Pudil et al. 2014). This would give the beetles sufficient time for hatching and larval development; prior to that any competing drosophilids and other mycophagous flies react in series on the developing smells of the gleba on the exposed caps, occupy the sporophores, and quickly consume the mushrooms (Leschen 1999).

VIII. Fungal Mycelia and Invertebrates

Interactions between invertebrates and *Agaricomycetes* are multiple. Mycophagous invertebrates may live and feed on fruiting bodies (**macromycophages**) as exemplified above (Sects. IV.B, V, and VII) or on mycelium (**micromycophages**). Further distinctions are possible: **obligatory macromycobionts** live within mushrooms and are feeding on them, **obligatory sapromycobionts** inhabit decomposing fruiting bodies and feed by **saprophagy**, **facultative mycobionts** live on the surface of fruiting bodies and mycelium (**epibionts**) and feed either by **micromycophagy** or possibly by **saprosporophagy**, and **eurobionts** live in association with fungal substrates of different stages and feed by **zoophagy** or **necrophagy** on living or dead animals (Krivosheina 2008; Yamashita et al. 2015). The following concentrates on communication of *Agaricomycetes* via VOCs in connection with micromycophagy and breeding in fungal-infested substrates.

A. Decomposer Communities

Substantial amounts of VOCs in very broad and complex and in qualitatively and quantitatively dynamically changing combinations are released during plant litter and wood decomposition. VOCs originate from the various members of the **decomposer communities** and from the decaying plant material and the soil (biotic and abiotic), while the roots of resident local vegetation and their symbiotic and pathogenic associates further intermingle into this (Ramirez et al. 2010; Peñuelas et al. 2014; Kanchiswamy et al. 2015a, b; Isidorov et al. 2016; van Dam et al. 2016; Mäki et al. 2017; Massalha

et al. 2017). It is thus very hard in these complex multi-trophic environments with multiple biotic and abiotic influences from the soil, plant litter, and decaying wood to define the individual origins of the various kinds of VOCs emitted by organisms and establish further the possible effects which the VOCs can have on other individuals in the communities.

Mostly, simplified laboratory systems are used to define for individual species their VOC profiles and potential ecological VOC functions. Antibiotic and toxic activities as biological defense functions are frequently attributed to fungal VOCs detected in such manner, while analyses of potential functions of VOCs as signals in organismal communication are still scarce (Kramer and Abraham 2012).

Many *Agaricomycetes*, especially white-rot species (species of *Bjerkandera*, *Hypholoma*, *Lepista*, *Phellinus*, *Pholiota*, and *Stropharia*, *Mycena epipterygia*, *Oudemansiella mucida*, *Peniophora pseudopini*, *Phylloporia ribis*, *T. versicolor*, and others), produce volatile antifungal chlorinated aromatic compounds, e.g., chlorinated anisyl metabolites, such as 3,5-dichloro-*o*-anisyl alcohol (Fig. 9) by *Hypholoma elongatum* (Field et al. 1995; Swarts et al. 1997; Boustie et al. 2005). Fungistatic activity against phytopathogenic *Ascomycetes* by *Hypsizygus marmoreus* bases on 2-methylpropanoic acid 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester (Oka et al. 2015; Fig. 9). The white-rot *Anthracophyllum discolor* produces a blend of sesquiterpenes (α-bisabolene, bulnesene; Fig. 8) combined with antimicrobial chlorinated aromatic compounds (1,5-dichloro-2,3-dimethoxybenzene, 3,5-dichloro-4-methoxybenzaldehyde, 3-chloro-4-methoxybenzaldehyde; Fig. 9). This blend showed strong antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum* from the *Ascomycetes* and against *Mucor miehei* from the *Mucoromycotina* (Schalchli et al. 2015). VOC mixtures with strong antifungal and also nematicidal activities of the wood-decay fungus *Schizophyllum commune* contain as main compound β-bisabolol (Fig. 8) and VOC blends of the white-rot *T. versicolor* a sesquiterpene alcohol (Schalchli et al. 2011; Pimenta et al. 2017). The mycorrhizal *Lactarius vellereus* produces (in the fruiting bodies) the antibacterial sesquiterpenes velleral and isovelleral (Sterner et al. 1985; Fig. 8) and *Stereum* species and *Cortinarius odorifer* in their mycelia the antibacterial (+)-torreyol (Egli et al. 1988; Ainsworth et al. 1990; Solis et al. 2004; Fig. 8).

Inter- and intra-specific interactions are however inevitable where niches overlap such as for decomposers (Boddy 2000; Crowther et al. 2012). Saprophagous invertebrate communities comprise mostly of generalists and consume large amounts of plant litter. They form together with saprotrophic fungi and bacteria decomposer communities. Saprotrophic mycelial and distinctly the cord-forming-*Agaricomycetes* are drivers for enzymatic litter decomposition, of leaves, needles, wood debris, and other plant material. At the same time, however, they present also coincidental and treasured food sources for various plant-litter-feeding invertebrates which are part of the overall decomposer communities (Hättenschwiler et al. 2005; Osono 2007; A'Bear et al. 2014a; David 2014). This can have consequences for the individual fungi in their niche, their decomposition output, nutrient cycling, and carbon storage of their ecosystems. Outcomes are differentially determined by feeding and food preferences of the kind of grazing of macroinvertebrates (e.g., millipedes, wood lice, springtails, oribatid mites, nematodes, oligochaete worms, gastropods) and the agaricomycete species (e.g., *Hypholoma fasciculare*, *P. impudicus*, *Phanerochaete velutina*, and *Resinicium bicolor*) grazed on (Crowther et al. 2011b; A'Bear et al. 2014b). Outcomes are further influenced by the state of the decomposing substrate, the successions of animals and fungal decomposers (colonization history) and soil food web changes related to, organismal complexity of the system, and various environmental parameters such as temperature, moisture, and light conditions (Boddy 2000; Osono 2007; Crowther et al. 2013; A'Bear et al. 2014c, d; Jacobsen et al. 2015; Ulyshen 2016; Hiscox et al. 2016b, 2017; Seibold et al. 2016; Kirchenbauer et al. 2017).

Grazing of the millipede *Blaniulus guttulus* on mycelium of the wood-decay fungus *H. fasciculare*, for example, resulted in block of mycelial extension growth, while grazing by the nematode *Porcellio scaber* stimulated fungal growth. *H. fasciculare* is a species rich in awkward secondary metabolites and is considered unpalatable for many invertebrates, but *B. guttulus* is apparently able to cope with this (Crowther et al. 2011a; A'Bear et al. 2014b). Consumption by *B. guttulus* was selective on

H. fasciculare as a less competitive species in combative interactions with the white-rot-species *R. bicolor* and *P. velutina* as other *Agaricomycetes*. In resource partitioning, the woodlouse *Oniscus asellus* and the nematode-*Panagrellus redivivus* in contrast selectively grazed on the palatable mycelium of the dominant decay fungus *R. bicolor*. *O. asellus* thereby could eliminate entire mycelial cord systems of *R. bicolor* (Rotheray et al. 2009; Crowther et al. 2011a, b) in favor of other fungal species. In other cases of invertebrate grazing, damaged fungi tried to compensate losses by increased mycelial growth, and competitive growth hierarchies between different decay fungi were converted (Bretherton et al. 2006; Rotheray et al. 2011; Crowther et al. 2012; A'Bear et al. 2014a, 2012). Grazing by invertebrates influenced production of fungal enzymes, positively as recorded in *H. fasciculare* and *P. velutina* or negatively in *R. bicolor* and *Stereum hirsutum* (Dyer et al. 1992; Crowther et al. 2011c, 2012; A'Bear et al. 2014b, c, d; Hiscox and Boddy 2017), and with it the overall wood decomposition rates (Crowther et al. 2011b, 2012; A'Bear et al. 2014b, d). Grazing by invertebrates can thus alter the ecological success of competitive fungal species. Invertebrate grazing helps to maintain fungal multi-species communities and hinders loss of weaker fungal competitors such as *H. fasciculare* which may otherwise be outcompeted by stronger growing fungal decomposers (A'Bear et al. 2013, 2014b).

Competition between saprotrophic fungi includes competition for space, in soil as well as in wood (Donnelly and Boddy 1998; Boddy 2000; A'Bear et al. 2014b; Hiscox et al. 2015b, 2017; Hiscox and Boddy 2017). Depending on species, climatic conditions, and state of plant litter and wood decay, antagonistic interactions between two colliding fungi can result in **deadlock** where neither of the fungus gains territory or in **replacement** where either one fungus overgrows in parts or fully the other or both fungi mutually overgrow each other (Boddy 2000; Osono 2007; Evans et al. 2008; Hiscox et al. 2015b, 2016b; El Ariebi et al. 2016). Antagonistic reactions between agaricomycetous decomposer fungi come along with production of VOCs or also **diffusible organic compounds** (**DOCs**) (Boddy 2000; Hynes et al. 2007; Evans et al. 2008; Peiris et al. 2008; El Ariebi et al. 2016). Production of VOCs is induced in species-dependent manners when colonies of different *Agaricomycetes* meet each other (Hiscox and Boddy 2017). Some first evidences for fungal communication over distances via VOCs are emerging in interactions of *Agaricomycetes* which can stimulate certain species (*T. versicolor*, *Bjerkandera adusta*) in their growth (Evans et al. 2008).

Between *H. fasciculare* and *R. bicolor* appears to be no long-distance antagonism which would manifest in mycelial morphological changes (Boddy 2000). However, when they come into direct physical contact, specific pigments are synthesized by both species, there is bidirectional partial replacement growth, and between the two antagonists, ten different VOCs were produced (on artificial malt extract agar medium). Among the VOCs were several (toxic) sesquiterpenes identified as α- and γ-muurolene, α- and γ-cadinene, α- and γ-amorphene, and α- and γ-bulgarene (Fig. 8) which are possibly emitted for chemical defense and to halt growth extension of competitors (Hynes et al. 2007; Kramer and Abraham 2012; Hiscox and Boddy 2017). In a newer study on beech (*Fagus sylvatica*) wood, (*E*)-germacene D, isolongifolene, β-chamigrene, β-patchoulene, eremophilene, and γ-cadinene (Fig. 8) were encountered. (*E*)-Germacene D and eremophilene reoccurred in pairings between *H. fasciculare* and *P. velutina* and between *R. bicolor* and *H. fasciculare*. α-Bulnesene was encountered between pairings of *P. velutina*, *R. bicolor*, and *P. impudicus*. Moreover, the monoterpene (C10 terpenoid) limonene (Fig. 14) and the ketone 3-octanone (Fig. 2a) appeared in all interactions (El Ariebi et al. 2016; Hiscox and Boddy 2017). In other work on malt extract agar, eight interaction-specific VOCs were produced between *Stereum gausapatum* and *T. versicolor*. No sesquiterpene was identified but the monoterpene α-myrcene (Fig. 14) and a number of benzenoids were found (Evans et al. 2008).

VOC production upon physical contact between different fungi might play a role in informing each other on their competitive nature under space limitations. VOC production may help by VOC toxicity characters to

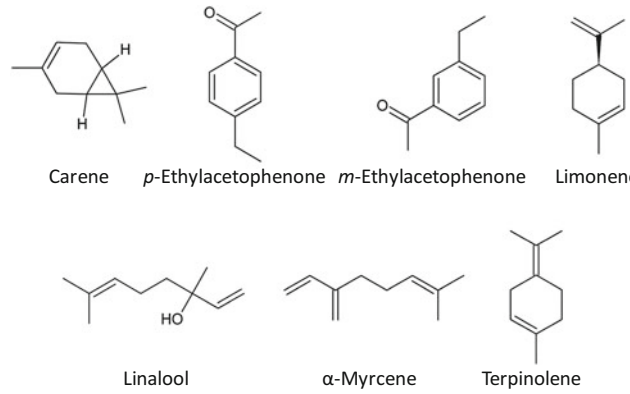


Fig. 14 Monoterpenes from *Agaricomycetes* with possible signaling functions

define winners in the fungal antagonistic interactions (Peñuelas et al. 2014), while VOCs might even been used further by fungi as carbon sources (Cale et al. 2016). Above the fungal interactions, VOC production may have further consequences for the ecosystem within and across ecological niches. For example, mediated by the variously produced sesquiterpenes and monoterpenes, VOCs may serve secondarily in invertebrate antifeedant defense. In other instances, VOCs from species-specific-combative interactions may address insect communication systems for either attraction or repellency and may possibly also influence interactions with other microbes (e.g., bacteria, other type of fungi) and possibly also protists in the decomposer communities (Leather et al. 2014; Hung et al. 2015; Geisen et al. 2016). The sesquiterpenes α- and γ-muurolene (Fig. 8) contribute such to attraction of the moth *Helicoverpa armigera* (cotton bollworm), while they are also considered as parts of antifeedant defense responses (Hartlieb and Rembold 1996; Geervliet et al. 1997; Hynes et al. 2007). Antennae of the diamondback moth *Plutella xylostella* reacted on (*E*)-germacene D, (*E*)-β-caryophyllene (Fig. 8), and linalool (Fig. 14) and antennae of the tobacco budworm moth *Heliothis virescens* on (*E*)-germacene D (Mozuraitis et al. 2002; Wee et al. 2016).

In support of these ideas, there are also interactions and induced VOC production between strains of a same species in the space-limited environments. The reactions on self-competition are however lower with less complex mixtures of VOCs which as compared to interspecies interactions are generally released in reduced amounts. They do not always have visible effects on hyphal growth and structure (Hynes et al. 2007; El Ariebi et al. 2016; Hiscox and Boddy 2017). α-Bulnesene (Fig. 8) and terpinolene (Fig. 14) were repeatedly detected in self-confrontations of white-rot fungi, while *H. fasciculare* had the most complex VOC compositions including further sesquiterpenes (Hynes et al. 2007; El Ariebi et al. 2016). The sesquiterpene alcohol (+)-torreyol (Fig. 8) is produced at the mycelial fronts in confrontations between antagonistic *Stereum hirsutum* strains and between strains of other *Stereum* species (Ainsworth et al. 1990). Fungus gnats can be specifically attracted for grazing to mycelial confrontation zones of intraspecies antagonisms provided in *Stereum* through **heterogenic incompatibility** (Boddy et al. 1983; Esser 2016). Torreyol is regarded as an insect pheromone in courtship found on wings of females of the northern blue butterfly *Lycaeides argyrognomon* (Lundgren and Bergström 1975).

B. Wood as Substrate

Many saproxylic insects are specialized on wood of specific conditions which include tree species, size of wood pieces, fresh state, decay stage, and occurrence of fungi in general or of distinct fungal species (Grove 2002; Holighaus 2012; Seibold et al. 2014). Wood-decaying fungi concentrate nutrients about 10 times as compared to their substrate and may provide fungivorous insects with better feed than what the

substrate wood presents (Jonsell and Nordlander 2004). Certain insects are however also themselves capable to digest wood by own lignocellulolytic enzymes (Martin 1991; Watanabe and Tokuda 2010; Calderon-Cortes et al. 2012; Ulyshen 2016), while efficient wood-decaying fungi can still bring superior nutritional conditions as support to wood-inhabiting and wood-feeding insects (Kasson et al. 2016). Similar as there are successions in deadwood of fungi, there are successions in highly specialized insects on the resource wood. Early arrivers on wood of both, fungi and insects, might still be at random. However, early arrivers of both kinds of organisms can much influence by **priority effects** later colonizations by other organisms of the own type and by that of the other kingdom (Weslien et al. 2011; Strid et al. 2014; Ottosson et al. 2014; Hiscox et al. 2015a, 2016a; Jacobsen et al. 2015; Wende et al. 2017).

Fungal spores for wood infestation might arrive by air or be vectored and inoculated by invertebrates. In animal transfer, long-distance and short-range attraction to the substrate wood will be of importance for success. Volatiles emitted from the pure wood, from a fungus (mycelium or mushroom), and from decaying wood and combinations thereof can be detected by invertebrates and lead them to their favored habitats and ecological niches. Reactions might be more general or specific with respect to the tree, a fungus, and an invertebrate (Lindelöw et al. 1991; Fäldt et al. 1999; Weissbecker et al. 2004; Holighaus and Schütz 2006; Holighaus 2012; Leather et al. 2014).

As long as wood odors still outweigh revealing fungal VOCs, during early stage of wood decay and on longer distance, wood odors seem to be still more important for attraction of saproxylic species such as of the *Heteroptera* suborder of insects (Seibold et al. 2014). Olfactory cues from wood appear to direct the saproxylic fungivorous heteropteran *Aradus obtectus* over long distance to the macrohabitat with deadwood, while *A. obtectus* seems to locate patches in trunks with its fungal host by mycelial odors of the decay fungus *F. pinicola*. Tested perennial fruiting bodies of *F. pinicola* in contrast conveyed repellent effects, and the conks might have been not of the right age (e.g., too old) for appealing *A. obtectus* or already been populated by other insects (Seibold et al. 2014; Koban et al. 2016).

Fine scaling in order to find fungal mycelium in the wood can be quite difficult when respective mycelial patches are still quite small or, in the cases of specialists, when they are positioned among patches of other fungal species on the same wood piece. It might then be much easier to locate host mycelium and wood decayed by the respective fungi by distinctive odors of their fruiting bodies. Sporophores tend to correlate with larger mycelial patches in the wood (Ovaskainen et al. 2013; Leather et al. 2014; Seibold et al. 2014) and may therefore be the better guide to the mycelium in the decaying wood. Seeking for mycophagous prey in the wood through finding fungal fruiting bodies can then be also favorable for antagonistic animals by a higher likelihood of detection of higher numbers of potential prey in the decaying wood (see, e.g., the case of the generalistpredator rove beetle *L. lunulatus* and *F. pinicola* sporocarps in Sect. IV.B; Johansson et al. 2006).

While some insects such as *L. lunulatus* are specifically attracted to fruiting bodies of wood-decaying *Agaricomycetes*, others discriminate between fruiting bodies, wood, and wood with mycelium and seek distinctively for the mycelium in the wood. The specialist bark beetle-*Dryocoetes autographus* is thus attracted to wood of spruce (*Picea abies*) which is specifically infested by the brown-rot *Fomitopsis rosea*, while wood infested with the white-rot *Phellinus chrysoloma* is largely ignored (Johansson et al. 2006).

As shown for both the white-rot *T. versicolor* and for the brown-rot *Fomitopsis palustris*, odor compositions emitted from artificial medium (potato dextrose medium was used) and from the host substrate (*Fagus crenata* wood chips were used) can much distinguish. Each fungus and each medium had their specific resulting odor composition, with restricted overlaps in between. The majority of VOCs on wood chips were observed with proceeding wood decay. *T. versicolor* produced 22 unique VOCs in a total of 41

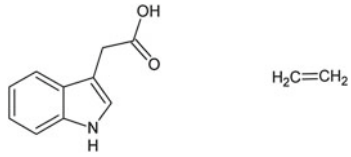
compounds on wood and *F. palustris* 38 unique VOCs in a total of 58 compounds. *T. versicolor* on wood released eight sesquiterpenes [in order of appearance: cuparene, longifolene, cadinene, valencene, and cedrene (Fig. 8) after 28 days of incubation; thujopsene and selinene (Fig. 8) after 56 days of incubation; longipinene (Fig. 8) after 112 days of incubation] and no monoterpene. *F. palustris* on wood gave rise to 22 different monoterpenes and 8 sesquiterpenes [in order of appearance: copaene, isoledene, cedrene, longifolene, and thujopsene (Fig. 8) after 56 days of incubation; longipinene and isosativene (Fig. 8) after 84 days of incubation; cadinene (Fig. 8) after 112 days of incubation], i.e., 52% of total VOCs produced by the fungus on wood were terpenoids (Konuma et al. 2015).

Early successional saproxylic beetles arriving on fresh wood use wood-emittedethanol and α-pinene as signals (Schroeder 1992; Jonsell et al. 2003) and possibly in addition also the monoterpenes 3-carene and terpinolene (Byers 1992; Fig. 14). Decaying wood with active decay fungi emits larger amounts of ethanol and monoterpenes than virgin wood. These increased concentrations direct the pioneer mountain pine beetle *Dendroctonus ponderosae* to wood with decay fungi (Gara et al. 1993). Johansson et al. (2006) speculate that this might similarly be the case for *D. autographus*. A window of a specific wood decay state and the responsible fungal species can play roles. Oviposition of the European hardwood ambrosia beetle *Trypodendron domesticum* correlated with a short early phase of emissions of phenolic wood degradation compounds such as 2-methoxy-phenole and 1,2-dimethoxybenzene originating from fermenting *F. sylvaticus* wood shortly after felling and 2-methoxy-phenole and 4-methoxy-phenole originating from wood with beginning white-rot. The beetle did however not react on any of the sesquiterpenes released by the fungus *T. versicolor* at the same time (Holighaus and Schütz 2006; Holighaus 2012). Comparing different fungal species on beech wood at an early infection state (1 week) revealed a hierarchy of emission for typical mushroom odoreight-carbon compounds (Fig. 2a) of white-rot *T. versicolor* > brown-rot*Postia placenta* > brown-rot *Gloeophyllum trabeum*, while *G. trabeum* gave rise to the largest family of sesquiterpenes (Thakeow 2008).

IX. *Amylostereum* and Its Symbiotic Wood Wasps

A tight symbiotic relationship has been established between *Amylostereum areolatum* as a white-rot fungus and the European wood wasp-*Sirex noctilio*. Female wood wasps drill ca 1.5 cm deep holes into the sapwood of living pine trees for oviposition of their eggs, delivery of phytotoxic mucus into the tree, and inoculation with fungal arthrospores which are transported by the wood wasps in their mycangia. Germinated fungal mycelia will attack the pine wood and provide food and a suitable humid environment for the hatched larvae by lignocellulose degradation (Slippers et al. 2003, 2015; Thompsen et al. 2014; Kües et al. 2016a). *S. noctilio* females are attracted to potential oviposition places by blends of wood-emitted volatiles, mainly (+)-3-carene, (−)-α-pinene, (−)-β-pinene, and ethanol (Simpson 1976; Simpson and McQuilkin 1976; Böroczky et al. 2012; Martínez et al. 2014; Erbilgin et al. 2017). They further use fungal VOCs likely as synomones to differentiate previously attacked trees as preferred stressed hosts with weakened defense reactions (Fernández et al. 2015).

The *Hymenoptera Ibalia leucospoides* is a parasitoid of the wood wasps. It finds its concealed insect hosts (eggs and larvae) within the wood by fungal odors, at best when the eggs and larvae in the boreholes are still reachable for the parasitoid, prior to that larvae start burrowing deeper into the wood (Madden 1968; Spradbery 1974, 1977; Martínez et al. 2006). Concentration differences of fungal volatiles are appointed by *I. leucospoides* for patch-choice decisions at a distance to assess relative host densities for parasitization (Martínez et al. 2006; Fischbein et al. 2012). In laboratory cultures, VOC blends of acetone, acetaldehyde, ethanol, and 2,2,8-trimethyltricyclo[6.2.2.01,6] dodec-5-ene (TMTD; Fig. 8) were released by the fungus in amounts which individually varied with cultural age (Jofré et al. 2016). *I. leucospoides* was specifically attracted to VOCs emitted by the fungus at a cultural age of 2 weeks and slightly after (Jofré et al. 2016). *I. leucospoides* showed an antennal response to acetaldehyde (Madden 1968), while acetalde-



Indole-3-acetic acid (IAA) Ethylene

Fig. 15 Phytohormones produced by *Agaricomycetes*

hyde was not detected in the cultural blends which were attractive to the cynipid wasps (Jofré et al. 2016). Wasp attraction correlated however with onset of TMDT production. Though, a semiochemical function of TMTD is so far not proven. Moreover, volatile attractants to *I. leucospoides* could have been overlooked in the study by methodical limits in sampling of types of volatiles (Jofré et al. 2016). Other researchers reported production of linalool (Fig. 14) and *p*- and *m*-ethylacetophenone (Fig. 14) in older cultures of *A. areolatum* and *I. leucospoides* antenna reactions on these compounds. The aromatic *p*-anisaldehyde (Fig. 9) and mixtures of the aromates *p*- and *m*-ethylacetophenone were attractive to the wood wasp in bioassays. Antenna reactions on linalool were female-specific, and application of *p*-anisaldehyde elicited oviposition reactions (Bryant 2010; Cucura 2013).

A further example for a tight mutualism between insects and a newly described white-rot fungus has recently been reported between saproxylic ambrosia beetles of the genera *Ambrosiodmus* and *Ambrosiophilus* and the polyporic arthrosporic strictly symbiotic *Flavodon ambrosius* which exists in nature above continents probably only as a single haplotype. The beetles transport the highly efficient aggressive white-rot fungus within their mycangia to galleries in trees (Kasson et al. 2016; Simmons et al. 2016; You et al. 2016; Li et al. 2017). Beetles of the genus *Ambrosiophilus* exhibit a further interesting unique behavior—**mycocleptism** (fungus stealing)—in which they overtake galleries of larger ambrosia beetles of the genus *Beaverium* and establish their galleries from the foreign tunnels into which the *Beaverium* fungal symbionts might directly grow (Hulcr and Cognato 2010). Types of communications in these diverse symbiotic and parasitic interactions are so far not studied (Hulcr and Stelinski 2017). Also not yet exploited in experimental work, intimate communication should also occur in the highly specified symbiotic interaction between termites and their within-nest agriculture of the white-rot fungus *Termitomyces*, associated bacteria and related microbe-mediated nest hygiene (Aanen 2006; Mathew et al. 2012; Poulsen et al. 2014; Kües et al. 2016a).

X. Mycorrhiza and Root Pathogens

Fungal interactions with roots can be beneficial for plants such as by the symbiotic mycorrhiza or harmful when pathogens are involved. Communication of fungi with plant roots can start prior to any physical contacts in a pre-colonization phase (Ditengou et al. 2015; Cordovez et al. 2017). Very few innovative studies are so far available on communication of *Agaricomycetes* with their plant hosts via VOCs.

The plant pathogen *Rhizoctonia solani* emits cocktails of VOCs which manipulate from a distance phytohormone and oxylipin productions by *Arabidopsis thaliana* (auxin up, ethylene and jasmonate down), accelerate plant development with production of more biomass and higher numbers of flowers, increase the root biomass of the plant and change the root architecture, and simultaneously make the plant more sensitive to the herbivore moth *Mamestra brassicae*, possibly as a predisposition for easier fungal infection. The fungal oxylipins 1-octen-3-ol and 3-octanone (Fig. 2a) were tested negatively as being responsible for the effects of *R. solani* VOC blends on the plant (Cordovez et al. 2017).

Phytohormones, i.e., diffusible auxin (indole-3-acetic acid, IAA) and volatile ethylene (Fig. 15), can be provided by ectomycorrhizal fungi (shown for the ascomycete *Tuber borchii* and the basidiomycetes *Hebeloma cylindrosporum*, *Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius*, and *Tricholoma vaccinum*) as fungi which have inducing effects on root formation of their tree hosts (Karabaghli et al. 1998; Karabaghli-Degron et al. 1998; Tranvan et al. 2000; Niemi et al. 2002; Splivallo et al. 2009; Krause et al. 2015; Vayssières et al. 2015). However, effects by fungal produced diffusible auxin on host roots need close distances of the fungi to the roots (Felton et al. 2009). Fungal VOCs in contrast might overcome longer distances. Accordingly, the ectomycorrizhal species *L. bicolor* emits specific bouquets of VOCs

which induce lateral root formation on its host tree poplar (*Populus* × *canescens*) and also on *A. thaliana* as a nonhost plant. Sesquiterpenes were found to be responsible and (−)-thujopsene (Fig. 8) to be sufficient to stimulate outgrowth of lateral roots. ß-Caryophyllene (Fig. 8) as another fungal sesquiterpene emitted by *L. bicolor* and found at first emitted by the also ectomycorrhizal species *P. involutus* was in contrast non-effective (Müller et al. 2013; Ditengou et al. 2015). ß-Caryophyllene has alone or as compound in VOC mixtures antimicrobial and insecticidal activities (Huang et al. 2012; Kudalkar et al. 2012; Fantaye et al. 2015), attracts nematodes which prey on root-damaging insect larvae (Rasmann et al. 2005), and it is considered a candidate product of *L. bicolor* for enhancing host root resistance against fungal pathogens (Ditengou et al. 2015).

Overall in a comparative study on mycelial cultures performed by Müller et al. (2013), the bouquet of VOCs emitted from the symbiotic *L. bicolor* distinguished from that of *P. involutus*, but much more from the VOC mixtures emitted by the opportunistic root pathogens *Armillaria mellea* and *Pholiota squarrosa* and the saprotrophic *Stropharia rugosoannulata*. The VOC mixtures of the pathogens shared as common sesquiterpenes γ-muurolene, α-bisabolene and epizonarene (Fig. 8) and the bicyclic alcohol geosmin (Fig. 9) while emission of α-muurolene and γ-selinene (Fig. 8) was unique to the saprotroph *S. rugosoannulata*. It is open whether these and other unique VOCs in the fungal scents have any relevance to the respective fungal life styles (Müller et al. 2013; Kanchiswamy et al. 2015a, b).

XI. Conclusions

We have collected in this chapter a vivid potpourri full of fascinating observations on *Agaricomycetes* in which communication between fungi and other individuals via VOCs should play a prominent role. Sessile fungi can so contact over shorter and longer distances other organisms and confer messages to these for them to react upon. Rarely, we however know yet or not for sure the actual chemical compound or compounds used by the fungi for signaling. Recent technical advances in VOC collection and detection and the ever increasing metabolite mass-spec databases with time will put things right (Kramer and Abraham 2012; Sect. II).

From the observations presented, ideas are emerging on why fungi may send out signals and to whom, who may benefit from direct communication and who as eavesdropper may take possible advantage from fungal signals. It is clear that fungi communicate on all levels, between own cells and with individuals of the own species and of other species in the own and in foreign kingdoms (see also the previous chapter 5 "Communication of Fungi on Individual, Species, Kingdom and above Kingdom Levels" in the first edition of Mycota XV, Kües and Navarro-Gonzaléz 2009).

In this chapter on communication of *Agaricomycetes* via VOCs, we have used the term signal not necessarily in its strictest biological definition, i.e., a means evolved for actively conveying information and influencing the behavior of the receivers (Lehmann et al. 2014; van't Padje et al. 2016). As one reason, this is because we still know much too little on true signals send out by the fungi. Cues distinguish from biological signals as passive, non-evolving biological and environmental traits which inherently provide the eavesdropping observer with information. A cue can however evolve into a signal for more advanced communication (Lehmann et al. 2014; van't Padje et al. 2016). Discussed examples in this chapter (e.g., in Sects. VI.D and VIII.A) document that chemical defense systems can interlink with communication and defense functions can adopt signaling functions. Signaling and communication in fungi resemble much in principles what is seen in plants (Blande and Glinwood 2016). As in the plants, good candidates for changing from a pre-existing status of cues into true biological signals are, for example, sesquiterpenes and other terpenoids (Fig. 8), next to using a diverse range of alcohols, ketones, aromates and more kind of VOCs.

There is a wealth of different sesquiterpenes with a vast structural diversity identified in the *Agaricomycetes* (Fig. 8), several of which are

unique to the fungi and even to individual fungal species. Only a few of them have been assigned clear functions to such as antibacterial, antifungal, insecticidal, and nematicidal. Mixtures of sesquiterpenes are common in the fungi which may provide them more easily with simultaneous defense measures against many different predators, parasites and competitors (Kramer and Abraham 2012; Kües and Badalyan 2017; see above Sects. V and VIII). Several of the fungal sesquiterpenes appear to have adopted dual roles. Signaling by sesquiterpenes offers advantages to confer on the one hand multiple different signals by individual and specific VOCs and on the other hand by their manifold possible combinations to provide matchless odor diversities, in order to distinguish from other senders and to address other specific organisms in required spatiotemporal patterns. In complex environmental systems of multiple interspecies communications and interactions in complicated networks with inestimable numbers of different signals from further sources, confusion might be avoided in this manner by the highest specificity that individual sesquiterpene signals and respective mixtures can provide. With a very high chemical specificity of signals, effects can be taxon-specific. Moreover, only low signal concentrations are needed under such circumstances for balancing out and conferring a message. Developing very specific own VOC production systems can thus be cost-effective.

In comparison to other fungal VOCs, sesquiterpenes and other terpenoids have comparably high vapor pressures. Such compounds will therefore be good conveyers as signals in shorter-distance communication. In long-distance communication, other less specific and more fugitive VOCs with lower vapor pressures and cheap production costs will perform better, such as the more general eight-carbon compounds as oxidation products of fatty acids (Sect. IV) or the sulfur compounds as typical biotic decay odors appointed, e.g., by the stinkhorns (Sect. VII). This is, for example, also reflected in the broad reaction spectrum on these VOCs seen distributed over the whole animal kingdom, the rather attraction of generalists, and the broader range of different ecological and developmental tasks which are controlled by VOCs like 1-octen-3-ol (Fig. 2a) for the fungi. Among are autoregulatory functions which neatly fit into and overlap with the ongoing broader communications within the complexer environments. The autoregulator 1-octen-3-ol combines again signaling and defense functions to optimally ponder decisions for the own fungal survival (Sect. IV.A). In organismal interactions, 1-octen-3-ol can have functions both as attractant and as repellent (Sect. IV.B). Mostly in studies of finding reactive VOCs, attention is given to attractants, looking at positive reactions. However, also other volatile compounds than 1-octen-3-ol may have dual roles for attracting the right target organism at the right time and in repellency of unwanted organisms [compare observations on α- and γ-muurolene (Fig. 8) presented in Sect. VIII.A]. As possibly documented in the examples of discrimination of fruiting bodies of different fungal species by different invertebrates (Sects. VI.A and VIII.B), combinations of VOCs are probably ideal to confer at the same time signals attractive to some organisms and hostile to others.

Acknowledgments Wolfgang Rohe and Martin Gabriel are thanked for scientific discussions. KW acknowledges a PhD scholarship awarded by the Royal Thai Government.

References

A'Bear DA, Boddy L, Jones TH (2012) Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. Glob Chang Biol 18:1823–1832

A'Bear AD, Murray W, Webb R, Boddy L, Jones TH (2013) Contrasting effects of elevated temperature and invertebrate grazing regulate multispecies interactions between decomposer fungi. PLoS One 8:e77610

A'Bear AD, Jones TH, Boddy L (2014a) Potential impacts of climate change on interactions among saprotrophic cord-forming fungal mycelia and grazing soil invertebrates. Fungal Ecol 10:34–43

A'Bear AD, Jones TH, Boddy L (2014b) Size matters: what have we learnt from microcosm studies of decomposer fungus-invertebrate interactions? Soil Biol Biochem 78:274–283

A'Bear AD, Jones TH, Kandeler E, Boddy L (2014c) Interactive effects of temperature and soil-moisture on fungal-mediated wood decomposition and extracellular enzyme activity. Soil Biol Biochem 70:151–158

A'Bear AD, Boddy L, Kandeler E, Ruess L, Jones TH (2014d) Effects of isopod population density on woodland decomposer microbial community function. Soil Biol Biochem 77:112–120

Aanen DK (2006) As you reap, so shall you sow: coupling of harvesting and inoculation stabilizes the mutualism between termites and fungi. Biol Lett 2:209–212

Abdullah AH, Adom AH, Shakaff AYM, Ahmad MN, Saad MA, Tan ES, Fikri NA, Markom MA, Zakaria A (2011) Electronic nose system for *Ganoderma* detection. Sens Lett 9:353–358

Abraham J, Opuni-Frimpong E, Weissbecker B, Schütz S, Angeli S (2014) Olfactory cues of mahogany trees to female *Hypsipyla robusta*. Bull Insect 67:21–30

Agger S, Lopez-Gallego F, Schmidt-Dannert C (2009) Diversity of sesquiterpene synthases in the basidiomycete *Coprinus cinereus*. Mol Microbiol 72:1181–1195

Ainsworth AM, Rayner ADM, Broxholme SJ, Beeching JR, Pryke JA, Scard PR, Berriman J, Powell KA, Floyd AJ, Branch SK (1990) Production and properties of the sesquiterpene, (+)-torreyol, in degenerate mycelial interaction between strains of *Stereum*. Mycol Res 94:799–809

Albuquerque P, Casadevall A (2012) Quorum sensing in fungi – a review. Med Mycol 50:337–345

Almaguer M, Rojas-Flores TI, Rodríguez-Rajo FJ, Aira M-J (2014) Airborne basidiospores of *Coprinus* and *Ganoderma* in a Caribbean region. Aerobiologia 30:197–204

Alpha CJ, Campos M, Jacobs-Wagner C, Strobel SA (2015) Mycofumigation by the volatile organic compound-producing fungus *Muscodor albus* induces bacterial cell death through DNA damage. Appl Environ Microbiol 81:1147–1156

Amare MG, Keller NP (2014) Molecular mechanisms of *Aspergillus flavus* secondary metabolism and development. Fungal Genet Biol 66:11–18

Anslan A, Bahram M, Tedersoo L (2016) Temporal changes in fungal communities associated with guts and appendages of Collembola as based on culturing and high-throughput sequencing. Soil Biol Biochem 96:152–159

Aprea E, Biasoli F, Carlin S, Versini G, Märk TD, Gasperi F (2007) Rapid white truffle headspace analysis by proton transfer reaction mass spectrometry and comparison with solid-phase microextraction coupled with gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 21:2554–2572

Arora D (1986) Mushrooms demystified: a comprehensive guide to the fleshy fungi. Ten Speed Press, Berkeley, CA

Arrarte E, Garmendia G, Rossini C, Wisniewski M, Vero S (2017) Volatile organic compounds produced by Antarctic strains of *Candida sake* play a role in the control of postharvest pathogens of apples. Biol Control 109:14–20

Ashkannejhad S, Horton TR (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. New Phytol 169:345–354

Ashmore L, Craske JD, Srzednicki G (2014) Volatile compounds in fresh, cooked fresh, dried and cooked dried *Agaricus bisporus* using ambient temperature vacuum distillation. Int Food Res J 21:263–268

Assaf S, Hadar Y, Dosoretz CG (1995) Biosynthesis of 13-hydroperoxylinoleate, 10-oxo-8-decenioc acid, and 1-octen-3-ol from linoleic acid by a mycelial-pellet homogenate of *Pleurotus pulmonarius*. J Agric Food Chem 43:2173–2178

Assaf S, Hadar Y, Dosoretz CG (1997) 1-Octen-3-ol and 13-hydroperoxylinoleate are products of distinct pathways in the oxidative breakdown of linoleic acid by *Pleurotus pulmonarius*. Enzym Microb Technol 21:484–490

Avbelj M, Zupan J, Raspor P (2016) Quorum-sensing in yeast and its potential in wine making. Appl Microbiol Biotechnol 100:7841–7852

Baby S, Johnson AJ, Govindan B (2015) Secondary metabolites from *Ganoderma*. Phytochemistry 114:66–101

Baietto M, Wilson AD, Bassi D, Ferrini F (2010) Evaluation of three electronic noses for detecting incipient wood decay. Sensors 10:1062–1092

Balakrishnan K, Holighaus G, Weissbecker B, Schütz S (2017) Electroantennographic responses of red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) to volatile organic compounds. J Appl Entomol 141:477–486

Ballari SA, Barrios-García MN (2013) A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. Mammal Rev 44:124–134

Basden EB (1952) Some drosophilids (Diptera) of the British Isles. Entomol Monthly Mag 88:200–201

Baubet E, Bonenfant C, Brandt S (2004) Diet of the wild boar in the French Alps. Galemys 16:101–113

Beck JJ, Vannette RL (2017) Harnessing insect-microbe chemical communications to control insect pests of agricultural systems. J Agric Food Chem 65:23–28

Beck JJ, Torto B, Vannette RL (2017) Eavesdropping on plant-insect-microbe chemical communications in agricultural ecology: a virtual issue on semiochemicals. J Agric Food Chem 65:5101–5103

Bedoya-Pérez MA, Isler I, Banks PB, McArthur C (2014) Roles of volatile terpene, 1,8-cineole, in plant-herbivore interactions: a foraging odor cue as well as a toxin? Oecologia 174:827–837

Beenken L, Sainge MN, Kocyan A (2016) *Lactarius megalopterus*, a new angiocarpous species from a tropical rainforest in Central Africa, shows adap-

tations to endozoochorous spore dispersal. Mycol Prog 15:58
Belda I, Ruiz J, Esteban-Fernández A, Navascués E, Marquina D, Santos A, Moreno-Arribas MV (2017) Microbial contribution to wine aroma and its intended use for wine quality improvement. Molecules 22:189
Beltran-Garcia MJ, Estarron-Espinosa M, Ogura T (1997) Volatile compounds secreted by the oyster mushroom (*Pleurotus ostreatus*) and their antibacterial activities. J Agric Food Chem 45:4049–4052
Benjamin DR (1995) Mushrooms: poisons and panaceas. A handbook for naturalist, mycologists and physicians. W.H. Freeman and Company, New York
Bennett JW, Inamdar AA (2015) Are some fungal volatile organic compounds (VOCs) mycotoxins? Toxins 7:3785–3804
Benucci GMN, Bonito GM (2016) The truffle microbiome: species and geography effects on bacteria associated with fruiting bodies of hypogeous Pezizales. Microb Ecol 72:4–8
Berendsen RL, Kalkhove SIC, Lugones LG, Wösten HAB, Bakker PAHM (2012) Germination of *Lecanicillium fungicola* in the mycosphere of *Agaricus bisporus*. Environ Microbiol Rep 4:227–233
Berendsen RL, Kalkhove SIC, Lugones LG, Baars JJP, Wösten HAB, Bakker PAHM (2013) Effects of the mushroom-volatile 1-octen-3-ol on dry bubble disease. Appl Microbiol Biotechnol 97:5535–5543
Berger KJ, Guss DA (2005a) Mycotoxins revisited: Part I. J Emerg Med 28:53–62
Berger KJ, Guss DA (2005b) Mycotoxins revisited: Part II. J Emerg Med 28:175–183
Bernardo D, Cabo AP, Novaes-Ledieu M, Mendoza CG (2004) *Verticillium* disease or "dry bubble" of cultivated mushrooms: the *Agaricus bisporus* lectin recognizes and binds *Verticillium fungicola* cell wall glucogalactomannan. Can J Microbiol 50:729–735
Bertolino S, Vizzini A, Wauters LA, Tosi G (2004) Consumption of hypogeous and epigeous fungi by the red squirrel (*Sciurus vulgaris*) in subalpine conifer forests. For Ecol Manag 202:227–233
Betancourt DA, Krebs K, Moore SA, Martin SM (2013) Microbial volatile organic compound emissions from *Stachybotrys chartarum* growing on gypsum wallboard and ceiling tile. BMC Microbiol 13:283
Bitas V, Kim HS, Bennett JW, Kang S (2013) Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol Plant-Microbe Interact 26:835–843
Blande JD, Glinwood R (eds) (2016) Deciphering chemical language of plant communication. Springer, Cham
Blaschke H, Bäumler W (1989) Mycophagy and spore dispersal by small mammals in Bavarian forests. For Ecol Manag 26:237–245
Bloomfield BJ, Alexander M (1967) Melanins and resistance of fungi to lysis. J Bacteriol 93:1276–1280
Boa ER (2004) Wild edible fungi – a global overview of their use and importance to people. Food and Agriculture Organization of the United Nations, Rome, Italy, http://www.fao.org/docrep/007/y5489e/y5489e00.htm
Boddy L (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol 31:185–194
Boddy L, Coates D, Rayner ADM (1983) Attraction of fungus gnats to zones of intraspecific antagonism on agar plates. Trans Br Mycol Soc 81:149–151
Borg-Karlson AK, Englund FO, Unelius CR (1994) Dimethyl oligosulphides, major volatiles released from *Sauromatum guttatum* and *Phallus impudicus*. Phytochemistry 35:321–323
Böroczky K, Zylstra KE, McCartney NB, Mastro VC, Tumlinson JH (2012) Volatile profile differences and associated *Sirex noctilio* activity in two host tree species in the Northeastern United States. J Chem Ecol 38:213–221
Boswell GP (2012) Modelling combat strategies in fungal mycelia. J Theor Biol 304:226–234
Boustie J, Rapior S, Fortin H, Tomasi S, Besserie JM (2005) Chemotaxonomic interest of volatile components in *Lepista inversa* and *Lepista flaccida* distinction. Cryptogam Mycol 26:27–35
Bozok F, Zarifikhosroshahi N, Kafkas E, Taşkin H, Büyükalaca S (2015) Comparison of volatile compounds of fresh *Boletus edulis* and *B. pinophilus* in Marmara region of Turkey. Not Bot Hort Agrobot Cluj-Naoica 43:192–195
Bozok F, Dogan HH, Taşkin H, Kaskas E, Büyükalaca S (2017) Volatile constituents of the edible *Tricholoma terreum* in Marmara region of Turkey. J Essent Oil Bear Plants 20:253–258
Braunsdorf C, Mailänder-Sanchez D, Schaller M (2016) Fungal sensing of host environment. Cell Microbiol 18:1188–1200
Bresinsky A, Besl H (1990) A colour atlas of poisonous fungi. Wolfe Publishing, London
Bretherton S, Tordoff GM, Jones TH, Boddy L (2006) Compensatory growth of *Phanerochaete velutina* mycelial systems grazed by *Folsomia candida* (Collembola). FEMS Microbiol Ecol 58:33–40
Brodhun F, Feussner I (2011) Oxylipins in fungi. FEBS J 278:1047–1063
Bryant PW (2010) Kairomonal attraction of the parasitoid *Ibalia leucospoides* (Hymenoptera: Ibaliidae) to *Amylostereum areolatum*, an obligate mycosymbiont of the European woodwasp, *Sirex noctilio*. Master thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY
Buller AHR (1909) Researches on fungi, vol I. Longmans, Green, London
Buller AHR (1922) Researches on fungi, vol II. Longmans, Green, London
Buller AHR (1931) Researches on fungi, vol IV. Longmans, Green, London

Burla H, Bächli G, Huber H (1991) *Drosophila* reared from the stinkhorn, *Phallus impudicus*, near Zurich, Switzerland. J Zool Syst Evol Res 29:97–107

Bruns TD (1984) Insect mycophagy in the Boletales: Fungivore diversity and the mushroom habitat. In: Wheeler Q, Blackwell M (eds) Fungus-insect relationships: perspectives in ecology and evolution. Columbia University Press, New York, pp 91–129

Burr B, Barthlott W, Westerkamp C (1996) *Stahelio-myces* (Phallales) visited by *Trigona* (Apidae): melittophily in spore dispersal of an Amazon stinkhorn? J Trop Ecol 12:441–445

Buzzini P, Gasparetti C, Turchetti B, Cramarossa MR, Vaughan-Martini A, Martini A, Pagnoni UM, Forti L (2005) Production of volatile organic compounds (VOCs) by yeasts isolated from the ascocarps of black (*Tuber melanosporum* Vitt.) and white (*Tuber magnatum* Pico) truffles. Arch Microbiol 184:187–193

Byers JA (1992) Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. J Chem Ecol 18:2385–2402

Çağlarirmak N (2008) Determination of nutrient and volatile constituents of *Agaricus bisporus* (brown) at different stages. J Sci Food Agric 89:634–638

Calderon-Cortes N, Quesada M, Watanabe H, Cano-Camacho H, Oyama K (2012) Endogenous plant cell wall digestion: a key mechanism in insect evolution. Annu Rev Ecol Evol Syst 43:45–71

Caldwell OR, Vernes K, Bärlocher F (2005) The northern flying squirrel (*Glaucomys sabrinus*) as a vector for inoculation of red spruce (*Picea rubens*) seedlings with ectomycorrhizal fungi. Sydowia 57:166–178

Cale JA, Collignon RM, Klutsch JG, Kanekar SS, Hussain A, Erbilgin N (2016) Fungal volatiles can act as carbon sources and semiochemicals to mediate interspecific interactions among bark beetle-associated fungal symbionts. PLoS One 11: e0162197

Callac P, Guinberteau J (2005) Morphological and molecular characterization of two novel species of *Agaricus* section *Xanthodermatei*. Mycologia 97:416–424

Calonje M, Mendoza CG, Cabo AP, Bernardo D, Novaes-Ledieu M (2000) Interaction between the mycoparasite *Verticillium fungicola* and the vegetative mycelial phase of *Agaricus bisporus*. Mycol Res 104:988–992

Camazine S (1983) Mushroom chemical defense: food aversion learning induced by hallucinogenic toxin, muscimol. J Chem Ecol 9(11):1473–1481

Camazine S (1985) Olfactory aposematism: association of food toxicity with naturally occurring odor. J Chem Ecol 11:1289–1285

Carazo P, Font E (2013) 'Communication breakdown': the evolution of signal unreliability and deception. Anim Behav 87:17–22

Cardona ME, Collinder E, Stern S, Tjellström B, Norin E, Midtvedt T (2005) Correlation between faecal iso-butyric and iso-valeric acids in different species. Microb Ecol Health Dis 17:177–182

Carey AB, Colgan W, Trappe JM, Molina R (2002) Effects of forest management on truffle abundance and squirrel diets. Northwest Sci 76:148–157

Carlquist M, Gibson B, Yuceer YK, Paraskevopoulou A, Sandell M, Angelov AI, Gotcheva V, Angelov AD, Etschmann M, de Billerbeck GM, Lidén G (2015) Process engineering for bioflavour production with metabolically active yeasts - a mini-review. Yeast 32:123–143

Carrau F, Gaggero C, Aguilar PS (2015) Yeast diversity and native vigor for flavor phenotypes. Trends Biotechnol 33:148–154

Castello JD, Shaw CG, Furnuss MM (1976) Isolation of *Cryptoporus volvatus* and *Fomes pinicola* from *Dendroctonus pseudotsugae*. Phytopathology 66:1431–1434

Castillo-Guevara C, Sierra J, Galindo-Flores G, Cuautle M, Lara C (2011) Gut passage of epigeous ectomycorrhizal fungi by two opportunistic mycophagous rodents. Curr Zool 57:283–299

Cespedes CL, Aqueveque PM, Avila JG, Alarcon J, Kubo I (2015) New advances in chemical defenses of plants: researches in Calceolariaceae. Phytochem Rev 14:367–380

Chaisaena W (2008) Light effects on fruiting body development of wildtype in comparison to light-insensitive mutant strains of the basidiomycete *Coprinopsis cinerea*, grazing of mites (*Tyrophagus putrescentiae*) on the strains and production of volatile organic compounds during fruiting body development. PhD thesis, University of Goettingen, Goettingen, Germany

Champavier Y, Pommier MT, Arpin N, Voiland A, Pellon G (2000) 10-Oxo-*trans*-8-decenoic acid (ODA): production, biological activities, and comparison with other hormone-like substances in *Agaricus bisporus*. Enzym Microb Technol 26:243–251

Chen J, Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohols. Genes Dev 20:1150–1161

Chen CC, Wu CM (1984) Studies on the enzymic reduction of 1-octen-3-one in mushroom *Agaricus bisporus*. J Agric Food Chem 32:1342–1344

Chen S, Qiu C, Huang T, Zhou W, Qi Y, Gao Y, Shen J, Qiu L (2013) Effect of 1-aminocyclopropane-1-carboxylic acid deaminase producing bacteria on the hyphal growth and primordium initiation of *Agaricus bisporus*. Fungal Ecol 6:110–118

Chen G, Zhang R-R, Liu Z, Sun W-B (2014) Spore dispersal of fetid *Lysurus mokusin* by feces of mycophagous insects. J Chem Ecol 40:893–899

Chen J, Zhao RL, Psarra LA, Guelly AK, De Kesel A, Rapior S, Hyde KD, Chukeatirote E, Callac P (2015) *Agaricus* section *Brunneopicti*: a phyloge-

netic reconstruction with descriptions of four new taxa. Phytotaxa 192:145–168
Chiron N, Michelot D (2005) Mushroom odors, chemistry and role in the biotic interactions – a review. Cryptogam Mycol 26:299–364
Cho IH, Kim SY, Choi HK, Kim YS (2006) Characterization of aroma-active compounds in raw and cooked pine-mushrooms (*Tricholoma matsutake* Sing.). J Agric Food Chem 54:6332–6335
Cho IH, Namgung HJ, Choi HK, Kim YS (2008) Volatiles and key odorants in the pileus and stipe of pine-mushroom (*Tricholoma matsutake* Sing.). Food Chem 106:71–76
Claridge AW, Tanton MR, Seebeck JH, Cork SJ, Cunningham RB (1992) Establishment of ectomycorrhiza on the roots of two species of *Eucalyptus* from fungal spores contained in the faeces of the long-nosed potoroo (*Potorous tridactylus*). Aust J Ecol 17:207–217
Claridge AW, Tanton MT, Cunningham RB (1993) Hypogeal fungi in the diet of the long-nosed potoroo (*Potorous tridactylus*) in mixed-species and regrowth eucalypt forest stands in south-eastern Australia. Wildl Res 20:321–337
Cloonan KR, Andreadis SS, Baker TC (2016a) Attraction of female fungus gnats, *Lycoriella ingenua*, to mushroom-growing substrates and the green mold *Trichoderma aggressivum*. Entomol Exp Appl 159:298–304
Cloonan KR, Andreadis SS, Chen H, Jenkins NE, Baker TC (2016b) Attraction, oviposition and larval survival of the fungus gnat, *Lycoriella ingenua*, on fungal species isolated from adults, larvae, and mushroom compost. PLoS One 11:e0167074
Clotuche G, Navajas M, Mailleux AC, Hance T (2013) Reaching the ball or missing the flight? Collective dispersal in the two-spotted spider mite *Tetranychus urticae*. PLoS One 8:e77573
Cloyd RA, Marley KA, Larson RA, Dickinson A, Arieli B (2011) Repellency of naturally occurring volatile alcohols to fungus gnat *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae) adults under laboratory conditions. J Econ Entomol 104:1633–1639
Colgan W III, Claridge AW (2002) Mycorrhizal effectiveness of *Rhizopogon* spores recovered from feacal pellets of small forest-dwelling mammals. Mycol Res 106:314–320
Combet E, Henderson J, Eastwood DC, Burton KS (2006) Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. Mycoscience 47:317–326
Combet E, Henderson J, Eastwood DC, Burton K (2009) Influence of sporophore development, damage, storage, and tissue specificity on the enzymic formation of volatiles in mushrooms (*Agaricus bisporus*). J Agric Food Chem 57:3709–3717
Cooper T, Vernes K (2011) Mycophagy in the larger bodied skinks of the genera *Tiliqua* and *Egernia*: are there implications for ecosystem health? Aust Zool 35:681–684
Cope RB (2007) Peer-reviewed – mushroom poisoning in dogs. Vet Med 102:95–100
Corcuff R, Mercier J, Tweddell R, Arul J (2011) Effect of water activity on the production of volatile organic compounds by *Muscodor albus* and their effect on three pathogens in stored potato. Fungal Biol 115:220–227
Cordovez V, Mommer L, Moisan K, Lucas-Barbosa D, Pierek R, Mumm R, Carrion VJ, Raaijmakers JM (2017) Plant phenotypic and transcriptional changes induced by volatiles from the fungal root pathogen *Rhizoctonia solani*. Front Plant Sci 8:1262
Cork SJ, Kenagy GJ (1989) Rates of gut passage and retention of hypogeous fungal spores in two forest-dwelling rodents. J Mammal 70:512–519
Costa R, Tedone L, De Grazia S, Dugo P, Mondello L (2013) Multiple headspace-solid-phase microextraction: an application to quantification of mushroom volatiles. Anal Chim Acta 770:1–6
Cottier F, Mühlschlegel FA (2012) Communication in fungi. Int J Microbiol 2012:351832
Courtney SP, Kibota TT, Singleton TS (1990) Ecology of mushroom-feeding Drosophilidae. Adv Ecol Res 20:225–275
Crowther TW, Boddy L, Jones TH (2011a) Outcomes of fungal interactions are determined by soil invertebrate grazers. Ecol Lett 14:1134–1142
Crowther TW, Boddy L, Jones TH (2011b) Species-specific effects of soil fauna on fungal foraging and decomposition. Oecologia 167:535–545
Crowther TW, Jones TH, Boddy L, Baldrian P (2011c) Invertebrate grazing determines enzyme production by basidiomycete fungi. Soil Biol Biochem 43:2050–2068
Crowther TW, Boddy L, Jones TH (2012) Functional and ecological consequences of saprotrophic fungus-grazer interactions. ISME J 6:1992–2001
Crowther TW, Stanton DWG, Thomas SM, A'Bear AD, Hiscox J, Jones TH, Voříšková J, Baldrian P, Boddy L (2013) Top-down control of soil fungal community composition by a globally distributed keystone consumer. Ecology 94:2518–2528
Cruz C, Noël-Suberville C, Montury M (1997) Fatty acid content and some flavor compound release in two strains of *Agaricus bisporus*, according to three stages of development. J Agric Food Chem 45:64–67
Cucura DMT (2013) Kairomonal attraction of the native parasitoid, *Ibalia leucospoides* (Hymenoptera: Ibaliidae), to *Amylostereum areolatum*, a mycosymbiont of *Sirex noctilio*. Master thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY
Culleré L, Ferreira V, Chevret B, Venturini ME, Sánchez-Gimeno AC, Blanco D (2010) Characterisation of aroma active compounds in black truffles (*Tuber melanosporum*) and summer truffles (*Tuber aestivum*) by gas chromatography–olfactory. Food Chem 122:300–306
Culleré L, Ferreira V, Marco P, Venturini ME, Marco P, Blanco D (2013) Potential aromatic compounds as markers to differentiate between *Tuber melanos-*

porum and *Tuber indicum* truffles. Food Chem 141:105–110
Culleré L, Ferreira V, Marco P, Venturini ME, Blanco D (2017) Does the host tree exert any influence on the aromatic composition of the black truffle (*Tuber melanosporum*)? Flavour Fragr J 32:133–140
Dardaillon M (1986) Seasonal variations in habitat selection and spatial distribution of wild boar (*Sus scrofa*) in the Camargue, Southern France. Behav Process 13:251–268
Das S, Gupta-Bhattacharya S (2012) Monitoring and assessment of airborne fungi in Kolkata, India, by viable and non-viable air sampling methods. Environ Monit Assess 184:4671–4684
David JF (2014) The role of litter-feeding macroarthropods in decomposition processes: a reappraisal of common views. Soil Biol Biochem 76:109–118
Davis TS, Crippen TL, Hofstetter RW, Tomberlin JK (2013) Microbial volatile emissions as insect semiochemicals. J Chem Ecol 39:840–859
De Pinho PG, Ribeiro B, Goncalves RF, Baptista P, Valentao P, Seabra RM, Andrade PB (2008) Correlation between the pattern volatiles and the overall aroma of wild edible mushrooms. J Agric Food Chem 56:1704–1712
Delaunois B, Jeandet P, Clement C, Bailieul F, Dorey S, Cordelier S (2014) Uncovering plant-pathogen crosstalk through apoplastic proteomic studies. Front Plant Sci 5:249
Di Francesco A, Ugolini L, Lazzeri L, Mari M (2015) Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. Biol Control 81:8–14
Diaz P, Ibanez E, Reglero G, Senorans FJ (2009) Optimization of summer truffle aroma analysis by SPME: comparison of extraction with different polarity fibres. LWT Food Sci Technol 42:1253–1259
Dicke M, Sabelis MW (1988) Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? Funct Ecol 2:131–139
Dicke M, van Lenteren JC, Boskamp GFP, van Dongen-van Leeuwen E (1984) Chemical stimuli in host-habitat location by *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae), a parasite of *Drosophila*. J Chem Ecol 10:695–712
Dickschat JS (2014) Capturing volatile natural products by mass spectrometry. Nat Prod Rep 31:836–861
Dickschat JS (2017) Fungal volatiles – a survey from edible mushrooms to moulds. Nat Prod Rep 34:310–328
Dijkstra FY, Wikén TO (1976) Studies on mushroom flavors. 1. Organoleptic significance of constituents of the cultivated mushroom, *Agaricus bisporus*. Z Lebensm Unters-Forsch 160:255–262
Ding X, Hou YL (2012) Identification of genetic characterization and volatile compounds of *Tricholoma matsutake* from different geographical origins. Biochem Syst Ecol 44:233–239
Ditengou FA, Müller A, Rosenkranz M, Felten J, Lasik H, Miloradovic van Doorn M, Legué V, Palme K, Schnitzler J-P, Polle A (2015) Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat Commun 6:6279
Dixon EF, Hall RA (2015) Noisy neighbourhoods: quorum sensing in fungal-polymicrobial infections. Cell Microbiol 17:1431–1441
Djekic I, Vunduk J, Tomašević I, Kozarski M, Petrovic P, Niksic M, Pudja P, Klaus A (2017) Application of quality function deployment on shelf-life analysis of *Agaricus bisporus* Portobello. LWT Food Sci Technol 78:82–90
Donaldson R, Stoddart M (1994) Detection of hypogeous fungi by Tasmanian bettong (*Bettongia gaimardi*: Marsupialia; Macropodoidea). J Chem Ecol 20:1201–1207
Donnelly D, Boddy L (1998) Developmental and morphological responses of mycelial systems of *Stropharia caerulea* and *Phanerochaete velutina* to soil nutrient enrichment. New Phytol 138:519–531
Dressaire E, Yamada L, Song B, Roper M (2015) Mushroom spore dispersal by convectively driven winds. arXiv:1512.07611
Dressaire E, Yamada L, Song B, Roper M (2016) Mushrooms use convectively created airflows to disperse their spores. Proc Natl Acad Sci USA 113:2833–2838
Drickamer LC (1972) Experience and selection behavior in food habits of *Peromyscus* – use of olfaction. Behaviour 41:269–286
Drilling K, Dettner K (2009) Electrophysiological responses of four fungivorous Coleoptera to volatiles of *Trametes versicolor*: implications for host selection. Chemoecology 19:109–115
Drissen G, Hemerik L, van Alphen JJM (1990) *Drosophila* species, breeding in the stinkhorn (*Phallus impudicus* Pers.) and their larval parasitoids. Neth J Zool 40:409–427
Dyer HC, Boddy L, Preston-Meek CM (1992) Effect of the nematode *Panagrellus redivivus* on growth and enzyme production by *Phanerochaete velutina* and *Stereum hirsutum*. Mycol Res 96:1019–1028
Eastwood D, Burton K (2002) Mushrooms – a matter of choice and spoiling oneself. Microbiol Today 29:18–18
Eastwood DC, Herman B, Noble R, Dobrovin-Pennington A, Sreenivasaprasad S, Burton KS (2013) Environmental regulation of reproductive phase change in *Agaricus bisporus* by 1-octen-3-ol, temperature and CO_2. Fungal Genet Biol 55:54–66
Effmert U, Kalderas J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in soil. J Chem Ecol 38:665–703
Egli S, Gfeller H, Bigler P, Schlunegger U-P (1988) Isolierung und Identifikation des Sesquiterpenalkohols (+/−)-Torreyol aus Reinkulturen des Ektomykorrhizapilzes *Cortinarius odorifer* Britz. Eur J For Pathol 18:351–356

El Ariebi N, Hiscox J, Scriven SA, Müller CT, Boddy L (2016) Production and effects of volatile organic compounds during interspecific interactions. Fungal Ecol 20:144–154

Endara L, Grimaldi DA, Roy BA (2010) Lord of the flies: pollination of *Dracula* orchids. Lankesteriana 10:1–11

Epps MJ, Arnold AE (2010) Diversity, abundance and community network structure in sporocarp-associated beetle communities of the central Appalachian mountains. Mycologia 102:785–802

Erbilgin N, Stein JD, Accivatti RE, Gilette NE, Mori SR, Bischel K, Cale JA, Carvalho CR, Wood DL (2017) A blend of ethanol and (−)-α-pinene were highly attractive to native siricid woodwasps (Siricidae, Siricinae) infesting conifers of the Sierra Nevada and the Allegheny Mountains. J Chem Ecol 43:172–179

Esser K (2016) Heterogenic incompatibility in fungi. In: Wendland J (ed) Growth, differentiation and sexuality. The mycota, vol I, 3rd edn. Springer, Cham, pp 103–131

Evans JA, Eyre CA, Rogers HJ, Boddy L, Müller CT (2008) Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. Fungal Ecol 1:57–68

Fäldt J, Jonsell M, Nordlander G, Borg-Karlson AK (1999) Volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* and their functions as insect attractants. J Chem Ecol 25:567–590

Fäldt J, Eriksson M, Valterová I, Borg-Karlson AK (2000) Comparison of headspace techniques for sampling volatile natural products in a dynamic system. Z Naturforsch C 55:180–188

Fantaye CA, Köpke D, Gershenzon J, Degenhardt J (2015) Restoring (*E*)-β-caryophyllene production in a non-producing maize line compromises its resistance against the fungus *Colletotrichum graminicola*. J Chem Ecol 41:213–223

Farre-Armengol G, Filella I, Llusia J, Peñuelas J (2016) Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. Trends Plant Sci 21:854–860

Felton J, Kohler A, Morin E, Bhalerao RP, Palme K, Martin F, Ditengou FA, Legué V (2009) The ectomycorrhizal fungus *Laccaria bicolor* stimulates lateral root formation in poplar and *Arabidopsis* through auxin transport and signaling. Plant Physiol 151:1991–2005

Fernández AA, Martínez AS, Villacide JM, Corley JC (2015) Behavioural responses of the woodwasp *Sirex noctilio* to volatile emissions of its fungal symbiont. J Appl Entomol 139:654–659

Fiecchi A, Kienle MG, Scala A, Cabella P (1967) Bismethylthiomethane, an odorous substance from white truffle, *Tuber magnatum* Pico. Tetrahedron Lett 8:1681–1682

Field JA, Verhagen FJM, de Jong E (1995) Natural organohalogen production by basidiomycetes. Trends Biotechnol 13:451–456

Fischbein D, Bettinelli J, Bernstein C, Corley JC (2012) Patch choice from a distance and use of habitat information during foraging by the parasitoid *Ibalia leucospoides*. Ecol Entomol 37:161–168

Fischer GJ, Keller NP (2016) Production of cross-kingdom oxylipins by pathogenic fungi: an update on their role in development and pathogenicity. J Microbiol 54:254–264

Flegg PB (1979) Effect of temperature on sporophore initiation and development in *Agaricus bisporus*. Mushroom Sci 10:595–602

Flegg PB, Wood DA (1985) Growth and fruiting. In: Flegg PB, Spence DM, Wood DA (eds) The biology and technology of the cultivated mushroom. Wiley, Chichester, pp 141–178

Fogel R, Trappe JM (1978) Fungus consumption (mycophagy) by small animals. Northwest Sci 52:1–31

Fons F, Rapior S, Eyssartier G, Bessière JM (2003) Volatile compounds in the *Cantharellus*, *Craterellus* and *Hydnum* genera. Cryptogam Mycol 24:367–376

Forbes SL, Perrault KA (2014) Decomposing odour profiling in the air and soil surrounding vertebrate carrion. PLoS One 9:e95107

Forti L, Di Mauro S, Cramarossa MR, Filipucci S, Turchetti B, Buzzini P (2015) Non-conventional yeasts whole cells as efficient biocatalysators for the production of flavors and fragrances. Molecules 20:10377–10398

Fraatz MA, Riemer SJL, Stöber R, Kaspera R, Nimtz M, Berger RG, Zorn H (2009a) A novel oxygenase from *Pleurotus sapidus* transforms valencene to nootkatone. J Mol Catal B Enzym 61:202–207

Fraatz MA, Berger RG, Zorn H (2009b) Nootkatone – a biotechnological challenge. Appl Microbiol Biotechnol 83:35–41

Frank JL, Anglin S, Carrington EM, Taylor DS, Viratos N, Southworth D (2009) Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks on *Quercus garryana*. Botany 87:821–829

Frazier K, Ligett A, Hines M, Styer E (2000) Mushroom toxicity in a horse with meningioangiomatosis. Vet Hum Toxicol 42:166–167

Fuijoka K, Shimizu N, Manome Y, Ikeda K, Yamamoto K, Tomizawa Y (2013) Discrimination method of the volatiles from fresh mushrooms by an electronic nose using a trapping system and statistical standardization to reduce sensor value variation. Sensors 13:15532–15548

Galante TE, Horton TR, Swaney DP (2011) 95% of basidiospores fall within 1 m of the cap: a field- and modelling-based study. Mycologia 103:1175–1183

Gara RI, Littke WR, Rhoades DF (1993) Emissions of ethanol and monoterpenes by fungal infected lodgepole pine trees. Phytochemistry 34:987–990

Garbeva P, Hordijk C, Gerards S, de Boer W (2014) Volatiles produced by the mycophagus soil bacterium *Collimonas*. FEMS Microbiol Ecol 87:639–649

Garcia-Alcega S, Nasir ZA, Ferguson R, Whitby C, Dumbrell AJ, Colbeck I, Gomes D, Tyrrel S, Cou-

lon F (2017) Fingerprinting outdoor air environment using microbial volatile organic compounds (MVOCs) – a review. TrAC Trends Anal Chem 86:75–83

García-Estrada C, Martín JF (2016) Biosynthetic gene clusters for relevant secondary metabolites produced by *Penicillium roqueforti* in blue cheeses. Appl Microbiol Biotechnol 100:8303–8313

Garnica S, Weiss M, Walther G, Oberwinkler F (2007) Reconstructing the evolution of agarics from nuclear gene sequences and basidiospore ultrastructure. Mycol Res 111:1019–1029

Garibay-Orijel R, Ramírez-Terrazo A, Ordaz-Velázquez M (2012) Woman care about local knowledge, experiences from ethnomycology. J Ethnobiol Ethnomed 8:25

Geervliet JBF, Posthumus MA, Vet LEM, Dicke M (1997) Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species. J Chem Ecol 23:2935–2954

Geisen S, Koller R, Hünninghaus M, Dumack K, Urich T, Bonkowski M (2016) The soil food web revisited: Diverse and widespread mycophagous soil protists. Soil Biol Biochem 94:10–18

Genard M, Lescourret F, Durrieu G (1988) Mycophagie chez le sanglier et hypothèses sur son rôle dans la dissémination des spores de champignons hypogés. Can J Zool 66:2324–2327

Genov P (1981) Food composition of wild boar in North-eastern and Western Poland. Acta Theriol 26:185–205

Gioacchini AM, Menotta M, Bertini L, Rossi I, Zeppa S, Zambonelli A, Piccoli G, Stocchi V (2005) Solid-phase microextraction gas chromatography/mass spectrometry: a new method for species identification of truffles. Rapid Commun Mass Spectrom 19:2365–2370

Gioacchini AM, Menotta M, Guescini M, Saltarelli R, Ceccaroli P, Amicucci A, Barbieri E, Giomoro G, Stocchi V (2008) Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds. Rapid Commun Mass Spectrom 22:3147–3153

Gkatzionis K, Yunita D, Linforth RST, Dickinson M, Dodd CER (2014) Diversity and activities of yeast from different parts of a Stilton cheese. Int J Food Microbiol 177:109–116

Gonmori K, Fujita H, Yokoyama K, Watanabe K, Suzuki O (2011) Mushroom toxins: a forensic toxicological review. Forensic Toxicol 29:85–94

Grimaldi D, Jeanike J (1984) Competition in natural populations of mycophagous *Drosophila*. Ecology 65:1113–1120

Grosshauser S, Schieberle P (2013) Characterization of the key odorants in pan-fried white mushrooms (*Agaricus bisporus* L.) by means of molecular sensory science: comparison with the raw mushroom tissue. J Agric Food Chem 61:3804–3813

Grove SJ (2002) Saproxylic insect ecology and the sustainable management of forests. Annu Rev Ecol Syst 33:1–23

Grove JF, Blight MM (1983) The oviposition attractant for the mushroom phorid *Megaselia halterata*: the identification of volatiles present in mushroom house air. J Sci Food Agric 34:181–185

Guevara R, Hutcheson KA, Mee AC, Rayner ADM, Reynolds SE (2000a) Resource partitioning of the host *Coriolus versicolor* by two ciid beetles: the role of odour compounds and host ageing. Oikos 91:184–194

Guevara R, Rayner ADM, Reynolds SE (2000b) Orientation of specialist and generalist fungivorous ciid beetles to host and non-host odours. Physiol Entomol 25:288–295

Guevara R, Rayner ADM, Reynolds SE (2000c) Effects of fungivory by two specialist ciid beetles (*Octotemnus glabriculus* and *Cis boleti*) on the reproductive fitness of their host fungus, *Coriolus versicolor*. New Phytol 145:137–144

Günther CS, Goddard MR, Newcomb RD, Buser CC (2015) The context of chemical communication driving a mutualism. J Chem Ecol 41:929–936

Hackman W, Meinander M (1979) Diptera feeding as larvae on macrofungi in Finland. Ann Zool Fenn 16:50–83

Hågvar S (1999) Saproxylic beetles visiting living sporocarps of *Fomitopsis pinicola* and *Fomes fomentarius*. Norw J Entomol 46:25–32

Hågvar S, Steen R (2013) Succession of beetles (genus *Cis*) and oribatid mites (genus *Carabodes*) in dead sporocarps of the red-banded polypore fungus *Fomitopsis pinicola*. Scan J For Res 28:436–444

Hågvar S, Amundsen T, Økland B (2014) Mites of the genus *Carabodes* (Acari, Oribatida) in Norwegian coniferous forests: occurrence in different soils, vegetation types and polypore hosts. Scan J For Res 29:629–638

Halbwachs H, Bässler C (2015) Gone with the wind – a review on basidiospores of lamellate agarics. Mycosphere 6:78–112

Halbwachs H, Brandl R, Bässler C (2015) Spore wall traits of ectomycorrhizal and saprotrophic Agarics may mirror their distinct lifestyles. Fungal Ecol 17:197–204

Halbwachs H, Simmel J, Bässler C (2016) Tales and mysteries of fungal fruiting: how morphological and physiological traits affect a pileate lifestyle. Fungal Biol Rev 30:36–61

Hall IR, Brown GT, Zambonelli A (2007) Taming the truffle: the history, lore, and science of the ultimate mushroom. Timber Press, Portland, Oregon

Halpern GM (2007) Healing mushrooms. Square One Publishers, Garden Park City, NY

Hammond JBW, Nichols R (1975) Changes in respiration and soluble carbohydrates during the post-harvest storage of mushrooms (*Agaricus bisporus*). J Sci Food Agric 26:835–842

Hanski I (1989) Fungivory: fungi, insects and ecology. In: Wilding N, Collins NM, Hammond PM, Webber JF (eds) Insect-fungus interactions. Academic Press, London, pp 25–68

Hanson AM, Hodge KT, Porter LM (2003) Mycophagy among primates. Mycologist 17:6–1

Hanson AM, Hall MB, Porter LM, Lintzenich B (2006) Composition and nutritional characteristics of fungi consumed by *Callimico goeldii* in Pando, Brazil. Int J Primatol 27:323–346

Hartlieb E, Rembold H (1996) Behavioral response of female *Helicoverpa* (*Heliothis*) *armigera* HB. (Lepidoptera: Noctuidae) moths to synthetic pigeonpea (*Cajanus cajan* L.) kairomone. J Chem Ecol 22:821–837

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36:191–218

Hayashi N, Honda K, Hara S, Idzumihara H, Mikata K, Komae H (1996) The chemical relationship between fungus and beetles on *Ponderosa* pine. Z Naturforsch 51c:813–817

Hayes WA (1978) Biological nature. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushrooms. Academic Press, New York, NY, pp 192–217

Hedlund K, Vet LEM, Dicke M (1996) Generalist and specialist parasitoid strategies of using odours of adult drosophilid flies when searching for larval hosts. Oikos 77:390–398

Heitmann M, Zannini E, Axel C, Arendt R (2017) Correlation of flavor profile to sensory analysis of bread produced with different *Saccharomyces cerevisiae* originating from the baking and beverage industry. Cereal Chem 94:746–751

Hirst MB, Richter CL (2016) Review of aroma formation through metabolic pathways of *Saccharomyces cerevisiae* in beverage fermentations. Am J Enol Vitic 67:361–370

Hiscox J, Boddy L (2017) Armed and dangerous – chemical warfare in wood decay communities. Fungal Biol Rev 31:169–184

Hiscox J, Savoury M, Müller CT, Lindahl BD, Rogers HJ, Boddy L (2015a) Priority effects during fungal community establishment in beech wood. ISME J 9:2246–2260

Hiscox J, Savoury M, Vaughan IP, Müller CT, Boddy L (2015b) Antagonistic fungal interactions influence carbon dioxide evolution from decomposing wood. Fungal Ecol 14:24–32

Hiscox J, Savoury M, Johnston SR, Parfitt D, Müller CT, Rogers HJ, Boddy L (2016a) Location, location, location: priority effects in wood decay communities may vary between sites. Environ Microbiol 18:1954–1969

Hiscox J, Clarkson G, Savoury M, Powell G, Savva I, Lloyd M, Shipcott J, Choimes A, Cumbriu XA, Boddy L (2016b) Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. Fungal Ecol 21:32–42

Hiscox J, Sacoury M, Toledo S, Kingscott-Edmunds J, Bettridge A, Waili NA, Boddy L (2017) Threesomes destabilize certain relationships: multispecies interactions between wood decay fungi in natural resources. FEMS Microbiol Ecol 93. https://doi.org/10.1093/femsec/fix014

Holighaus G (2012) Odour signals relevant to beetles in deadwood habitats. PhD thesis, University of Goettingen, Goettingen, Germany

Holighaus G, Rohlfs M (2016) Fungal allelochemicals in insect pest management. Appl Microbiol Biotechnol 100:5681–5689

Holighaus G, Schütz S (2006) Odours of wood decay as semiochemicals for *Trypodendron domesticum* L. (Col., Scolytidae). Mitt Dtsch Ges Allg Angew Ent 15:161–165

Holighaus G, Weißbecker B, von Fragstein M, Schütz S (2014) Ubiquitous eight-carbon volatiles of fungi are infochemicals for a specialist fungivore. Chemoecology 24:57–66

Horton TW, Swaney DP, Galante TE (2013) Dispersal of ectomycorrhizal basidiospores: the long and the short of it. Mycologia 105:1623–1626

Hosaka K, Uno K (2012) A preliminary survey on larval diversity in mushroom fruit bodies. Bull Natl Mus Nat Sci Ser B 38:77–85

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan W III, Domínguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Spatafora JW, Trappe JM (2006) Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. Mycologia 98:949–959

Howard WE, Marsh RE, Cole RE (1968) Food detection by deer mice using olfactory rather than visual cues. Anim Behav 16:13–17

Hsueh YP, Gronquist MR, Schwarz EM, Nath RD, Lee CH, Gharib S, Schroeder FC, Sternberg PW (2017) Nematophagous fungus *Arthrobotrys oligospora* mimics olfactory cues of sex and food to lure its nematode prey. elife 6:e20023

Huang M, Sanchez-Moreiras AM, Abel C, Sohrabi R, Lee S, Gershenzon J, Tholl D (2012) The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (*E*)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytol 193:997–1008

Hulcr J, Cognato AL (2010) Repeated evolution of crop theft in fungus-farming ambrosia beetles. Evolution 64:3205–3212

Hulcr J, Stelinski LL (2017) The ambrosia symbiosis: from evolutionary ecology to practical management. Annu Rev Entomol 62:285–303

Hung R, Lee S, Bennett JW (2015) Fungal volatile organic compounds and their role in ecosystems. Appl Microbiol Biotechnol 99:3395–3340

Husson F, Bompas D, Kermasha S, Belin JM (2001) Biogeneration of 1-octen-3-ol by lipoxygenase and hydroperoxide lyase activities of *Agaricus bisporus*. Process Biochem 37:177–182

Hutchings ML, Alpha-Cobb CJ, Hiller DA, Berro J, Strobel SA (2017) Mycofumigation through production of the volatile DNA-methylating agent *N*-methyl-*N*-nitrosoisobutyramide by fungi in the genus *Muscodor*. J Biol Chem 292:7358–7371

Hüttermann A, Majcherczyk A (2007) Conversion of biomass to fodder for ruminants or: how to get wood edible? In: Kües U (ed) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, Goettingen, Germany, pp 537–554

Hynes J, Müller CT, Jones TH, Boddy L (2007) Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. J Chem Ecol 33:43–57

Inga B (2007) Reindeer (*Rangifer tarandus tarandus*) feeding on lichens and mushrooms: traditional ecological knowledge among reindeer-herding Sami in northern Sweden. Rangifer 27:93–106

Ingold CT (1953) Dispersal in fungi. Clarendon Press, Oxford

Isidorov V, Tyszkiewicz Z, Pirożnikow E (2016) Fungal succession in relation to volatile organic compounds emissions from scots pine and Norway spruce leave litter-decomposing fungi. Atmos Environ 131:301–306

Jacobsen RM, Birkemoe T, Sverdrop-Thygeson A (2015) Priority effects of early successional insects influence late successional fungi in dead wood. Ecol Evol 5:4896–4905

Jacobsen RM, Kauserud H, Sverdrup-Thygeson A, Markussen Bjorbækmo M, Birkemoe T (2017) Wood-inhabiting insects can function as targeted vectors for decomposer fungi. Fungal Ecol 29:76–84

Jakolev J (2012) Fungal hosts of mycetophilids (Diptera: Sciaroidea excluding Sciaridae): a review. Mycology 3:11–23

Janssen A, van Alphen JJM, Sabelis MW, Bakker K (1995a) Specificity of odour-mediated avoidance of competition in *Drosophila* parasitoids. Behav Ecol Sociobiol 36:229–235

Janssen A, van Alphen JJM, Sabelis MW, Bakker K (1995b) Odour-mediated avoidance of competition in *Drosophila* parasitoids: the ghost of competition. Oikos 73:356–366

Jeleń HH (2003) Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. Lett Appl Microbiol 36:263–267

Ji T, Kang M, Baik BK (2017) Volatile organic compounds of whole-grain soft winter wheat. Cereal Chem 94:594–601

Jinks A, Laing DG (1999) A limit in the processing of components in odour mixtures. Perception 28:395–404

Jinks A, Laing DG (2001) The analysis of odor mixtures by humans: evidence for a configurational process. Physiol Behav 72:51–63

Jofré N, Pildain MB, Cirigliano AM, Cabrera GM, Corley JC, Martínez AS (2016) Host selection by *Ibalia leucospoides* based on temporal variations of volatiles from the hosts' fungal symbiont. J Appl Entomol 140:736–743

Johnson CN (1996) Interactions between mammals and ectomycorrhizal fungi. Trees 11:503–507

Johnson SD, Jürgens A (2010) Convergent evolution and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus. S Afr J Bot 76:796–807

Johansson T, Olsson J, Hjältén J, Jonsson BG, Ericson L (2006) Beetle attraction to sporocarps and wood infected with mycelia of decay fungi in old-growth spruce forests of northern Sweden. For Ecol Manag 237:335–341

Jones SE, Elliott MA (2017) *Streptomyces* exploration: competition, volatile communication and new bacterial behaviours. Trends Microbiol 25:523–532

Jones SE, Ho L, Rees CA, Hill JE, Nodwell JR, Elliot MA (2017) *Streptomyces* exploration is trigged by fungal interactions and volatile signals. elife 6:e21738

Jonsell N, Nordlander G (1995) Field attraction of *Coleoptera* to odours of the wood-decaying polypores *Fomitopsis pinicola* and *Fomes fomentarius*. Ann Zool Fenn 32:391–402

Jonsell M, Nordlander G (2002) Insects in polypore fungi as indicator species: a comparison between forest sites differing in amounts of dead wood. For Ecol Manag 157:101–118

Jonsell M, Nordlander G (2004) Host selection patterns in insects breeding on bracket fungi. Ecol Entomol 29:697–705

Jonsell M, Nordlander G, Johnsson M (1999) Colonization patterns of insects breeding in wood-decaying fungi. J Insect Conserv 3:145–161

Jonsell M, Nordlander G, Ehnström B (2001) Substrate associations of insects breeding in sporocarps of wood-decaying fungi. Ecol Bull 49:173–194

Jonsell M, Schroeder M, Larson T (2003) The saproxylic beetle *Bolitophagus reticulans*: its frequency on managed forests, attractive to volatiles and flight period. Ecography 26:421–428

Jonsell M, Alonso CG, Forshage M, van Achterberg C, Komonen A (2016) Structure of insect community in the fungus *Inonotus radiatus* in riparian boreal forests. J Nat Hist 50:1613–1631

Jonsson M, Kindvall O, Jonsell M, Nordlander G (2003) Modelling mating success of saproxylic beetles in relation to search behavior, population density and substrate abundance. Anim Behav 65:1069–1076

Jürgens A, Dötterl S, Meve U (2006) The chemical nature of fetid floral odors in stapeliads (Apocynbaceae-Asclepiadoideae-Ceropegieae). New Phytol 172:452–468

Jürgens A, Wee SL, Shuttleworth A, Johnson SD (2013) Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. Ecol Lett 16:1157–1167

Kadowaki K (2010a) Species coexistence patterns in a mycophagus insect community inhabiting the wood-decaying bracket fungus *Cryptoporus volva-*

tus (Polyporaceae: Basidiomycota). Eur J Entomol 107:89–99

Kadowaki K (2010b) Behavioral observation of two fungivorous beetles (Coleoptera: Tenebrionidae) on wood-decaying bracket fungus *Cryptoporus volvatus*. Entomol Sci 13:159–161

Kadowaki K, Inouye BD (2015) Habitat configuration affects spatial pattern of β diversity of insect communities breeding in oyster mushrooms. Ecosphere 6:72

Kadowaki K, Leschen RAB, Beggs JR (2011) Competition-colonization dynamics of spore-feeding beetles on the long-lived bracket fungi *Ganoderma* in New Zealand native forest. Oikos 120:776–786

Kaiser R (1993) On the scent of orchids. In: Teranishi R, Buttery RG, Sugisawa H (eds) Bioactive volatile compounds from plants. American Chemical Society, Washington, DC, pp 240–268

Kaiser R (2006) Flowers and fungi use scents to mimic each other. Science 311:806–807

Kamle M, Bar E, Lewinsohn D, Shavit E, Roth-Bejerano N, Kagan-Zur V, Barak Z, Guy O, Zaady E, Lewinsohn D, Sitrit Y (2017) Characterization of morphology, volatile profile, and molecular markers in edible desert truffles from the Negev desert. J Agric Food Chem 65:2977–2983

Kamra DN, Zadražil F (1986) Influence of gaseous phase, light and substrate pretreatment on fruitbody formation, lignin degradation and *in vitro* digestibility of wheat straw fermented with *Pleurotus* spp. Agric Wastes 18:1–17

Kanchiswamy CN, Malnoy M, Maffei ME (2015a) Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci 20:206–211

Kanchiswamy CN, Malnoy M, Maffei ME (2015b) Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front Plant Sci 6:151

Karabaghli C, Frey-Klett P, Sotta B, Bonnet M, Le Tacon F (1998) *In vitro* effect of *Laccaria bicolor* S238N and *Pseudomonas fluorescens* strain BBc6 on rooting of de-rooted shoot hypocotyls of Norway spruce. Tree Physiol 18:103–111

Karabaghli-Degron C, Sotta B, Bonnet M, Le Tacon F (1998) The auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) inhibits the stimulation of *in vitro* lateral root formation and the colonization of the tap-root cortex of Norway spruce (*Picea abies*) seedlings by the ectomycorrhizal fungus *Laccaria bicolor*. New Phytol 140:723–733

Kasson MT, Wickert KL, Staider CM, Macias AM, Berger MC, Simmons DR, Short DPG, DeVallance DB, Hulcr J (2016) Mutualism with aggressive wood-degrading*Flavodon ambrosius* (Polyporales) facilitates niche expansion and communal social structure in *Ambrosiophilus* ambrosia beetles. Fungal Ecol 23:86–96

Kataržyte M, Kutorga E (2011) Small mammal mycophagy in hemiboreal forest communities of Lithuania. Cent Eur J Biol 6:446–456

Kearney JN, Shorrocks B (1981) The utilization of naturally occurring yeasts by *Drosophila* species, using chemically defined substrates. Biol J Linn Soc 15:39–56

Kinzner M-C, Tratter M, Bächli G, Kirchmair M, Kaufmann R, Arthofer W, Schlick-Steiner BC, Steiner FM (2016) Oviposition substrate of the mountain fly *Drosophila nigrospersa* (Diptera: Drosophilidae). PLoS One 11:e165743

Kirchenbauer T, Fartmann T, Bässler C, Löffler F, Müller J, Strätz C, Seibold S (2017) Small-scale positive response of terrestrial gastropods to dear-wood addition is mediated by canopy openness. For Ecol Manag 396:85–90

Kleofas V, Popa F, Niedenthal E, Rühl M, Kost G, Zorn H (2015) Analysis of the volatilome of *Calocybe gambosa*. Mycol Prog 14:93

Koban MB, Gossner MM, Müller J, Steidle JLM, Bässler C, Hothorn T, Unsocker SB, Seibold B (2016) Short-distance attraction of saproxylic Heteroptera to olfactory cues. Insect Conserv Div 9:254–257

Kobayashi M, Kitabayashi K, Tuno N (2017) Spore dissemination by mycophagus adult drosophilids. Ecol Res 32:621–626

Komonen A, Kouki J (2005) Occurrence and abundance of fungus-dwelling beetles (Ciidae) in boreal forests and clearcuts: habitat associations at two spatial scales. Anim Biodic Conserv 28:137–147

Komonen A, Jonsell M, Økland B, Sverdrup-Thygeson A, Thunes K (2004) Insect assemblage associated with the polypore *Fomitopsis pinicola*: a comparison across Fennoscandia. Entomol Fenn 15:102–112

Konuma R, Umezawa K, Mizukoshi A, Kawarada K, Yoshida M (2015) Analysis of microbial volatile organic compounds produced by wood-decay fungi. Biotechnol Lett 37:1845–1852

Korpi A, Jarnberg J, Pasanen AL (2009) Microbial volatile organic compounds. Crit Rev Toxicol 39:139–193

Kou Y, Naqvi NI (2016) Surface sensing and signaling networks in plant pathogenic fungi. Semin Cell Dev Biol 57:84–92

Kramer R, Abraham W-R (2012) Volatile sesquiterpenes from fungi: what are they good for? Phytochem Rev 11:15–37

Krause K, Henke C, Asiimwe T, Ulbricht A, Klemmer S, Schachtschabel D, Boland W, Kothe E (2015) Biosynthesis and secretion of indole-3-acetic acid and its morphological effects on *Tricholoma vaccinum*-spruce ectomycorrhiza. Appl Environ Microbiol 81:7003–7011

Krivosheina NP (2008) Macromycete fruit bodies as a habitat for dipterans (Insecta, Diptera). Entomol Rev 88:778–792

Krügener S, Krings U, Zorn H, Berger RG (2010) A dioxygenase of *Pleurotus sapidus* transforms (+)-valenceneregio-specifically to (+)-nootkatone via a stereo-specifically allylic hydroperoxidation. Bioresour Technol 101:457–462

Kudalkar P, Strobel G, Riyaz-Hassan S, Geary B, Sears J (2012) *Muscodor sutura*, a novel endophytic fungus with volatile antibiotic activities. Mycoscience 53:319–325

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U (2015) From two to many: multiple mating types in basidiomycetes. Fungal Biol Rev 29:126–152

Kües U, Badalyan SM (2017) Making use of genomic information to explore the biotechnological potential of medicinal mushrooms. In: Agrawal DC, Tsay H-S, Shyur L-F, Wu Y-C, Wang S-Y (eds) Medicinal plants and fungi: recent advances in research and development. Springer, Berlin, pp 397–458

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kües U, Martin F (2011) On the road of understanding truffles in the underground. Fungal Genet Biol 48:555–560

Kües U, Navarro-Gonzaléz M (2009) Communication of fungi on individual, species, kingdom and above kingdom levels. In: Anke T, Weber S (eds) Physiology and genetics. Selected basic and applied aspects. The mycota, vol XV. Springer, Berlin, pp 79–106

Kües U, Navarro-Gonzaléz M (2015) How do Agaricomycetes shape their fruiting bodies? 1. Morphological aspects of development. Fungal Biol Rev 29:63–97

Kües U, Künzler M, Bottoli APF, Walser PJ, Granado JD, Liu Y, Bertossa RC, Ciardo D, Clergeot P-J, Loos S, Ruprich-Robert G, Aebi M (2003) Mushroom development in higher basidiomycetes: implications for human and animal health. In: Kushwaha RKS (ed) Fungi in human and animal health. Scientific Publishers (India), Jodhpur, pp 431–469

Kües U, James TY, Heitman J (2011) Mating type in basidiomycetes: unipolar, bipolar, and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J (eds) Evolution of fungi and fungal-like organisms. The mycota, vol XIV. Springer, Berlin, pp 97–160

Kües U, Badalyan SM, Gießler A, Dörnte B (2016a) Asexual sporulation in Agaricomycetes. In: Wendland J (ed) Growth, differentiation and sexuality. The mycota, vol I, 3rd edn. Springer, Cham, pp 269–328

Kües U, Subba S, Yu Y, Sen M, Khonsuntia W, Singhaduang W, Lange K, Lakkireddy K (2016b) Regulation of fruiting body development in *Coprinopsis cinerea*. Mushroom Sci 19:318–322

Kuribayashi T, Kaise H, Uno C, Hara T, Hayakawa T, Joh T (2002) Purification and characterization of lipoxygenase from *Pleurotus ostreatus*. J Agric Food Chem 50:1247–1253

Lacy RC (1984) Predictability, toxicity and trophic niche breadth in fungus-feeding Drosophilidae (Diptera). Ecol Entomol 9:43–54

Laing DG, Francis GW (1989) The capacity of humans to identify odors in mixtures. Physiol Behav 46:809–814

Lakkireddy K, Kües U (2017) Bulk isolation of basidiospores from wild mushrooms by electrostatic attraction with low risk of microbial contaminations. AMB Express 7:28

Lane GA, Fraser K (1999) A comparison of phenol and indole flavor compounds in fat, and of phenols in urine of cattle fed pasture or grain. N Z J Agric Res 42:289–296

Lauchli R, Potzer J, Kitto RZ, Kalbarczyk KZ, Rabe KS (2014) Improved selectivity of an engineered multi-product terpene synthase. Org Biomol Chem 12:4013–4020

Launchbaugh KL, Urness PJ (1992) Mushroom consumption (mycophagy) by North American cervids. Great Basin Nat 52:321–327

Le Roux C, Tournier E, Lies A, Sanguin H, Chevalier G, Duponnois R, Mousain D, Prin Y (2016) Bacteria of the genus *Rhodopseudomonas* (Bradyrhizobiaceae): obligate symbionts in mycelial cultures of the black truffles *Tuber melanosporum* and *Tuber brumale*. Springerplus 5:1085

Leach JE, Triplett LR, Argueso CT, Trivedi P (2017) Communication in the phytobiome. Cell 169:587–596

Leather SR, Baumgart EA, Evans HF, Quicke DLJ (2014) Seeing the trees for the wood – beech (*Fagus sylvatica*) decay fungal volatiles influence the structure of saproxylic beetle communities. Insect Conserv Div 7:314–326

Lee SO, Kim HY, Choi GJ, Lee HB, Jang KS, Choi YH, Kim JC (2009) Mycofumigation with *Oxyporus latemarginatus* EF069 for control of postharvest apple decay and *Rhizoctonia ropo* rot on moth orchid. J Appl Microbiol 106:1213–1219

Lee LW, Cheong MW, Curran P, Yu B, Liu SQ (2016) Modulation of coffee aroma via the fermentation of green coffee beans with *Rhizopus oligosporus*: I. Green coffee. Food Chem 211:916–924

Lehmann KDS, Goldman BW, Dworkin BW, Bryson DM, Wagner AP (2014) From cues to signals: evolution of interspecific communication via aposematism and mimicry in a predator-prey system. PLoS One 9:e91783

Lehmkuhl JF, Gould LE, Cázares E, Hosford DR (2004) Truffle abundancy and mycophagy by northern flying squirrels in eastern Washington forests. For Ecol Manag 200:49–65

Lemfack MC, Nickel J, Dunkel M, Preissner R, Piechulla B (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42:D744–D748

Leonhardt RH, Plagemann I, Linke D, Zelena K, Berger RG (2013) Orthologous lipoxygenases of *Pleurotus* spp. a comparison of substrate specificity and sequence homology. J Mol Catal B Enzym 97:189–195

Leschen RAB (1999) Systematics of Nitidulinae (Coleoptera; Nitidulidae): phylogenetic relationships, convexity and the origin of phallalophagy. Invertebr Taxon 13:845–882

Li DW (2005) Release and dispersal of basidiospores from *Amanita muscaria* var. *alba* and their infiltration onto a residence. Mycol Res 109:1235–1242

Li S, Nie Y, Ding Y, Zhao J, Tang X (2015) Effects of pure and mixed koji cultures with *Saccharomyces cerevisiae* on apple homogenate cider fermentation. J Food Process Preserv 39:2421–2430

Li N, Alfiky A, Vaughan MM, Kang S (2016a) Stop and smell the fungi: fungal volatile metabolites are overlooked signals involved in fungal interaction with plants. Fungal Biol Rev 30:134–144

Li Q, Zhang L, Li W, Li X, Huang W, Yang H, Zheng L (2016b) Chemical compositions and volatile compounds of *Tricholoma matsutake* from geographical areas at different stages of maturity. Food Sci Biotechnol 25:71–77

Li Y, Bateman CC, Skelton J, Jusino MA, Nolen ZJ, Simmons DR, Hulcr J (2017) Wood decay fungus *Flavodon ambrosius* (Basidiomycota Polyporales) is widely farmed by two genera of ambrosia beetles. Fungal Biol 121:984–989

Liarzi O, Bar E, Lewinsohn E, Ezra D (2016) Use of the endophytic fungus *Daldinia* cf. *concentrica* and its volatiles as bio-control agents. PLoS One 11: e0168242

Lilleskov EA, Bruns TD (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97:762–769

Lim TM (1977) Production, germination and dispersal of basidiospores of *Ganoderma pseudoferreum* on *Hevea*. J Rubber Res Inst Malays 25:93–99

Lin TY, Chen CY, Chien SC, Hsiao WW, Chu FH, Li WH, Lin CC, Shaw JF, Wang SY (2011) Metabolite profiles for *Antrodia cinnamomea* fruiting bodies harvested at different culture ages and from different wood substrates. J Agric Food Chem 59:7626–7635

Lin YL, Ma LT, Lee YR, Shaw JD, Wang SW, Chu FH (2017) Differential gene expression network in terpenoid synthesis of *Antrodia cinnamomea* in mycelia and fruiting bodies. J Agric Food Chem 65:1874–1866

Lindelöw Å, Risberg B, Sjödin K (1991) Attraction during night flight of scolytids and other bark- and wood-dwelling beetles to volatiles from fresh and stored spruce wood. Can J For Res 22:224–228

Liu C, Li Y (2017) Evaluation of the volatile profile of *Tuber liyuanum* by HS-SPME with GC-MS. Nat Prod Res 31:972–975

Liu RS, Jin GH, Xiao DR, Li HM, Bai FW, Tang YJ (2015) Screening of the key volatiles organic compounds of *Tuber melanosporum* fermentation by aroma sensory evaluation combination with principle component analysis. Sci Rep 5:17954

Livne-Luzon S, Avidan Y, Weber G, Migael H, Bruns T, Ovadia O, Shemesh H (2017) Wild boars as spore dispersal agents of ectomycorrhizal fungi: consequences for community composition at different habitat types. Mycorrhiza 27:165–174

Lopez-Gallego F, Agger SA, Abate-Pella D, Distefano MD, Schmidt-Dannert C (2010a) Sesquiterpene synthases Cop4 and Cop6 from *Coprinus cinereus*: catalytic promiscuity and cyclization of farnesyl pyrophosphate geometric isomers. Chembiochem 11:1093–1106

Lopez-Gallego F, Wawrzyn GT, Schmidt-Dannert C (2010b) Selectivity of fungal sesquiterpene synthases: role of the active site's H-1α loop in catalysis. Appl Environ Microbiol 76:7723–7733

Love DE (1976) The activities of various Diptera at the stinkhorn *Phallus impudicus*. Irish Nat J 18:301–303

Łuczaj L, Nieroda Z (2011) Collecting and learning to identify edible fungi in southeastern Poland: age and gender differences. Ecol Food Nutr 50:319–336

Łuczaj L, Stawarczyk K, Kosiek T, Pietras M, Kujawa A (2015) Wild food plants and fungi used by Ukrainians in the western part of Maramures region in Romania. Acta Soc Bot Pol 84:339–346

Lundgren L, Bergström G (1975) Wing scents and scent-released phases in the courtship behavior of *Lycaeides argyrognomon* (Lepidoptera: Lycaenidae). J Chem Ecol 1:399–412

Lynd ZR, Schlenke TA, Morran LT, de Roode JC (2017) Ethanol confers differential protection against generalist and specialist parasitoids of *Drosophila melanogaster*. PLoS One 12:e180182

Madden JL (1968) Behavioral responses of parasites to the symbiotic fungus associated with *Sirex noctilio*. Nature 218:189–190

Madelin MF (1956) Studies on the nutrition of *Coprinus lagopus* Fr., especially as affecting fruiting. Ann Bot 20:307–330

Maga JA (1981) Mushroom flavor. J Agric Food Chem 29:1–4

Magan N, Evans P (2000) Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. J Stored Prod Res 36:319–340

Mäki M, Heinonsalo J, Hellén H, Bäck J (2017) Contribution of understorey vegetation and soil processes to boreal forest isoprenoid exchange. Biogeosciences 14:1055–1073

Malheiro R, de Ponho PG, Soares S, Ferreira ACD, Baptista P (2013) Volatile biomarkers for wild mushrooms species discrimination. Food Res Int 54:186–194

Manimohan P, Thomas KA, Nisha VS (2007) Agarics on elephant dung in Kerala State, India. Mycotaxon 99:147–157

Maraun M, Augustin D, Müller J, Bässler C, Scheu S (2014) Changes in the community composition and trophic structure of microarthropods in sporocarps of the wood decaying fungus *Fomitopsis pinicola* along an altitudinal gradient. Appl Soil Ecol 84:16–23

March RE, Richards DS, Ryan RW (2006) Volatile compounds from six species of truffle – head-space analysis and vapor analysis at high mass resolution. Int J Mass Spectrom 249:60–67

Mari M, Bautista-Baños S, Sivakumar D (2016) Decay control in the postharvest system: role of microbial and plant volatile organic compounds. Postharvest Biol Technol 122:70–81

Martin MM (1991) The evolution of cellulose digestion in insects. Philos Trans R Soc B Biol Sci 333:281–288

Martin SH, Steenkamp ET, Wingfield MJ, Wingfield BD (2013) Mate-recognition and species boundaries in the ascomycetes. Fungal Divers 58:1–12

Martínez Blanco X, Tejera L, Beri Á (2016) First volumetric record of fungal spores in the atmosphere of Montevideo City, Uruguay: a 2-year survey. Aerobiologia 32:317–333

Martínez AS, Fernández-Arhex V, Corley JC (2006) Chemical information from the fungus *Amylostereum areolatum* and host-foraging behaviour in the parasitoid *Ibalia leucospoides*. Physiol Entomol 31:336–340

Martínez AS, Villacide J, Fernández Ajo AA, Martinson SJ, Corley JC (2014) *Sirex noctilio* flight behavior: toward improving current monitoring techniques. Entomol Exp Appl 152:135–140

Maser C, Maser Z (1988) Interactions among squirrels, mycorrhizal fungi, and coniferous forests in Oregon. Great Basin Nat 48:358–369

Maser C, Nussbaum RA, Trappe JM (1978) Fungal small mammal interrelationships with emphasis on Oregon coniferous forests. Ecology 59:799–809

Maser Z, Maser C, Trappe JM (1985) Food-habits of the northern flying squirrel (*Glaucomys sabrinus*) in Oregon. Can J Zool 63:1084–1088

Maser C, Maser Z, Witt JW, Hunt G (1986) The northern flying squirrel. A mycophagist in southwestern Oregon. Can J Zool 64:2086–2089

Massalha H, Korenblum E, Tholl D, Aharoni A (2017) Small molecules below-ground: the role of specialized metabolites in the rhizosphere. Plant J 90:788–807

Mathew GM, Ju YM, Lai CY, Mathew DC, Huang CC (2012) Microbial community analysis in the termite gut and fungus comb of *Odontotermes formosanus*: the implication of *Bacillus* as mutualist. FEMS Microbiol Lett 79:504–517

Mattson DJ, Podruzny SR, Haroldson MA (2002) Consumption of fungal sporocarps by Yellowstone grizzly bears. Ursus 13:95–103

Mau JL, Beelman RB (1996) Role of 10-oxo-trans-8-decanoic acid in the cultivated mushroom, *Agaricus bisporus*. In: Royse DJ (ed) Mushroom biology and mushroom products. Pennsylvania State University, University Park, pp 553–562

Mau JL, Beelman RB, Ziegler GR (1992) 1-Octen-3-ol in the cultivated mushroom, *Agaricus bisporus*. J Food Sci 57:704–706

Mau JL, Beelman RB, Ziegler GR (1993) Factors affecting 1-octen-3-ol in mushrooms at harvest and during postharvest storage. J Food Sci 58:331–334

Mau JL, Chyau CC, Li JY, Tseng YH (1997) Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. J Agric Food Chem 45:4726–4729

Mau JL, Lin YP, Chen PT, Wu YH, Peng JT (1998) Flavor compounds in king oyster mushrooms *Pleurotus eryngii*. J Agric Food Chem 46:4587–4591

Mau JL, Lin HC, Ma JT, Song SF (2001) Non-volatile taste components of several specialty mushrooms. Food Chem 73:461–466

McQuade DB, Williams EH, Eichenbaum HB (1986) Cues used for localizing food by the gray squirrel (*Sciurus carolinensis*). Ethology 72:22–30

Mehli H, Skuterud L (1998) The influence of fungi on long-term behavior of radiocaesium in Norwegian sheep. Sci Total Environ 224:9–17

Meng D, Shen L, Yang R, Zhang X, Sheng J (2014) Identification and active site analysis of the 1-aminocyclopropane-1-carboxylic acid oxidase catalysing the synthesis of ethylene in *Agaricus bisporus*. Biochim Biophys Acta 1840:1201–1228

Meyer RT, Weir A, Horton TR (2015) Small-mammal consumption of hypogeous fungi in the central Adirondacks of New York. Northeast Nat 22:648–651

Micheluz A, Manente S, Rovea M, Slanzi D, Varese GC, Ravagnan G, Formenton G (2016) Detection of volatile metabolites of moulds isolated from a contaminated library. J Microbiol Methods 128:34–41

Mokkonen M, Lindstedt C (2016) The evolutionary ecology of deception. Biol Rev 91:1020–1035

Money NP (1998) More g´s than the space shuttle: ballistospore discharge. Mycologia 90:547

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge

Morawicki RO, Beelman RB, Peterson D, Ziegler G (2005) Biosynthesis of 1-octen-3-ol and 10-oxi-trans-8-decenoic acid using a crude homogenate of *Agaricus bisporus*. Optimization of the reaction: kinetic factors. Process Biochem 40:131–137

Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, Bailey AM, Billette C, Coutinho PM, Deakin G, Doddapaneni H, Floudas D, Grimwood J, Hildén K, Kües U, LaButti KM, Lapidus A, Lindquist EA, Lucas SM, Murat C, Riley RW, Salamov AA, Schmutz J, Subramanian V, Wösten HAB, Xu J, Eastwood DC, Cullen D, de Vries RP, Lundell T, Hibbett DS, Henrissat B, Burton KS, Kerrigan RW, Challen MP, Grigoriev

IV, Martin F (2012) Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to humic-rich ecological niche. Proc Natl Acad Sci USA 109:17501–17506

Mozuraitis R, Stranden M, Ramirez MI, Borg-Karlson AK, Mustaparta H (2002) (−)-Germacrene D increases attraction and oviposition by the tobacco budworm moth *Heliothis virescens*. Chem Senses 27:505–509

Müller A, Faubert P, Hagen M, Castell WZ, Polle A, Schnitzler JP, Rosenkranz M (2013) Volatile profiles of fungi – chemotyping of species and ecological functions. Fungal Genet Biol 54:25–33

Nagy LG, Walther G, Házi J, Vágvölgyi C, Papp T (2011) Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the Psathyrellaceae. Syst Biol 60:303–317

Nagy LG, Házi J, Szappanos B, Kocsubé S, Bálint B, Rákhely G, Vágvölgyi C, Papp T (2012) The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (Agaricales, Fungi). Syst Bot 61:595–607

Nakamori T, Suzuki A (2005) Spore-breaking capabilities of collembolans and their feeding habitat within sporocarps. Pedobiologia 49:261–267

Nakamori T, Suzuki A (2007) Defensive role of cystidia against Collembola in the basidiomycetes *Russula bella* and *Strobilurus ohshimae*. Mycol Res 111:1345–1351

Nakamori T, Suzuki A (2008) Surface properties of the mushroom *Strobilurus ohshimae* result in food differentiation by collembolan species. Eur J Soil Biol 44:478–482

Nakamori T, Suzuki A (2010) Spore resistance and gut-passage time of macrofungi consumed by *Ceratophysella denisana* (Collembola: Hypogastruridae). Fungal Ecol 3:38–42

Nakamori T, Suzuki A (2012) Occurrence and gut contents of flatworms on fungal sporocarps. J Nat Hist 46:2763–2767

Nakano M, Ochiai A, Kamata K, Nakamori T (2017) The preference of *Morulina alata* (Collembola: Neanuridae) feeding on some fungal sporocarps and the effects of passage through the gut on spores. Eur J Soil Biol 81:116–119

Navarro-Gonzaléz M (2008) Growth, fruiting body development and laccase production of selected coprini. PhD thesis, University of Goettingen, Goettingen

Nidiry ESJ (2001) Structure-fungitoxicity relationships of some volatile flavor constituents of the edible mushrooms *Agaricus bisporus* and *Pleurotus florida*. Flavour Fragr J 16:245–248

Niederpruem DJ (1963) Role of carbon dioxide in fruiting *of Schizophyllum commune*. J Bacteriol 85:1300–1308

Niemi K, Vuorinen T, Ernstsen A, Häggman H (2002) Ectomycorrhizal fungi and exogenous auxins influence root and mycorrhiza formation of scots pine hypocotyl cuttings in vitro. Tree Physiol 22:1231–1239

Nieminen T, Neubauer P, Sivelä S, Vatamo S, Silfverberg P, Salkinoja-Salonen M (2008) Volatile compounds produced by fungi grown in strawberry jam. LWT Food Sci Technol 41:2051–2056

Noble R, Dobrovin-Pennington A, Hobbs PJ, Pederby J, Rodger A (2009) Volatile C8 compounds and pseudomonads influence primordium formation of *Agaricus bisporus*. Mycologia 101:583–591

Noble R, Fermor TR, Lincoln S, Dobrovin-Pennington A, Evered C, Mead A, Li R (2017) Primordia initiation of mushroom (*Agaricus bisporus*) strains on axenic casing materials. Mycologia 95:620–629

Noël-Suberville C, Cruz C, Guinberteau J, Montury M (1996) Correlation between fatty acid content and aromatic release in fresh blewit (*Lepista nuda*). J Agric Food Chem 44:1180–1183

Nosaka S, Miyazawa M (2014) Characterization of volatile components and odor-active compounds in the oil of edible mushroom *Boletopsis leucomelas*. J Oleo Sci 63:577–583

Nuñez MA, Hayward J, Horton TR, Amico GC, Dimarco RD, Barrios-Garcia MN, Simberloff D (2013) Exotic mammals disperse exotic fungi that promote invasion by exotic trees. PLoS One 8(6): e66832

Nuske SJ, Vernes K, May TW, Claridge AW, Congdon BC, Krockenberger A, Abell SE (2017a) Redundancy among mammalian fungal dispersers and the importance of declining specialists. Fungal Ecol 27:1–13

Nuske SJ, Vernes K, May TW, Claridge AW, Congdon BC, Krockenberger A, Abell SE (2017b) Data on the fungal species consumed by mammal species in Australia. Data Brief 12:251–260

Nuss I (1982) Die Bedeutung der Proterosporen: Schlußfolgerungen aus Untersuchungen an *Ganoderma* (*Basidiomycetes*). Plant Syst Evol 141:53–76

Oei P (2003) Mushroom cultivation, 3rd edn. Bakhuys Publishers, Leiden, The Netherlands

Oka K, Ishihara A, Sakaguchi N, Nishino S, Parada SY, Nakagiri A, Otani H (2015) Antifungal activity of volatile compounds produced by an edible mushroom *Hypsizygus marmoreus* against phytopathogenic fungi. J Phytopathol 163:11–12

Okull DO, Beelman RB, Gourama H (2003) Antifungal activity of 10-oxo-trans-8-decenoic acid and 1-octen-3-ol against *Penicillium expansum* in potato dextrose agar medium. J Food Prot 66:1503–1505

Olberg S, Andersen J (2000) Field attraction of beetles (Coleoptera) to the polypores *Fomes fomentarius* and *Phellinus* spp (Fungi: Aphyllophorales) in Northern Norway. Entomol Gen 24:217–236

Oldroyd GED (2013) Speak, friend and enter: signaling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

Oliveira FC, Barbosa FG, Mafezoli J, Oliveira MCF, Camelo ALM, Longhinotti E, Lima ACA, Camara MPS, Conçalves FJT, Freire FCO (2015) Volatile

organic compounds from filamentous fungi: a chemotaxonomic tool of the Botryosphaeriaceae family. J Braz Chem Soc 26:2189–2194

Omarini A, Henning C, Ringuelet J, Zygadlo JA, Albertó E (2010) Volatile composition and nutritional quality of the edible mushroom *Polyporus tenuiculus* grown on different agro-industrial waste. Int J Food Sci Technol 45:1603–1609

Orledge GM, Reynolds SE (2005) Fungivore host-use groups from cluster analysis: patterns of utilisation of fungal fruiting bodies by ciid beetles. Ecol Entomol 30:620–641

Osono T (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecol Res 22:955–974

Ottosson E, Nordén J, Dahlberg A, Edman M, Jönsson M, Larsson KH, Olsson J, Penttilä R, Stenlid J, Ovaskainen O (2014) Species associations during the succession of wood-inhabiting fungal communities. Fungal Ecol 11:17–28

Ouzouni PK, Koller WD, Badeka AV, Riganakos KA (2009) Volatile compounds from the fruiting bodies of the three *Hygrophorus* mushroom species from Northern Greece. Int J Food Sci Technol 44:854–859

Ovaskainen O, Schigel D, Ali-Kovero H, Auvinen P, Paulin L, Nordén B, Nordén J (2013) Combining high-throughput sequencing with fruit body surveys reveals contrasting life-history strategies in fungi. ISME J 7:1696–1709

Överås J, Ulvund MJ, Bakkevig S, Eiken R (1979) Poisoning in sheep induced by the mushroom *Cortinarius speciosissimus*. Acta Vet Scand 20:148–150

Paczkowski S, Schütz S (2011) Post-mortem volatiles of vertebrate tissue. Appl Microbiol Biotechnol 91:917–935

Paczkowski S, Maibaum F, Paczkowska MH, Schütz S (2012) Decaying mouse volatiles perceived by *Calliphora vicina*Rob.-Desv. J Forensic Sci 57:1497–1506

Paczkowski S, Nicke S, Ziegenhagen H, Schütz S (2015) Volatile emission of decomposing pig carcasses (*Sus scrofa domesticus* L.) as an indication for *postmortem* interval. J Forensic Sci 60:S130–S137

Pan LQ, Zhang W, Zhu N, Mao SB, Tu K (2014) Early detection and classification of pathogenic fungal disease in post-harvest strawberry fruit by electronic nose and gas chromatography-mass spectrometry. Food Res Int 62:162–168

Pannebakker BA, Garrido NRT, Zwaan BJ, van Alphen JJM (2008) Geographic variation in host-selection behavior in the *Drosophila* parasitoid *Leptopilina clavipes*. Entomol Exp Appl 127:48–54

Pantoja LDM, do Nascimento RF, de Araújo Nunes AB (2016) Investigation of fungal volatile organic compounds in hospital air. Atmos Pollut Res 7:659–663

Papaj DR, Vet LEM (1990) Odor learning and foraging success in the parasitoid, *Leptopilina heterotoma*. J Chem Ecol 16:3137–3150

Parafati L, Vitale A, Restuccia C, Cirvilleri G (2017) Performance evaluation of volatile organic compounds by antagonistic yeasts immobilized on hydrogel spheres against grey, green and blue postharvest decay. Food Microbiol 63:191–198

Park MS, Fong JJ, Lee H, Shin S, Lee S, Lee N, Lim YW (2014) Determination of coleopteran insects associated with spore dispersal of *Cryptoporus volvatus* (Polyporaceae: Basidiomycota) in Korea. J Asia Pac Entomol 17:647–651

Parkinson DR, Churchill TJ, Wady L, Pawliszyn J (2009) Investigation of mold growth in indoor school buildings by monitoring outgassed methyl benzoate as a MVOC biomarker. Indoor Built Environ 18:257–264

Peay KG, Schubert MG, Nouyen NH, Bruns TD (2012) Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. Mol Ecol 21:4122–4136

Pei F, Yang W, Ma N, Fang Y, Zhao L, An X, Xin Z, Hu Q (2016) Effect of the two drying approaches on the volatile profile of button mushroom (*Agaricus bisporus*) by headspace GC-MS and electronic nose. LWT Food Sci Technol 72:343–350

Peiris D, Dunn WB, Brown M, Kell DB, Roy I, Hedger JN (2008) Metabolic profiles of interacting mycelial fronts differ for pairings of the wood decay basidiomycete fungus, *Stereum hirsutum* with its competitors *Coprinus micaceus* and *Coprinus disseminatus*. Metabolomics 4:52–62

Pennerman KK, Yin G, Bennett JW (2015) Health effects of small volatile compounds from East Asian medicinal mushrooms. Mycobiology 43:9–13

Peñuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B, Schnitzler JP (2014) Biogenic volatile emissions from the soil. Plant Cell Environ 37:1866–1891

Pérez F, Castillo-Guevara C, Galindo-Flores G, Cuautle M, Estrada-Torres A (2012) Effect of gut passage by two highland rodent on spore activity and mycorrhiza formation of two species of ectomycorrhizal fungi (*Laccaria trichodermophora* and *Suillus tomentosus*). Botany 90:1084–1092

Perez-Leanos A, Ramirez Loustalot-Laclette M, Nazario-Yepiz N, Markow TA (2017) Ectoparasite mites and their *Drosophila* hosts. Fly 11:10–18

Peris JE, Rodriguez A, Peña L, Fedriani JM (2017) Fungal infestation boots fruit aroma and fruit removal by mammals and birds. Sci Rep 7:5646

Pettit GR, Meng Y, Pettit RK, Herald DL, Cichacz ZA, Doubek DL, Richert L (2010a) Antineoplastic agents. 556. Isolation and structure of Coprinastatin 1 from *Coprinus cinereus*. J Nat Prod 73:388–392

Pettit GR, Meng Y, Pettit RK, Herald DL, Hogan G, Cichacz ZA (2010b) Antineoplastic agents. 582. Part 1. Isolation and structure of a cyclobutane-type sesquiterpene cancer cell growth inhibitor from *Coprinus cinereus* (Coprinaceae). Bioorg Med Chem 18:4879–4883

Pfeil RM, Mumma RO (1992) Air sampling of volatiles from *Agaricus bisporus* in a mushroom facility and from mushroom compost. HortSci 27:416–419

Pfeil RM, Mumma RO (1993) Bioassay for evaluating attraction of the phorid fly, *Megaselia halteria* to compost colonized by the commercial mushroom, *Agaricus bisporus* and to 1-octen-3-ol and 3-octanone. Entomol Exp Appl 69:137–144

Piattoni F, Ori F, Morara M, Iotti M, Zambonelli A (2012) The role of wild boars in spore dispersal of hypogeous fungi. Acta Mycol 47:145–153

Piattoni F, Amicucci A, Iotti M, Ori V, Stocchi C, Zambonelli A (2014) Viability and morphology of *Tuber aestivum* spores after passage through the gut of *Sus scrofa*. Fungal Ecol 9:52–56

Piattoni F, Ori F, Amicucci A, Salerni E, Zambonelli A (2016) Interrelationships between wild boars (*Sus scrofa*) and truffles. Soil Biol 47:375–389

Pierce AM, Pierce HD, Borden JH, Oehlschlager AC (1991) Fungal volatiles: semiochemicals for scored-product beetles (Coleoptera, Cucujidae). J Chem Ecol 17:581–597

Pimenta L, Ferreira MA, Pedroso MP, Campos VP (2017) Wood-associated fungi produce volatile organic compounds toxic to root-knot nematode. Sci Agric 74:303–310

Piovano M, Garbarino JA, Sanchez E, Young ME (2009) Volatile organic components from fresh non-edible Basidiomycetes fungi. Nat Prod Commun 4:1737–1739

Pires EJ, Teixeira JA, Brányik T, Vicente AA (2014) Yeast: the soul of beer's aroma – a review of flavour-active esters and higher alcohols produced by brewing yeast. Appl Microbiol Biotechnol 98:1937–1949

Plagemann I, Zelena K, Arendt P, Ringel PD, Krings U, Berger RG (2013) LOX(Psa)1, the first recombinant lipoxygenase from a basidiomycete fungus. J Mol Catal B Enzym 87:99–104

Plagemann I, Krings U, Berger RG (2014) Isolation and characterization of wild-type lipoxygenase LOX (Psa)I from *Pleurotus sapidus*. Z Naturforsch C 69:148–154

Pöggeler S (2011) Function and evolution of pheromones and pheromone receptors in filamentous ascomycetes. In: Pöggeler S, Wöstemeyer J (eds) Evolution of fungi and fungal-like organisms, The mycota, vol XIV. Springer, Berlin, pp 73–96

Pointelli E, Santa-maria MA, Caretta G (1981) Coprophilous fungi of the horse. Mycopathologia 74:89–105

Põldmaa K, Kaasik A, Tammaru T, Kurina O, Jürgenstein S, Teder T (2016) Polyphagy on unpredictable resources does not exclude host specialization: insects feeding on mushrooms. Ecology 97:2824–2833

Policha T, Dacis A, Barnadas M, Dentinger BTM, Raguso RA, Roy BA (2016) Disentangling visual and olfactory signals in mushroom-mimicking-*Dracula* orchids using realistic three-dimensional printed flowers. New Phytol 210:1058–1071

Polizzi V, Adams A, Malysheva SV, De Saeger S, Van Peteghem C, Moretti A, Picco AM, De Kimpe N (2012) Identification of volatile markers for indoor fungal growth and chemotaxonomic classification of *Aspergillus species*. Fungal Biol 116:941–953

Polke M, Sprenger M, Scherlach K, Albán-Proaño MC, Martin R, Hertweck C, Hube B, Jacobsen ID (2017) A functional link between hyphal maintenance and quorum sensing in *Candida albicans*. Mol Microbiol 103:595–617

Poulsen M, Hu H, Li C, Chen Z, Xu L, Otani S, Nygaard S, Nobre T, Klaubauf S, Schindler PM, Hauser F, Pan H, Yang Z, Sonnenberg ASM, de Beer ZW, Zhang Y, Wingfield MJ, Grimmelikhuijzen CJP, de Vries RP, Korb J, Aanen DK, Wang J, Boosma JJ, Zhang G (2014) Complementary symbiont contributions to plant decomposition in a fungus-farming termite. Proc Natl Acad Sci USA 111:14500–14505

Pringle A, Patek SN, Fischer M, Stolze J, Money NP (2005) The captured launch of a ballistospore. Mycologia 97:866–871

Pringle A, Vellinga E, Peay K (2015) The shape of fungal ecology: does spore morphology give clues to a species´ niche? Fungal Ecol 17:213–216

Pudil F, Uvira R, Janda V (2014) Volatile compounds in stinkhorn (*Phallus impudicus* L. ex Pers.) at different stages of growth. Eur Sci J 10:163–171

Puschner B, Wegenast C (2012) Mushroom poisoning cases in dogs and cats: diagnosis and treatment of hepatotoxic, neurotoxic, gasteroenterotoxic, nephrotoxic, and muscarinic mushrooms. Vet Clin North Am Small Anim Pract 42:375–387

Pyare S, Longland WS (2001) Mechanisms of truffle detection by northern flying squirrels. Can J Zool 79:1007–1015

Pyysalo H (1976) Identification of volatile compounds in seven edible fresh mushrooms. Acta Chem Scand B 30:235–244

Quin MB, Flynn CM, Wawrzyn GT, Choudhary S, Schmidt-Dannert C (2013) Mushroom hunting by using bioinformatics: application of a predictive framework facilitates selective identification of sesquiterpene synthases in Basidiomycota. Chembiochem 14:2480–2491

Radványi D, Gere A, Jókai Z, Fodor P (2015) Rapid evaluation technique to differentiate mushroom disease-related moulds by detecting microbial volatile organic compounds using HS-SPME-GC-MS. Anal Bioanal Chem 407:537–545

Radványi D, Gere A, Sipos L, Kovács S, Jókai Z, Fodor P (2016) Discrimination of mushroom disease-related mould species based solely on unprocessed chromatograms. J Chemomet 30:197–202

Rahayu YY, Yoshizaki Y, Yamaguchi K, Okutsu K, Futagami T, Tamaki H, Sameshima Y, Takamine K (2017) Key volatile compounds in red kojishochu, a *Monascus*-fermented product, and their formation steps during fermentation. Food Chem 224:398–406

Ramirez KS, Lauber CL, Fierer N (2010) Microbial consumption and production of volatile organic

compounds at the soil-litter interface. Biogeochemistry 99:97–107
Ramsbottom J (1953) Mushrooms and toadstools. Collins, London
Rapior S, Breheret S, Talou T, Bessière JM (1997) Volatile flavor constituents of fresh *Marasmius alliaceus* (garlic *Marasmius*). J Agric Food Chem 45:820–825
Rapior S, Breheret S, Talou T, Pélissier Y, Milhau M, Bessière JM (1998) Volatile compounds of fresh *Agrocybe aegerita* and *Tricholoma sulfureum*. Cryptogam Mycol 19:15–23
Rapior S, Fons F, Bessière JM (2000) The fenugreek odor of *Lactarius helvus*. Mycologia 92:305–308
Rapior S, Breheret S, Talou T, Pélssier Y, Bessière JM (2002) The anise-like odor of *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essettei*. Mycologia 94:373–376
Rapior S, Fons F, Bessière JM (2003) Volatile flavor constituents of *Lepista nebularis* (clouded *Clitocybe*). Cryptogam Mycol 24:159–166
Rasmann S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434:732–737
Raudaskoski M, Kothe E (2015) Novel findings on the role of signal exchange in arbuscular and ectomycorrhizal symbioses. Mycorrhiza 25:243–252
Richardson MJ (2001) Diversity and occurrence of coprophilous fungi. Mycol Res 105:387–402
Richardson MJ (2002) The coprophilous succession. Fungal Divers 10:101–111
Robertson JA, McHugh JV, Whiting MF (2004) A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. Syst Entomol 29:173–187
Roháček J, Ševčík J (2013) Diptera associated with sporocarps of *Meripilus giganteus* in an urban habitat. Cent Eur J Biol 8:143–167
Rokni D, Hemmelder V, Kapoor V, Murthy VN (2014) An olfactory cocktail party: figure-ground segregation of odorants in rodents. Nat Neurosci 17:1225–1232
Rotheray TD, Boddy L, Jones TH (2009) Collembola foraging responses to interacting fungi. Ecol Entomol 34:125–132
Rotheray TD, Chanchellor M, Jones TH, Boddy L (2011) Grazing by collembolan affects the outcome of interspecific mycelial interactions of cord-forming basidiomycetes. Fungal Ecol 4:1–14
Rouquette J, Davis AJ (2003) *Drosophila* species (Diptera: Drosophilidae) oviposition patterns of fungi: the effect of allospecifics, substrate toughness, ovipositor structure and degree of specialization. Eur J Entomol 100:351–355
Ruan-Soto F, Caballero J, Martorelli C, Cifuentes J, González-Esquinca AR, Garibay-Orijel R (2013) Evaluation of the degree of mycophiliamycophobia among highland and lowland inhabitants from Chiapas, Mexico. J Ethnobiol Ethnomed 9:36
Rühl M, Kües U (2007) Mushroom production. In: Kües U (ed) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, Goettingen, pp 555–586
Ruther J, Meiners T, Steidle JML (2002) Rich in phenomena-lacking terms. A classification of kairomones. Chemoecology 12:161–167
Salvador AC, Baptista I, Barros AS, Gomes NCM, Cunha A, Almeida A, Rocha SM (2013) Can volatile organic metabolites be used to simultaneously assess microbial and mite contamination level in cereal grains and coffee beans? PLoS One 8:e59338
Sánchez Reyes E, Rodríguez de la Cruz D, Sánchez Sánchez J (2016) First fungal spore calendar of the middle-west of the Iberian Peninsula. Aerobiologia 32:529–538
Sawada A (2014) Mycophagy among Primates – what has been done and what can be done. Primate Res 30:5–21
Sawada A, Sato H, Inoue E, Otani Y, Hanya G (2014) Mycophagy among Japanese macaques in Yakushima: fungal species diversity and behavioral patterns. Primates 55:249–257
Sawahata T (2006) Hymenial area of agaric fruit bodies consumed by Collembola. Mycoscience 47:91–93
Sawahata T, Shimano S, Suzuki H (2008) *Tricholoma matsutake* 1-Ocen-3-ol and methyl cinnamate repel mycophagous *Proisotoma minuta* (Collembola: Insecta). Mycorrhiza 18(2):111–114
Sawoszczuk T, Sygula-Cholewińska J, del-Hoyo-Meléndez JM (2015) Optimization of headspace solid phase microextraction for the analysis of microbial volatile organic compounds emitted by fungi: application to historical objects. J Chromatogr A 1409:30–45
Sawoszczuk T, Sygula-Cholewińska J, del-Hoyo-Meléndez JM (2017a) Application of HD-SPME-GC-MS method for the detection of active moulds on historical parchment. Anal Bioanal Chem 409:2297–2307
Sawoszczuk T, Sygula-Cholewińska J, del-Hoyo-Meléndez JM (2017b) Application of solid-phase microextraction with gas chromatography and mass spectrometry for the early detection of active moulds on historical woolen objects. J Sep Sci 40:858–868
Sbarbati A, Osculati F (2006) Allelochemical communication in vertebrates: Kairomones, allomones and synomones. Cells Tissues Organs 183:206–219
Schalchli H, Hormazabal E, Becerra J, Birkett M, Alvear M, Vidal J, Quiroz A (2011) Antifungal activity of volatile metabolites emitted by mycelial cultures of saprophytic fungi. Chem Ecol 27:503–513
Schalchli H, Tortella GR, Rubilar O, Parra L, Hormazabal E, Quiroz A (2014) Fungal volatiles: an environmentally friendly tool to control pathogenic microorganisms in plants. Crit Rev Biotechnol 36:144–152

Schalchli H, Hormazábal E, Becerra J, Briceño G, Hernández V, Rubilar O, Diez MC (2015) Volatiles from white-rot fungi for controlling plant pathogenic fungi. Chem Ecol 31:754–763

Scheepmaker JWA, Geels FP, van Griensven LJLD, Smits PH (1996) Substrate dependent larval development and emergence of the mushroom pests *Lycoriella auripila* and *Megaselia halterata*. Entomol Exp Appl 79:329–334

Schickmann S, Urban A, Kräutler K, Nopp-Mayr U, Hackländer K (2012) The interrelationship of mycophagous small animals and ectomycorrhizal fungi in primeval, disturbed and managed Central European mountainous forests. Oecologia 170:395–409

Schigel DS (2012) Fungivory and host associations of Coleoptera: a bibliography and review of research approaches. Mycology 3:258–272

Schmidt R, Etalo DW, de Jager V, Gerards S, Zweers H, de Boer W, Garbeva P (2016) Microbial small talk: volatiles in fungal-bacterial interactions. Front Microbiol 6:1495

Schremmer F (1963) Wechselbeziehungen zwischen Pilzen und Insekten. Beobachtungen an der Stinkmorchel, *Phallus impudicus* L. ex. Pers. Österr Bot Z 110:380–400

Schroeder LM (1992) Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*. J Chem Ecol 18:1583–1593

Schuchardt S, Kruse H (2009) Quantitative volatile metabolite profiling of common indoor fungi: relevancy for indoor air analysis. J Basic Microbiol 49:350–362

Scott-Phillips TC (2008) Defining biological communication. J Evol Biol 21:387–395

Seibold S, Bässler C, Baldrian P, Thorn S, Müller J, Gossner MM (2014) Wood resource and not fungi attract early-successional saproxylic species of *Heteroptera* – an experimental approach. Insect Conserv Div 7:533–542

Seibold S, Bässler C, Baldrian P, Reinhard L, Thorn S, Ulyshen MD, Weiß I, Müller J (2016) Dead-wood addition promotes non-saproxylic epigeal arthropods but effects are mediated by canopy openness. Biol Conserv 204:181–188

Seljetun KO, von Krogh A (2017) Acute *Inocybe* mushroom toxicosis in dogs: 5 cases (2010–2014). J Vet Emerg Crit Care 27:212–217

Setsuda K (1995) Ecological study of beetles inhabiting *Cryptoporus volvatus* (PECK) SHEAR (II): relationship between development of the basidiocarps and life cycles of the five major species of beetle inhabiting the fungus, with discussion of the spore dispersal. Jpn J Entomol 63:609–620

Shaw DE, Roberts P (2002) Bees and phalloid exudate. Mycologist 16:109

Sherratt TN, Wilkinson DM, Bain RS (2005) Explaining Dioscorides' "double difference": why are some mushrooms poisonous, and do they signal unprofitability? Am Nat 166:767–775

Shorrocks B, Bingley M (1994) Priority effects and species coexistence: experiments with fungal-breeding *Drosophila*. J Anim Ecol 63:799–806

Shorrocks B, Charlesworth P (1982) A field study of the association between the stinkhorn *Phallus impudicus* Pers. and the British fungal-breeding-*Drosophila*. Biol J Linn Soc 17:307–318

Simpson RF (1976) Bioassay of pine oil components as attractants for *Sirex noctilio* (Hymenoptera: Siricidae) using electroantennogram techniques. Entomol Exp Appl 19:11–18

Simpson JA (2000) More on mycophagous birds. Australasian Mycol 19:49–51

Simpson RF, McQuilkin RM (1976) Identification of volatiles from felled *Pinus radiata* and electroantennograms they elicit from *Sirex noctilio*. Entomol Exp Appl 19:205–213

Singhadaung W, Navarro-Gonzaléz M, Plašil P, Schütz S, Kües U (2016) Mushroom mites in cultures of *Coprinopsis cinerea*. Mushroom Sci 19:126–129

Sivinski J (1981) Arthropods attracted to luminous fungi. Psyche 88:383–390

Sleeman DP, Jones P, Cronin JN (1997) Investigations of an association between the stinkhorn fungus and badger setts. J Nat Hist 31:983–992

Slippers B, Coutinho TA, Wingfield BD, Wingfield MJ (2003) A review of the genus *Amylostereum* and its association with woodwasps. S Afr J Sci 99:70–74

Slippers B, Hurley BP, Wingfield MJ (2015) Sirex woodwasps: a model for evolving management paradigms of invasive forest pests. Annu Rev Entomol 60:601–619

Smith KGV (1956) On the Diptera associated with the stinkhorn (*Phallus impudicus* Pers.) with notes on other insects and invertebrates found on this fungus. Proc R Ent Soc 31:49–55

Sodeikat G, Pohlmeyer K (2002) Temporary home range modifications of wild boar family groups (*Sus scrofa* L.) caused by drive hunts in Lower Saxony (Germany). Eur J Wildl Res 48:161–166

Soler-Rivas C, Jolivet S, Arpin N, Olivier JM, Wichers HJ (1999) Biochemical and physiological aspects of brown blotch disease of *Agaricus bisporus*. FEMS Microbiol Rev 23:591–614

Solis C, Becerra J, Flores C, Robledo J, Silva M (2004) Antibacterial and antifungal terpenes from *Pilgerodendron uviferum* (F. Don) Florin. J Chil Chem Soc 49:157–161

Simmons DR, You L, Bateman CC, Hulcr J (2016) *Flavodon ambrosius* sp. nov., a basidiomycetous mycosymbiont of *Ambrosiodmus* ambrosia beetles. Mycotaxon 131:277–285

Soteras F, Ibarra C, Geml J, Barrios-García MN, Domínguez S, Nouhra ER (2017) Mycophagy by invasive wild boar (*Sus scrofa*) facilitates dispersal of native and introduced mycorrhizal fungi in Patagonia, Argentina. Fungal Ecol 26:51–58

South A (1992) Terrestrial slugs. Biology, ecology and control. Chapman & Hall, London

Spakowicz DJ, Strobel SA (2015) Biosynthesis of hydrocarbons and volatile organic compounds by fungi:

bioengineering potential. Appl Microbiol Biotechnol 99:4943–4951
Spencer JFT, Spencer DM (1997) Ecology: where yeasts live. In: Spencer JFT, Spencer DM (eds) Yeast in natural and artificial habitats. Springer, Berlin, pp 33–58
Spiteller P (2008) Chemical defense strategies of higher fungi. Chemistry 14:9109–9110
Spiteller P (2015) Chemical ecology of fungi. Nat Prod Rep 32:971–993
Splivallo R, Culleré L (2016) The smell of truffles: from aroma biosynthesis to product quality. Soil Biol 47:393–407
Splivallo R, Ebeler SE (2015) Sulfur volatiles of microbial origin are key contributors to human-sense truffle aroma. Appl Microbiol Biotechnol 99:2583–2592
Splivallo R, Fischer U, Göbel C, Feussner I, Karlovsky P (2009) Truffles regulate plant root morphogenesis via the production of auxin and ethylene. Plant Physiol 150:2018–2029
Splivallo R, Novero M, Bertea CM, Bossi S, Bonfante P (2007a) Truffle volatiles inhibit growth and induce an oxidative burst in *Arabidopsis thaliana*. New Phytol 175:417–424
Splivallo R, Bossi S, Maffei M, Bonfante P (2007b) Discrimination by truffle fruiting body versus mycelial aromas by stir bar sorptive extraction. Phytochemistry 68:2584–2598
Splivallo R, Ottonello S, Mello A, Karlovsky P (2011) Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189:688–699
Splivallo R, Deveau A, Valdez N, Kirchhoff N, Frey-Klett P, Karlovsky P (2015) Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environ Microbiol 17:2647–2660
Spoerke DG (1994) Mushroom odors. In: Spoerke DG, Rumack BH (eds) Handbook of mushroom poisoning. Diagnosis and treatment. CRC Press, Boca Raton, FL, pp 399–418
Spooner B, Roberts R (2005) Fungi. Collins, London
Spradbery JP (1974) The responses of *Ibalia* species (Hymenoptera: Ibaliidae) to the fungal symbionts of siricid woodwasp hosts. J Entomol 48:217–222
Spradbery JP (1977) The oviposition biology of siricid woodwasps in Europe. Ecol Entomol 2:225–230
Spraker JE, Jewell K, Roze LV, Scherf J, Ndagano D, Beaudry R, Linz JE, Allen C, Keller NP (2014) A volatile relationship: profiling an interkingdom dialogue between two plant pathogens, *Ralstonia solanacearum* and *Aspergillus flavus*. J Chem Evol 40:502–513
Stavert JR, Drayton BA, Beggs JR, Gaskett AC (2014) The volatile organic compounds of introduced and native dung and carrion and their role in dung beetle foraging behaviour. Ecol Entomol 39:556–565
Sterner O, Bergman R, Kihlberg J, Wickberg B (1985) The sesquiterpenes of *Lactarius vellereus* and their role in a proposed chemical defense system. J Nat Prod 48:279–288
Stijve T (1998) Odours and pigments in stinkhorns. Aust Mycol Newsl 17:18–22
Stoffolano JG Jr, Yin C-M, Zou B-X (1989) Reproductive consequences for female black blowfly (Diptera: Calliphoridae) fed on the stinkhorn fungus, *Mutinus caninus*. Ann Entomol Soc Am 82:192–185
Stoffolano JG Jr, Zou B-X, Yin C-M (1990) The stinkhorn fungus, *Mutinus caninus*, as a potential food for egg development in the blowfly, *Phormia regina*. Entomol Exp Appl 55:267–273
Straatsma G, Sonnenberg ASM, van Griensven LJLD (2013) Development and growth of fruit bodies and crops of the button mushroom, *Agaricus bisporus*. Fungal Genet Biol 117:697–707
Streiblová E, Gryndlerová H, Gryndler M (2012) Truffle brûlé: an efficient fungal life style. FEMS Microbiol Ecol 80:1–8
Strid Y, Schroeder M, Lindahl B, Ihrmark K, Stenlid J (2014) Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections. Fungal Ecol 7:47–58
Strobel G (2006) Harnessing endophytes for industrial microbiology. Curr Opin Microbiol 9:240–244
Strobel G (2011) *Muscodor* species – endophytes with biological promise. Phytochem Rev 10:165–172
Strobel G (2014) The story of mycodiesel. Curr Opin Microbiol 19:52–58
Sulkowska J, Kaminski E (1974) Effects of different drying methods on quality and content of aromatic volatiles on dried mushrooms. Acta Aliment Pol 3:409–425
Susi P, Aktuganov G, Himanen J, Korpela T (2011) Biological control of wood decay against fungal infection. J Environ Manag 92:1681–1689
Suwannarach N, Kumla J, Bussaban B, Nuangmek W, Matsui K, Lumyong S (2013) Biofumigation with the endophytic fungus *Nodulisporium* spp. CMU-UPE34 to control postharvest decay of citrus fruit. Crop Prot 45:63–70
Suwannarach N, Kaewyana C, Yodmeeklin A, Kumla J, Matsui K, Lumyong S (2017) Evaluation of *Muscodor cinnamomi* as an egg biofumigant for the reduction of microorganisms on eggshell surfaces and its effects on egg quality. Int J Food Microbiol 244:52–61
Swarts HJ, Teunissen PJM, Verhagen FJM, Field JA, Wijnberg JBPA (1997) Chlorinated anisyl metabolites produced by basidiomycetes. Mycol Res 101:372–374
Szumny A, Adamski M, Winska K, Maczka W (2010) Identification of steroid compounds and essential oils from *Lycoperdon perlatum*. Przemysł Chemiczny 89:550–553
Talou T, Delmas M, Gaset A (1987) Principal constituents of black truffle (*Tuber melanosporum*) aroma. J Agric Food Chem 35:774–777
Talou T, Delmas M, Gaset A (1989a) Analysis of headspace volatiles from entire black truffle (*Tuber melanosporum*). J Sci Food Agric 48:57–62
Talou T, Delmas M, Gaset A (1989b) Direct capture of volatiles emitted from entire black Perigord truffle. J Essent Oil Res 1:281–286

Talou T, Gaset A, Delmas A, Kulifaj M, Montant C (1990) Dimethyl sulfide – the secret for black truffle hunting as animals. Mycol Res 94:277–278

Tarkka MT, Piechulla B (2007) Aromatic weapons: truffles attack plants by the production of volatiles. New Phytol 175:381–383

Taşkin H, Kadkas E, Büyükalaca S (2013) Comparison of various conditions in *Agaricus bisporus* by gas chromatography mass spectrometry (HS-GC/MS) technique. J Food Agric Environ 11:97–99

Teichert H, Dötterl S, Frame D, Kirejtshuk A, Gottsberger G (2012) A novel pollination mode, saprocantharophily, in *Duguetia cadaverica* (Annonaceae): a stinkhorn (Phallales) flower mimic. Flora 207:522–529

Thakeow P (2008) Development of a basic biosensor system for wood degradation using volatile organic compounds. PhD thesis, University of Goettingen, Goettingen, Germany

Thakeow P, Holighaus G, Schütz S (2007) Volatile organic compounds for wood assessment. In: Kües U (ed) Wood production, wood technology, and biotechnological impacts. Universitätsverlag Göttingen, Goettingen, pp 197–228

Thakeow P, Angeli S, Weißbecker B, Schütz S (2008) Antennal and behavioral responses of *Cis boleti* to fungal odor of *Trametes gibbosa*. Chem Senses 33:379–387

Thompsen BM, Bodart J, McEwen C, Gruner DS (2014) Adaptations for symbiont-mediated external digestion in *Sirex noctilio* (Hymenoptera: Siricidae). Ann Entomol Soc Am 107:453–460

Thorn S, Müller J, Bässler C, Gminder A, Brandl R, Heibl C (2015) Host abundance, durability, basidiome form and phylogenetic isolation determine fungivore species richness. Biol J Linn Soc 114:699–798

Thunes KH, Midtgaard F, Gjerde I (2000) Diversity of coleopteran of the bracket fungus *Fomitopsis pinicola* in a Norwegian spruce forest. Biodivers Conserv 9:833–852

Thunes KH, Willassen E (1997) Species composition of beetles (Coleoptera) in the bracket fungi *Piptoporus betulinus* and *Fomes fomentarius* (Aphyllophorales: Polyporaceae): an explorative approach with canonical correspondence analysis. J Nat Hist 31:471–486

Tibbles LL, Chandler D, Mead A, Jervis M, Boddy L (2005) Evaluation of the behavioural response of the flies *Megaselia halterata* and *Lycoriella castanescens* to different mushroom cultivation materials. Entomol Exp Appl 116:73–81

Toffano L, Fialho MB, Pascholati SF (2017) Potential of fumigation of orange fruits with volatile organic compounds produced by *Saccharomyces cerevisiae* to control citrus black spot disease at postharvest. Biol Control 108:77–82

Tomberlin JK, Crippen TL, Wu G, Griffin AS, Wood TK, Kilner RM (2017) Indole: an evolutionary conserved influencer of behavior across kingdoms. Bioessays 39:1600203

Tory MK, May TW, Keane PJ, Bennett AF (1997) Mycophagy in small mammals: A comparison of the occurrence and diversity of hypogeal fungi in the diet of the long-nosed potoroo *Potorous tridactylus* and the bush rat *Rattus fuscipes* from southwestern Victoria, Australia. Aust J Ecol 22:460–470

Tranvan H, Habricot Y, Jeannette E, Gay G, Sotta B (2000) Dynamics of symbiotic establishment between an IAA-overproducing mutant of the ectomycorrhizal fungus *Hebeloma cylindrosporum* and *Pinus pinaster*. Tree Physiol 20:123–129

Trappe JM, Claridge AW (2010) The hidden life of truffles. Sci Am 302:78–84

Tressl R, Bahri F, Engel KH (1982) Formation of 8-carbon and 10-carbon components in mushrooms (*Agaricus campestris*). J Agric Food Chem 30:89–93

Trierveiler-Pereira L, da Silveira RM, Hosaka K (2014) Multigene phylogeny of the Phallales (Phallomycetidae, Agaricomycetes) focusing on some previously unpresented genera. Mycologia 106:904–911

Trierveiler-Pereira L, Silva HCS, Funez LA, Baltazar JM (2016) Mycophagy by small mammals: new and interesting observations from Brazil. Mycosphere 7:297–304

Tsitsigiannis DI, Keller NP (2006) Oxylipins act as determinants of natural product biosynthesis and seed colonization in *Aspergillus nidulans*. Mol Microbiol 59:882–892

Tuno N (1998) Spore dispersal of *Dictyophora* fungi (Phallaceae) by flies. Ecol Res 13:7–15

Tuno N (1999) Insect feeding on spores of a bracket fungus, *Elfvingia applanata* (Pers.) Karst. (Ganodermataceae, Aphyllophorales). Ecol Res 14:97–103

Tuno N, Osawa N, Tanaka C (2009) Fungal toxins relevant to animals – The case of *Amanita*. In: Zhang W, Liu H (eds) Behavioral and chemical ecology. Nova Science, Hauppauge, NY, pp 235–250

Ugalde U, Rodriguez-Urra AB (2014) The Mycelium Blueprint: insights into the cues that shape the filamentous fungal colony. Appl Microbiol Biotechnol 98:8809–8819

Ugalde U, Rodriguez-Urra AB (2016) Autoregulatory signals in mycelial fungi. In: Wendland J (ed) Growth, differentiation and sexuality. The mycota, vol I, 3rd edn. Springer, Cham, pp 185–202

Ulyshen MD (2016) Wood decomposition as influenced by invertebrates. Biol Rev 91:70–85

Umar MH, Van Griensven LJLD (1997) Morphogenetic cell death in developing primordia of *Agaricus bisporus*. Mycologia 89:274–277

Urban A (2016) Truffles and small mammals. Soil Biol 47:353–373

Usami A, Nakaya S, Nakahashi H, Miyazawa M (2014) Chemical composition and aroma evaluation of volatile oils from edible mushrooms (*Pleurotus salmoneostraminus* and *Pleurotus sajor-caju*). J Oleo Sci 63:1323–1332

Usami A, Motooka R, Nakahashi H, Marumoto S, Miyazawa M (2015) Chemical composition and character impact odorants in volatile oils from edible mushrooms. Chem Biodivers 12:1734–1745

Vadatzadeh M, Deveau A, Splivallo R (2015) The role of the microbiome of truffle aroma formation: a meta-analysis approach. Appl Environ Microbiol 81:6946–6952

van Dam NM, Weinhold A, Garbeva O (2016) Calling in the dark: the role of volatiles for communication in the rhizosphere. Signal Commun Plants 26:175–210

van't Padje A, Whiteside MD, Kiers ET (2016) Signals and cues in the evolution of plant-microbe communication. Curr Opin Plant Biol 32:47–52

Vander Wall SB (2000) The influence of environmental conditions on cache recovery and cache pilferage by yellow chipmunks (*Tamias amoenus*) and deer mice (*Peromyscus maniculatus*). Behav Ecol 11:544–549

Vanhealen M, Vanhaelen-Fastré R, Geeraerts J (1980) Occurrence in mushrooms (Homobasidiomycetes) of cis- and trans-octa-1,5-dien-3-ol, attractants to the cheese mite *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae). Experientia 36:406–407

Varela C (2016) The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. Appl Microbiol Biotechnol 100:9861–9874

Vayssières A, Pěnčik A, Felten J, Kohler A, Ljung K, Martin F, Legué V (2015) Development of the poplar-*Laccaria bicolor* ectomycorrhiza modifies root auxin metabolism, signaling and response. Plant Physiol 169:890–902

Vélez-Pereira AM, De Linares C, Delgado R, Belmonte J (2016) Temporal trends of the airborne fungal spores in Catalonia (NE Spain), 1995–2013. Aerobiologia 32:23–37

Venkateshwarlu G, Chandravadana M, Tewari R (1999) Volatile flavour components of some edible mushrooms (Basidiomycetes). Flavour Fragr J 14:191–194

Verginer M, Leitner E, Berg G (2010) Production of volatile metabolites by grape-associated microorganisms. J Agric Food Chem 58:8344–8350

Verheggen F, Perrault KA, Caparros Medigo R, Dubois LM, Francis F, Haubruge E, Forbes SL, Focant J-F, Stefanuto P-H (2017) The odor of death: an overview of current knowledge on characterization and applications. Bioscience 67:600–613

Vernes K, Cooper T, Green S (2015) Seasonal fungal diets of small mammals in an Australian temperate forest ecosystem. Fungal Ecol 18:107–114

Vespermann KAC, Paulino BN, Barcelos MC, Pessôa MG, Pastore GM, Molina G (2017) Biotransformation of α- and β -pinene into flavor compounds. Appl Microbiol Biotechnol 101:1805–1817

Vet LEM (1982) Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym: Eucoilidae), a parasitoid of fungivorous *Drosophila*: the influence of conditioning. Neth J Zool 33:225–248

Vet LEM, de Jong AG, Franchi E, Papaj DR (1998) The effect of complete versus incomplete information on odour discrimination in a parasitic wasp. Anim Behav 55:1271–1279

Vita F, Taiti C, Pompeiano A, Bazihizina N, Lucarotti V, Mancuso S, Alpi A (2015) Volatile organic compounds in truffle (*Tuber magnatum* Pico): comparison of samples from different regions of Italy and from different seasons. Sci Rep 5:12629

Wallis IR, Claridge AW, Trappe JM (2012) Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy. Fungal Biol 116:590–602

Wang Y, Li Y, Yang J, Ruan J, Sun C (2016) Microbial volatile organic compounds and their application in microorganisms identification in foodstuff. TRAC 78:1–16

Warren JT, Mysterud I (1991) Fungi in the diet of sheep. Rangelands Arch 13:168–171

Wąsowicz E (1974) Identification of the volatile flavor compounds in mushroom *Agaricus bisporus*. Bull Acad Pol Sci 22:143–151

Wasser SP, Weis AL (1999) Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. Crit Rev Immunol 19:65–96

Watanabe H, Tokuda G (2010) Cellulolytic systems in insects. Annu Rev Entomol 55:609–632

Wawrzyn GT, Bloch SE, Schmidt-Dannert C (2012) Discovery and characterization of terpenoid biosynthetic pathways of fungi. Methods Enzymol 515:83–105

Wee SL, Oh HW, Park KC (2016) Antennal sensillum morphology and electrophysiological responses of olfactory receptor neurons in trichoid sensilla of the diamondback moth (Lepidoptera: Plutellidae). Fla Entomol 99:146–158

Weiss I, Rössler T, Hofferberth J, Brummer M, Ruther J, Stökl J (2013) A nonspecific defensive compound evolves into a competition avoidance cue and a female sex pheromone. Nat Commun 4:2767

Weissbecker B, Holighaus G, Schütz S (2004) Gas chromatography with mass spectrometric and electroantennographic detection: analysis of wood odorants by direct coupling of insect olfaction and mass spectrometry. J Chromatogr A 1056:209–216

Weisskopf L, Ryu C-M, Raaijmakers JM, Gerbeva P (2016) Editorial: smelly fumes: volatile-mediated communication between bacteria and other organisms. Front Microbiol 7:2031

Weldon PJ (2016) Receiver-error in deception, including mimicry: making the leap from intra- to interspecific domains. Biol J Linn Soc 120:717–728

Wende B, Gossner MM, Grass I, Arnstadt T, Hofrichter M, Floren A, Linsenmair KE, Weisser WW, Steffan-Dewenter I (2017) Trophic level, successional age and trait matching determine specialization of deadwood-based interaction networks of saproxylic beetles. Proc R Soc Biol Sci 284:1854

Wenke K, Kai M, Piechulla B (2010) Belowground volatiles facilitate interactions between plant roots and soil organisms. Planta 231:499–506

Werner S, Polle A, Brinkmann N (2016) Belowground communication: impacts of volatile organic compounds (VOCs) from soil fungi of other soil-inhabiting organisms. Appl Microbiol Biotechnol 100:8651–8665

Wertheim B, van Alphen JJM (2001) Species diversity in a mycophagous insect community: the case of spatial aggregation vs. resource partitioning. J Anim Ecol 69:335–351

Wertheim B, Vet LEM, Dicke M (2003) Increased risk of parasitism as ecological costs of using aggregation pheromones: laboratory and field study of *Drosophila-Leptopilina* interaction. Oikos 100:269–282

Wertheim B, Allemand R, Vet LEM, Dicke M (2006) Effects of aggregation pheromone on individual behavior and food web interactions: a field study on *Drosophila*. Ecol Entomol 31:216–226

Weslien J, Djupström LB, Schroeder M, Widenfalk O (2011) Long-term priority effects among insects and fungi colonizing decaying wood. J Anim Ecol 86:1155–1162

Wheatley RE (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek 81:357–364

Wheatley M (2007) Fungi in summer diets of northern flying squirrels (*Glaucomys sabrinus*) within managed forests of western Alberta, Canada. Northwest Sci 81:265–273

Wihlborg R, Pippitt D, Marsili R (2008) Headspace sorptive extraction and GC-TOFMS for the identification of volatile fungal metabolites. J Microbiol Methods 75:244–250

Wiskerke JSC, Dicke M, Vet LEM (1993) *Drosophila* parasitoid solves foraging problem through infochemical detour: the role of adult fly pheromone. Proc Exp Appl Entomol 4:79–84

Wongsuk T, Pumeesat P, Luplertlop N (2016) Fungal quorum sensing molecules. Role in fungal morphogenesis and pathogenicity. J Basic Microbiol 56:440–447

Wood WF, Largent DL (1999) Benzaldehyde and benzyl alcohol, the odour compounds from *Agaricus smithii*. Biochem Syst Ecol 27:521–522

Wood WF, Lefreve CK (2007) Changing volatile compounds from mycelium and sporocarps of American matsutake mushroom, *Tricholoma magnivelare*. Biochem Syst Ecol 35:634–636

Wood WF, Watson RL, Largent DL (1990) The odor of *Agaricus augustus*. Mycologia 82:276–278

Wood WF, Watson RL, Largent DL (1998) Phenol, the odour compound from *Agaricus praeclaresquamosus*. Biochem Syst Ecol 26:793–794

Wood WF, Archer CL, Largent DL (2001) 1-Octen-3-ol, a banana slug antifeedant from mushrooms. Biochem Syst Ecol 29:531–533

Wood WF, Clark TJ, Bradshaw DE, Foy BD, Largent DL, Thompsen BL (2004) Clitolactone: a banana slug antifeedant from *Clitocybe flaccida*. Mycologia 96:23–25

Wood JR, Dickie IA, Moeller HV, Peltzer DA, Bonner KI, Rattray G, Wilmshurst JM (2015) Novel interactions between non-native mammals and fungi facilitate establishment of invasive pines. J Ecol 103:121–129

Wöstemeyer J, Schimek C, Wetzel J, Burmester A, Voigt J, Schulz E, Ellenberger S, Siegmund L (2016) Pheromone action in the fungal groups Chytridiomycetes and Zygomycetes and in the Oophytes. In: Wendland J (ed) Growth, differentiation and sexuality. The mycota, vol I, 3rd edn. Springer, Cham, pp 203–234

Wu S, Zorn H, Krings U, Berger RG (2005a) Characteristic volatiles from young and aged fruiting bodies of wild *Polyporus sulfureus* (Bull.:Fr.) Fr. J Agric Food Chem 53:4525–4528

Wu S, Krings U, Zorn H, Berger RG (2005b) Volatile compounds from the fruiting body of beefsteak fungus *Fistulina hepatica* (Schaeffer: Fr.) Fr. Food Chem 92:221–226

Wurzenberger M, Grosch W (1982) The enzymatic breakdown of linoleic-acid in mushrooms (*Psalliota bispora*). Z Lebensm Untersuch Forsch 175:186–190

Wurzenberger M, Grosch W (1984a) Origin of the oxygen in the products of the enzymatic cleavage reaction of linoleic acid to 1-octen-3-ol and 10-oxo-trans-8-decenoic acid in mushrooms (*Psalliota bispora*). Biochim Biophys Acta 794:18–24

Wurzenberger M, Grosch W (1984b) The formation of 1-octen-3-ol from the 10-hydroperoxide dimer of linoleic acid by a hydroperoxide lyase in mushrooms (*Psalliota bispora*). Biochem Biophys Acta 794:25–30

Wurzenberger M, Grosch W (1984c) Stereochemistry of the cleavage of the 10-hydroperoxide isomer of linoleic acid to 1-octen-3-ol by a hydroperoxide lyase from mushrooms (*Psalliota bispora*). Biochim Biophys Acta 795:163–164

Wurzenberger M, Grosch W (1986) Enzymatic oxidation of linolenic acid to 1,Z-5-octadien-3-ol, Z-2,Z-5-octadien-1-ol and 10-oxo-E8-decenoic acid by a protein fraction from mushrooms (*Psalliota bispora*). Lipids 21:261–266

Yamashita S, Hijii N (2007a) Resource use pattern of a mycophagous beetle, *Neopallodes inermis* (Coleoptera, Nitidulidae) of soft fungi, *Collybia* spp. (Agaricales). Ann Entomol Soc Am 100:222–227

Yamashita S, Hijii N (2007b) The role of fungal taxa and developmental stages of mushrooms in determining the compostion of the mycophagous insect community in a Japanese forest. Eur J Entomol 104:225–233

Yamashita S, Ando K, Hoshina H, Ito N, Katayama Y, Kawanabe M, Maruyama M, Itioka T (2015) Food web structure of the fungivorous insect commu-

nity on bracket fungi in a Bornean tropical rain forest. Ecol Entomol 40:390–400
Yarden O (2014) Fungal association with sessile marine invertebrates. Front Microbiol 5:228
You L, Simmons DR, Bateman CC, Short DPG, Kasson MT, Rabaglia RJ, Hulcr J (2016) New fungus-insect symbioses: culturing, molecular, and histological methods determine saprotrophic Polyporales mutualist of *Ambrosiodmus* ambrosia beetles. PLoS One 11:e0147305
Zambonelli A, Ori F, Hall I (2017) Mycophagy and spore dispersal by vertebrates. In: Dighton J, White JF (eds) The fungal community. Its organization and role in ecosystems, 4th edn. CRC Press Taylor & Francis Group, Boca Raton, FL, pp 347–358
Zawirska-Wojtasiak R (2004) Optical purity of (R)-(−)-1-octen-3-ol in the aroma of various species of edible mushrooms. Food Chem 86:113–118
Zawirska-Wojtasiak R, Siwulski M, Wąsowicz E, Sobieralski K (2007) Volatile compounds of importance in the aroma of cultivated mushrooms *Agaricus bisporus* at different conditions of cultivation. Pol J Food Nutr Sci 57:367–372
Zeilinger S, Gupta VK, Dahms TES, Silva RN, Singh HB, Upadhyay RS, Vieira Gomes E, Tsui CK-M, Nyack SC (2016a) Friends or foe? Emerging insights from fungal interactions with plants. FEMS Microbiol Rev 40:182–207
Zeilinger S, Gruber S, Bansal R, Mukherjee PK (2016b) Secondary metabolism in *Trichoderma* – chemistry meets genomics. Fungal Biol Rev 30:74–90
Zeppa S, Gioacchini AM, Guidi C, Guescini M, Pierleoni R, Zambonelli A (2004) Determination of specific volatile organic compounds synthesized during *Tuber borchii* fruit body development by solid-phase microextraction and gas chromatography mass spectrometry. Rapid Commun Mass Spectrom 18:199–205
Zhang Z, Li G (2010) A review of advances and new developments in the analysis of biological volatile organic compounds. Microchem J 95:127–139
Zhang Z, Pawliszyn J (1993) Headspace solid-phase microextraction. Anal Chem 65:1843–1852
Zhang Z, Yang MJ, Pawliszyn J (1994) Solid-phase microextraction. A solvent-free alternative for sample preparation. Anal Chem 66:A844–A853
Zhang Y, Fraatz MA, Horlamus F, Quitman H, Zorn H (2014) Identification of potent odorants in a novel nonalcoholic beverage produced by fermentation of wort with shiitake (*Lentinula edodes*). J Agric Food Chem 62:4195–4203
Zhang C, Huang T, Shen C, Wang X, Qi Y, Shen J, Song A, Qiu L, Yi Y (2016a) Downregulation of ethylene production increases mycelial growth and primordia formation in the button culinary-medicinal mushroom *Agaricus bisporus* (Agaricomycetes). Int J Med Mushrooms 18:1131–1140
Zhang N, Chen H, Sun B, Mao X, Zhang Y, Zhou Y (2016b) Comparative analysis of volatile composition in Chinese truffles via GC × GC/HRTOF/MS and electric nose. Int J Mol Sci 17:412
Zhang ZM, Wu WW, Li GK (2008) A GC-MS study of the volatile organic composition of straw and oyster mushrooms during maturity and its relation to antioxidant activity. J Chromatogr Sci 46:690–696
Zhao GZ, Yao YP, Hao GF, Fang DS, Yin VX, Cao XH, Chen W (2015) Gene regulation in *Aspergillus oryzae* promotes hyphal growth and flavor formation in soy sauce koji. RCS Adv 5:24224–24230
Zhou JJ, Feng T, Ye R (2015) Differentiation of eight commercial mushrooms by electronic nose and gas chromatography-mass spectrometry. J Sensors 2015:374013

Endophytic Fungi, Occurrence, and Metabolites

Mysore V. Tejesvi[1], Anna Maria Pirttilä[1]

CONTENTS

I. Introduction

Endophytic fungi are defined as asymptomatically living inside plant tissue (Petrini 1986; Wilson 1995; Hardoim et al. 2015). Typically, grasses harbor systemic "clavicipitaceous" endophytes, colonizing throughout the plant tissues, whereas local infections are found in the majority of plant species by numerous non-clavicipitaceous endophytes (Carroll 1988; Stone et al. 2004). Species of the *Clavicipitaceae* occupy the shoot meristems of grasses and colonize the newly forming shoots intercellularly. The systemic colonization may advance to the floral organs from where the fungi are vertically transmitted in the seeds (Saikkonen et al. 2002). A host plant is often colonized by mainly one clavicipitacean species or genotype (Wille et al. 1999). The clavicipitaceous endophytes can have various beneficial effects on the host plant, such as induced growth, drought tolerance, and protection against animal and insect herbivory by production of toxic alkaloids (Clay 1988). Alkaloids produced by the clavicipitaceous fungi are the most well-known endophytic compounds. Lolitrems, neurotoxic indole-diterpenoid alkaloids, cause intoxication in cattle that graze on the endophyte-infected grass (Fletcher and Harvey 1981; Gallagher et al. 1984). Besides mammal herbivores, some alkaloids protect the host plant from insect herbivores (Bush et al. 1982; Siegel et al. 1990). New and very exciting findings regarding the symbiosis of Convolvulaceae with clavicipitaceous fungi are the subject of chapter "The Genus Periglandula and Its Symbiotum with Morning Glory Plants (Convolvulaceae)" by E. Leistner and U. Steiner in this volume.

The diversity of non-clavicipitaceous endophytes is rich, as well as their host selection, as these endophytes have been encountered in all lineages of land plants in all terrestrial ecosystems from the tropics to the tundra in both agricultural and natural environments (Arnold and Lutzoni 2007). The non-clavicipitaceous fungi are further divided into three groups, classes 2, 3, and 4, based on their patterns of host colonization, mechanism of transmission between generations, biodiversity, and ecological function in hosts (Rodriguez et al. 2009). The class 2 endophytes are found in all plant tissues, including roots, and can be transmitted via seed coats or rhizomes. Most of them are not very abundant in the soil and have high infection frequencies, up to 100%, in stressed host plants (Rodriguez et al. 2009). The diversity of class 2 endophytes in individual host plants is quite limited, comprising *Dikarya*,

[1] Ecology & Genetics, University of Oulu, Oulu, Finland; e-mail: am.pirttila@oulu.fi

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

Ascomycota, or *Basidiomycota*, and the individual species can colonize tissues extensively, although not to the extent of clavicipitaceous endophytes (Rodriguez et al. 2008). The class 2 endophytes include nematophagous fungi such as *Arthrobotrys* spp. and can benefit the host plant by deterring nematodes (Lopez-Llorca et al. 2006). Other beneficial effects reported are induction of host growth by production of plant hormones (Tudzynski and Sharon 2002) and protection of plant host against pathogens (Danielsen and Jensen 1999; Narisawa et al. 2002; Campanile et al. 2007). For example, class 2 endophytes, *Fusarium oxysporum* and *Cryptosporiopsis* sp., increase plant resistance toward pathogens in barley and larch, respectively (Schulz et al. 1999).

The fungi belonging to class 3 are the classical horizontally transmitted endophytes, which form highly localized infections of the size of couple mm^2 of leaf tissue and are extremely diverse by species richness (Rodriguez et al. 2009). One leaf of a tree can harbor a set of endophytes, which are not found in another leaf (Arnold et al. 2003), and individual plants can carry hundreds of different endophyte species. The endophytes in class 3 comprise mainly *Dikaryomycota* (*Ascomycota* or *Basidiomycota*), specifically Pezizomycotina, and are detected in the aerial parts of plants. Increasing body of evidence suggests that many foliar endophytes have species specificity. For example, Leotiomycetes are common to conifers, and Sordariomycetes are more typically found in tropical plants (Rodriguez et al. 2009). Furthermore, recent data show that the communities reflect the phylogeny (Solis et al. 2016) or genotype (Bálint et al. 2013) of the hosts. Within plant individuals, structures of the communities can change according to seasons (Martins et al. 2016). Some of these endophytes can reproduce through hyphal fragments, or by producing sexual or asexual spores on host plant tissue (Herre et al. 2005), which are released and carried by wind or rain to new host plants (Rodriguez et al. 2009) or transmitted by insects (Monk and Samuels 1990). The class 3 endophytes are extensively being studied for production of bioactive compounds against bacteria, fungi, and viruses. These endophytes are known for their capacity to produce a variety of compounds that have activity toward plant pathogens and herbivores. The compounds consist alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, and chlorinated compounds (Gunatilaka 2006; Weber 2009; Higginbotham et al. 2013; Tejesvi et al. 2011, 2013).

The class 4 endophytes are the so-called dark septate endophytes (DSE), which are found in plant roots. These endophytes mainly belong in Ascomycota and form melanized structures, inter- and intracellular hyphae and microsclerotia in the root tissues. These endophytes are not host-specific and have been described from more than 600 plant species in various ecosystems but mainly in high-stress environments (Jumpponen and Trappe 1998; Jumpponen 2001).

In this chapter, we discuss mainly the foliar, horizontally transferred, class 3 endophytes and review the new knowledge gathered on their diversity in host plants by new sequencing tools, their role in the host plant, and provide insights on their future use and potential.

II. Diversity of Endophytic Fungi Analyzed with the Next-Generation Sequencing Tools

Our understanding of microbial diversity has dramatically changed over the years due to next-generation sequencing (NGS) technology, which provides a more accurate picture compared to traditional methods or clone-based sequencing technologies. For example, the magnitude of endophytic diversity had been greatly underestimated using traditional methods. However, the techniques have limitations with regard to identification of species or, more specifically, definition of operational taxonomic units (OTUs). In general, NGS is cost-effective compared to culture-based techniques. There are various platforms used for NGS relying on different chemistries, such as Ion torrent Personal Genome Machine (PGM), 454 FLX Titanium, and Illumina Miseq and Hiseq (Caporaso et al. 2012). Ion torrent is a

semiconductor-based technology involving sequence by synthesis and measuring change in pH to acquire sequence data. The 454 technology is based on pyrosequencing, which uses ATP sulfurylase and luciferase enzymes. After incorporation of each nucleotide by DNA polymerase, a pyrophosphate is released, which produces a light proportional to the number of nucleotides incorporated (Mardis 2008). Illumina is the most commonly used sequencing platform for genome sequencing. The Illumina technology is based on fragmentation of DNA into 200 base strands, to which adapters are ligated. One strand with adapters is hybridized on a flow cell, and the amplification reaction occurs on the surface of the flow cell using fluorescent nucleotides. The primer on the one end of the molecules determines the sequence through light emission of nucleotides under microscope (Shendure and Ji 2008). With the NGS technologies, there will be challenges ahead to standardize sequence parameters for optimizing quality filtering and OTU thresholds. Without such optimization, there will be an accumulation of sequences of unknown taxonomic groups in public repositories.

A wealth of microbial diversity is found in the endophytic microbiomes of plants (Porras-Alfaro and Bayman 2011; Tejesvi et al. 2013; Hardoim et al. 2015). NGS-based community analysis has revealed an enormous hidden diversity of fungal endophytes. The NGS technology is a feasible method to assess and compare studies across communities and to estimate the actual diversity of these microbes (Jumpponen and Jones 2009; Hardoim et al. 2015). The majority of these fungi are uncultivable by traditional methods and have earlier been invisible without the NGS technology to the microbiologists, or scientists, in general (Glenn 2011). Despite the rapid progress in NGS technology, a vast number of studies still use culture-based approaches, leading to organismal bias and highly unlikely discovery of novel species. Another alternative approach is the direct cloning and sequencing (Tejesvi et al. 2010), but this is time-consuming and involves a primer-dependent bias with selection of clones for sequencing and identification. However, the rapid progress in the identification of species using next-generation sequencing has led to challenges such as data processing, quality of sequences, read lengths, archiving, and interpretation of data (Wooley et al. 2010; Kunin et al. 2010; Huse et al. 2010). The most commonly used databases such as GenBank, Greengenes, or Silva carry a substantial number of wrong identifications of species or, in some cases, minimal taxonomic information (Hibbett et al. 2011; Nagy et al. 2011).

An example of thorough plant microbiome studies has been done on *Eucalyptus grandis* endophytes (Kemler et al. 2013). *Eucalyptus* trees have earlier been found to be mainly dominated by Dothideomycetes and specifically the families Botryosphaeriaceae, Pleosporaceae, and Valsaceae using culture-dependent methods (Fisher et al. 1993; Smith et al. 1996). When the community composition was studied by NGS methods, Mycosphaerellaceae and Botryosphaeriaceae dominated, and new members within the Teratosphaeriaceae were found (Kemler et al. 2013). Another recent study employing NGS identified the European ash dieback pathogen *Hymenoscyphus fraxineus* as an endophyte, along with members of *Mycosphaerella* genus, in *Fraxinus mandshurica* trees growing in Far East Russia, as opposed to European ash where both of these endophytes were absent (Cleary et al. 2016). In beech, the diversity of fungal endophytes was found to reduce at higher elevations and in artificial environments (gardens) using NGS techniques (Unterseher et al. 2016). In *Pinus monticola*, inoculation of seedlings with antagonistic endophytic fungi reduced occurrence of pathogens in the foliar microbiome, which was mainly obtained from the neighboring *P. monticola* trees (Bullington and Larkin 2015). Similar to the study by Kemler et al. (2013), the species composition was completely different by NGS methods than by traditional methods. *Rhytismataceous* fungi have dominated previous culture-based studies of white pines (Ganley et al. 2004; Larkin et al. 2012), and NGS identified Davidiellaceae, Pleosporales, Tremellales, *Sydowia polyspora*, and *Preussia intermedia* as the core set of five naturally occurring western white pine endophytes, found in $\geq$90% of seedlings sampled (Bullington and Larkin 2015).

III. The Current Understanding on the Interaction of Endophytic Fungi with the Host

Generally, endophytes are seen as having roles ranging from latent pathogen to mutualistic symbiont (Schulz et al. 1999; Schulz and Boyle 2005; Saikkonen et al. 1998). Depending on host genotype, some endophytes may become pathogenic in stressed plants (Kogel et al. 2006; Bacon et al. 2008), but they can be beneficial in other conditions. For example, the pathogen *Fusarium verticillioides* can decrease the growth of another pathogen, *Ustilago maydis*, on maize and protect the host against disease (Rodriguez Estrada et al. 2012). There are speculations that endophytic fungi have evolved from pathogens, equally well to that pathogenic fungi have specialized from endophytes (Delaye et al. 2013; Xu et al. 2014).

However, transition from pathogen to endophyte and vice versa may not need a long evolution but can occur by deletion or mutation of a single gene (Redman et al. 1999; Tanaka et al. 2006). Earlier, *Colletotrichum magna* was transformed from pathogen to harmless endophyte by mutation of a single gene (Redman et al. 1999). On the other hand, the systemic grass endophyte *Epichloë festucae* became pathogenic to the host *Lolium perenne* by disruption of the NADPH oxidase gene (Tanaka et al. 2006). Even more transiently, an endophyte can become pathogenic by simply changing the growth conditions of the host by such subtle changes as intensity of lighting (Álvarez-Loayza et al. 2011). An endophyte of tropical palm *Iriartea deltoidea* is colonized by the endophyte *Diplodia mutila* asymptomatically in mature trees, but this fungus produces sporadically disease symptoms in seedlings. The asymptomatic growth was observed to occur in seedlings growing in low-light conditions, whereas high light induced pathogenic lifestyle in the fungus. The transition from endophyte to pathogen was suggested to result from light-induced production of reactive oxygen species in the fungus and further hypersensitivity reaction in the host plant, leading to cell death (Álvarez-Loayza et al. 2011). Similar as in *Epichloë festucae*, which had NAPDH oxidase disrupted and became pathogenic (Tanaka et al. 2006), ROS balance between the host and endophyte shows to play an important role in defining the nature of the interaction.

However, a very low proportion of endophytes are shown to become pathogenic to their host plant at specific conditions, and the more typical lifestyle is harmless or undefined. The horizontally transmitted foliar endophytes are especially enigmatic, because they infect only very small areas of the leaf and accumulate extremely low biomass, resulting in leaves carrying small patches of endophyte infections (Lodge et al. 1996; Arnold et al. 2003; Clay 2004). For these endophytes it is hard to imagine a dominating role in the plant, if any, given the small impact of each infection. In general, it is not even known whether all of these endophytes are metabolically active during the intercellular infection of the few cell area of the host. The sequenced genomes of horizontally transferred endophytes suggest a noninvasive but active metabolism and reflect the ability to exploit nutrients available within the intercellular niche of the host plant (Wang et al. 2015a; Gazis et al. 2016).

Regardless, already a few decades ago, the horizontally transmitted endophytes were suggested to act as a defense system for the host plant (Carroll 1988). Similar to the plant pathogens or insect herbivores, the life cycle of endophytes, in general, is several orders of magnitude shorter than the life cycle of their plant host. Therefore they can keep up with the evolution to resist pathogen and herbivore attacks by production of bioactive compounds. This hypothesis has been supported by numerous reports describing antiviral, antibacterial, antifungal, and insecticidal compounds from the horizontally transmitted endophytic fungi (Gunatilaka 2006; Weber 2009; Higginbotham et al. 2013; Tejesvi et al. 2011, 2013). Overall, endophytes are versatile secondary metabolite producers. Recently sequenced endophyte genomes demonstrate their great capacity for secondary metabolism producing a multitude

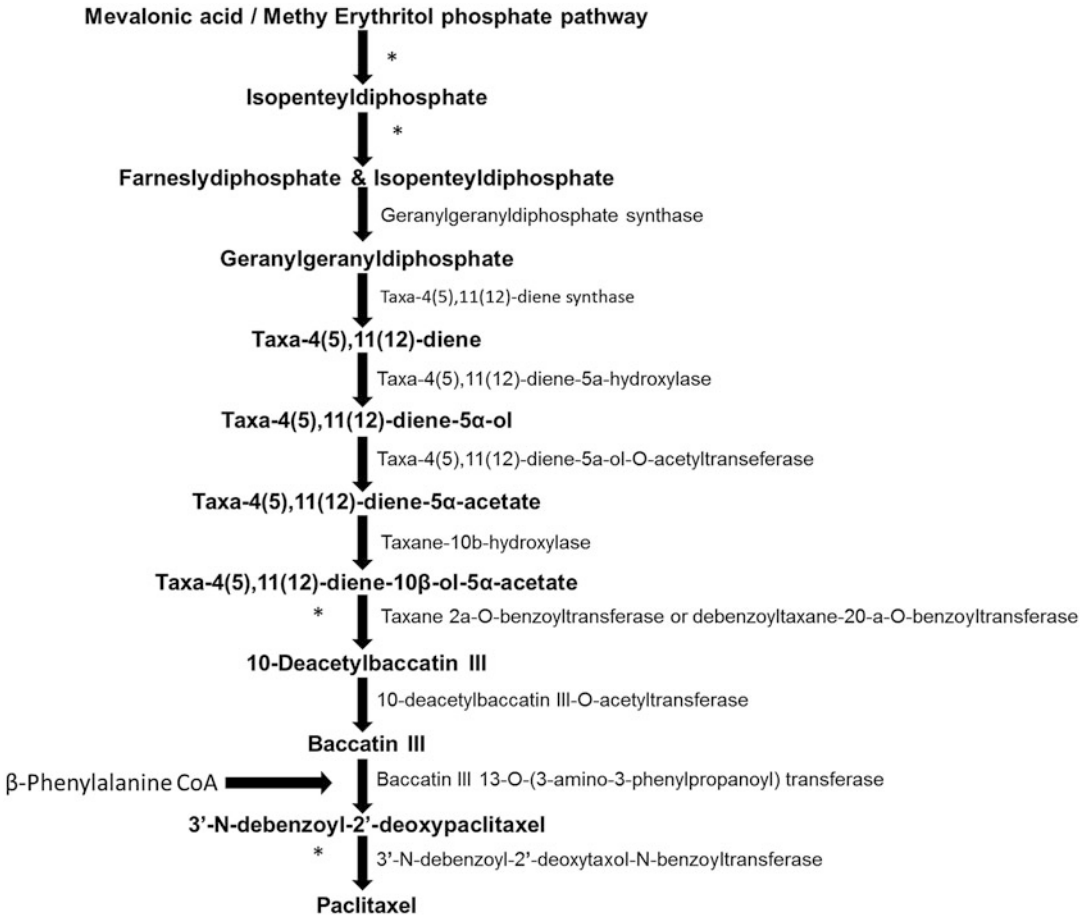


Fig. 1 Biosynthesis of paclitaxel is proposed to have 20 enzymatic steps in *Taxus* species. Multiple biosynthetic routes are indicated by asterisk sign (*)

of compounds (Wang et al. 2015a; Gazis et al. 2016). In the endophyte genomes, typically 7–29 polyketide synthase genes, 8–21 non-ribosomal peptide synthase genes, and 5–15 terpenoid synthase genes are found (Wang et al. 2015a; Gazis et al. 2016).

One of the most convincing researches showing that horizontally transmitted endophytes can protect the host plant has been done on *Phialocephala scopiformis,* which is an endophyte of white spruce (*Picea glauca*) (Miller et al. 2009). This endophyte deters and kills spruce budworm *Choristoneura fumiferana*, which is a serious pathogen on white spruce in Canada and Northern USA, by producing rugulosin (Fig. 1), an anti-insect compound (Sumarah et al. 2008). This endophyte was transmitted from host trees to white spruce seedlings at a rate as high as 40%, resulting in accumulation of the anti-budworm compound, rugulosin, in the seedlings in concentrations of 1 μg/g (Miller et al. 2009).

However, a large number of horizontally transmitted endophytes produce no bioactive compounds. Furthermore, because the endophytes infect only tiny areas of the host tissue, their role in the plant has been questioned. Considering the problem at the community level has brought some light to the dilemma. Cacao is very susceptible to diseases caused by

the pathogens belonging to *Phytophthora* spp., and a study on horizontally transmitted endophytes on cacao suggested that it is the competition for space between endophytic and pathogenic strains that might result in the benefit of the plant host and healthy growth, or disease, respectively (Arnold et al. 2003; Clay 2004; Arnold 2007). The study revealed that pathogens can colonize the tree leaf with equal probability to endophytes and that it is the balance between colonization by various species that matters. A recent report supports this, demonstrating that production of endophytic antimicrobial compounds is induced only in the presence of a pathogen (Combès et al. 2012).

Another type of plant protection suggested for endophytes is induction of resistance (Carroll 1991). It is well known that bacterial endophytes can protect the host from pathogens by activating the natural defense mechanisms, called induced systemic resistance (ISR), but endophytic fungi are rarely reported to protect their host by ISR (Vu et al. 2006; Blodgett et al. 2007; Bae et al. 2011). However, recent studies suggest that some endophytic fungi can elicit responses similar to those induced by wounding, herbivory, and pathogen invasion. Colonization of lima bean by endophytic *Fusarium* sp. induced production of jasmonic acid in the leaves, whereas levels of salicylic acid were lower after inoculation (Navarro-Meléndez and Heil 2014). When the endophyte *Chaetomium cochlioides* was inoculated into the host plant *Cirsium arvense*, changes in oxylipin profiles were detected (Hartley et al. 2015). Oxylipins generally act as hormonal signals in events such as wounding and herbivory, as well as ISR.

One potential mechanism of inducing plant resistance by fungal endophytes could be through siderophores. Siderophores are complex molecules that have a role in acquisition of iron from the soil by microbes (Powell et al. 1980; Schippers et al. 1987), act in pathogen-host interactions in animals (Bearden and Perry 1999; Schrettl et al. 2004), and are shown to elicit plant defense responses (van Loon et al. 2008). Siderophores have been isolated from fungal endophytes (Kajula et al. 2010; Johnson et al. 2013; Rosconi et al. 2013), but there are also strains, which lack siderophore biosynthesis (Kajula et al. 2010). Therefore it is hard to pinpoint an essential role for siderophores in plant-endophyte interaction. However, when extracellular siderophore biosynthesis of *Epichloë festucae* was disrupted, the symbiosis with ryegrass became antagonistic, which could be due to altered iron homeostasis in the plant-endophyte interaction (Johnson et al. 2013). Siderophores are important compounds in regulation of iron homeostasis and play a cytoprotective, antioxidant role by preventing Fenton's reaction (Hantke and Braun 2000). This indicates that siderophores may have a role in regulating the redox balance between the endophyte and the host.

The most dramatic changes in the host plant physiology by the endophyte occur via plant hormone production. Auxins and gibberellins are mainly produced by the root-associated endophytes (Khan et al. 2012). The endophyte *Colletotrichum* sp. of *Artemisia annua* (Lu et al. 2000), belonging to class 2, produces the auxin indole acetic acid (IAA). In bacteria, IAA has been shown to increase colonization efficiency (Suzuki et al. 2003) possibly through circumvention of the host defense (Navarro et al. 2006), and it may have such function in endophytic fungi as well. Cytokinin production is rare in endophytic fungi; however, it is identified in some species, such as the root-colonizing fungus *Piriformospora indica* (Vadassery et al. 2008). In *P. indica*, cytokinin plays a beneficial role for the plant, as deletion of cytokinin biosynthesis negatively affects host growth promotion by the endophyte (Vadassery et al. 2008).

On the other hand, endophyte infection may induce or alter secondary metabolism of the host plant. For example, the infection by the fungal endophyte *Paraphaeosphaeria* sp. significantly induces biosynthesis and accumulation of flavan-3-ols, phenolic acids, and oligomeric proanthocyanidins in leaves of bilberry (*Vaccinium myrtillus* L.) (Koskimäki et al. 2009).

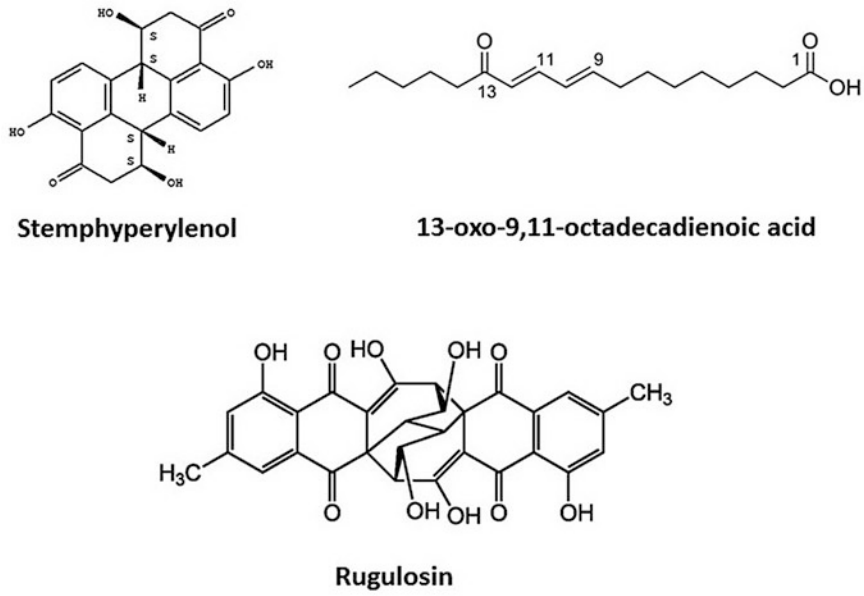


Fig. 2 Selected bioactive metabolite structures produced by endophytes

Another example of affecting the secondary metabolism of the plant host by an endophyte was made on bean (Jaber and Vidal 2009). The bean plants inoculated with the endophyte *Acremonium strictum* had significantly but transiently increased nectar production, and the number of extrafloral nectaries was higher than in uninoculated plants. The extrafloral nectaries were suggested to associate with plant defense, as endophyte inoculation reduced aphid fecundity on bean (Jaber and Vidal 2009). The same endophyte, *A. strictum*, inoculated on tomato, changed the volatile profiles of the host plants toward lower quantities of emitted terpenes and sesquiterpenes and higher quantities of trans-β-caryophyllene (Jallow et al. 2008). This was suggested to affect the host selection of the polyphagous moth *Helicoverpa armigera* for oviposition on tomato (Jallow et al. 2008). Some endophytes may even modify plant compounds further, as the horizontally transmitted endophyte *Paraconiothyrium variabile* is shown to manipulate the secondary metabolites of the host, *Cephalotaxus harringtonia* (Tian et al. 2014). The main compounds altered were glycosylated flavonoids, which were deglycosylated to the corresponding aglycone flavonoids, which induced hyphal growth of the fungus (Tian et al. 2014).

IV. Are Endophytic Fungi-Producing Host Compounds?

The demand for novel and natural metabolites to treat various ailments has outstretched to look for sources in new ecological niches. One such new source of novel structures and metabolites is endophytes within the plant microbiome (Gunatilaka 2006). Recently, through comparative genome analysis, it was found that a large number of fungal biosynthetic gene clusters (BGCs) are involved in the biosynthesis of small molecules earlier known to be produced by plants (Brakhage 2012; Keller 2015). The most widely studied such example is the production of paclitaxel (Taxol) by endophytes associated with *Taxus brevifolia* (Strobel et al. 1993; Zhang et al. 2009). Originally, paclitaxel, an anticancerous drug, was isolated and identified from *Taxus* species (Fig. 2). Due to the increased demand of paclitaxel, various sources have been explored, including *Taxus* cell cultures, fungal endophytes, and biosynthe-

sis using various precursors (Ramirez-Estrada et al. 2015). Recent studies have employed PCR amplification of paclitaxel biosynthetic sequences such as taxadiene synthase (TS), 10-deacetylbaccatin III-10-O-acetyltransferase (dbat), and C-13 phenylpropanoyl side chain-CoA acyltransferase (bapt) as markers to screen for paclitaxel production in endophytic fungi (Flores-Bustamante et al. 2010).

During the past two decades, the literature on the abundance of paclitaxel-producing endophytes has grown tremendously. More than 160 publications and patents have been written on paclitaxel production and biosynthesis of paclitaxel and related taxanes by endophytic fungi (Heinig et al. 2013). Paclitaxel is claimed to be produced by endophytic fungi in many genera, such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Monochaetia*, *Pestalotia*, *Pestalotiopsis*, *Pithomyces*, *Penicillium*, and *Xylaria* associated with *Taxus* and non-*Taxus* plants (Heinig et al. 2013). Paclitaxel is also claimed to be produced by several strains of endophytic bacteria, such as *Erwinia taxi*, *Micromonospora* sp., *Streptomyces* sp., *Kitasatospora* sp., *Bacillus cereus*, *B. megaterium*, *Sphingomonas taxi*, *B. subtilis*, *Pantoea* sp., and *Curtobacterium* sp. (Page and Landry 1996; Page et al. 2000; Caruso et al. 2000). It was thought that these endophytes could be used as paclitaxel producers in large fermenters to meet the increasing demand of the anticancer drug, but due to various challenges, these approaches have failed to bring paclitaxel widely available to the markets.

Under laboratory conditions, optimal utilization of endophytes is limited by various factors, including the presence of orphan biosynthetic pathways (Hertweck 2009). Biosynthesis of paclitaxel is known to be induced by methyl jasmonate (Onrubia et al. 2011), but more efficient induction is seen using the bacterial toxin coronatine, a jasmonic acid-isoleucine conjugate (Katsir et al. 2008). However, it is possible to manipulate endophytes by various biotechnological approaches, such as epigenetic modulation, chemical induction, metabolite remodeling, fermentation technologies, and medium engineering to scale up the production processes (Knappe et al. 2008; Schroeckh et al. 2009; Ul-Hassan et al. 2012). Dependence of endophytes on plant compounds for paclitaxel production can be eliminated or decreased, and the fermentation processes may be enhanced or optimized to increase the yield through environmentally friendly approaches, thus reducing production costs of paclitaxel. However, these techniques are still in infancy and associated with various challenges, which remain unfulfilled. This is mainly because of attenuation of metabolite production by endophytes by subsequent subculturing, lack of host stimulus in the culture media, or genes required for induction of secondary metabolites (Sachin et al. 2013; Gurudatt et al. 2010; Priti et al. 2009).

In general, the quantities of paclitaxel produced by endophytes remain much lower compared to the host plants, *Taxus* species. Alternative strategies such as heterologous expression can be used to improve the yield (Ramirez-Estrada et al. 2015; Heinig et al. 2013). The yields of paclitaxel production by heterologous expression in yeast are much higher as compared to the production in endophytic fungi. However, the lack of knowledge on endophytic genes involved in the biosynthesis of paclitaxel is a major limiting factor for the heterologous production (Heinig et al. 2013). Genes unique for formation of taxane and phenylpropanoyl transferase (BAPT) for acylation of the core structure of paclitaxel are essential for the biosynthesis. It has been speculated that during evolution, endophytes acquired genetic material through recombination from the host, even though experimental evidence is lacking (Venugopalan and Srivastava 2015). Potential paclitaxel biosynthesis genes from the endophyte EF0021 and the original endophyte *Taxomyces andreanae* (TA) of *Taxus brevifolia* have been thoroughly studied by various molecular biology techniques. Through conventional hybridization techniques, genomic phage libraries with 130- and 70-fold genome coverages of these endophytes, EF0021 and TA, respectively, were screened for paclitaxel biosynthesis genes using three probes specific for taxadiene synthase, taxane-5α-hydroxylase, and taxane-13α-hydroxylase (Wildung and Croteau 1996; Jennewein et al. 2001, 2004).

However, none of the sequences matched with the paclitaxel biosynthesis genes of *Taxus* species. When the genome sequences of the endophytes EF0021 and TA were examined to ascertain presence of biosynthesis genes of *Taxus* species, analysis of the contigs failed to confirm the presence of any biosynthesis genes similar with those of *Taxus* spp., and comparison of taxadiene synthase gene architecture revealed several major differences (Staniek et al. 2009; Heinig et al. 2013). Therefore, it was concluded that endophytic paclitaxel biosynthesis might have evolved independently.

Although it is well established in the case of gibberellins that the microbe and the host plant may differ in biosynthetic pathways of the compound (Bomke and Tudzynski 2009), the ability of endophytes to synthesize complex taxadiene structures is not possible without specific biosynthesis genes (Seemann et al. 2002; Felicetti and Cane 2004). As a result, it was concluded that EF0021 and TA are incapable of producing paclitaxel without those genes. It is assumed that paclitaxel and taxanes are highly lipophilic compounds and are accumulated in the endophyte cell walls during colonization in the host. These compounds can be stable for months, taken up by passive transport and stored in vesicles (Sharma and Straubinger 1994; Crosasso et al. 2000; Heinig et al. 2013). The above reason is valid for explaining detection of small amounts of paclitaxel in the fermentation products of endophytes and also the loss of paclitaxel production after subsequent subculturing of endophytic fungi. However, using genome sequencing and combinatorial chemistry, any independent taxane biosynthesis genes were not found in the endophytic fungus *Taxomyces andreanae* associated with *Taxus* trees, suggesting that orphan BGCs may be involved in paclitaxel biosynthesis (Heinig et al. 2013).

Sequencing of endophytic taxadiene synthase (TS) genes has revealed that endophytes possess biosynthetic pathways independent of the hosts (Staniek et al. 2009). Through genome sequencing of an endophytic fungus *Penicillium aurantiogriseum* NRRL 62431 (*P. expansum*), candidate genes for paclitaxel biosynthesis were identified by comparison with the 13 known paclitaxel biosynthetic genes in *Taxus*. However, the genes are clearly different from those of *T. baccata*, and it is unlikely that horizontal gene transfer has taken place. It was evident from the phylogenetic analysis of endophytic fungi and plants that they form separate clades indicating that no horizontal gene transfer from plant hosts to endophyte or vice versa has occurred, suggesting that *P. aurantiogriseum* NRRL 62431 may have unique enzymes catalyzing taxadiene synthesis (Yang et al. 2014).

To summarize, it has been well documented in the past few decades that endophytes can produce plant metabolites, but the quantities or concentrations of the compounds produced by endophytic fungi are insufficient for applications at industrial scale. It is important to study the complex interaction of endophytes in the host and the influence of various biotic and abiotic factors on the production of secondary metabolites, to optimize conditions for improved yields at industrial scale (Yang et al. 2014).

V. Industrial Applications on Endophytic Fungi

In general, endophytic research is growing enormously, as >7250 publications with the keyword "endophyte," and >2400 with the keywords "endophyte" and "metabolite" can be found within the last 25-year period (1991–2015) from Scopus (https://www.scopus.com). In general, over 33% of the articles report metabolites from endophytes, most of which are novel structures (Fig. 3). The number of patents filed and granted on endophytes has also increased during the 25-year period, and until April 2016, there were >2300 patents (http://www.freepatentsonline.com) filed or granted for new industrial applications or processes on endophytes. Furthermore, with the keywords "endophyte" and "metabolite," there were >1100 patent applications, more than 47% of which were related to novel structures or metabolites.

As of now, there are no products available in the market that are produced by or isolated

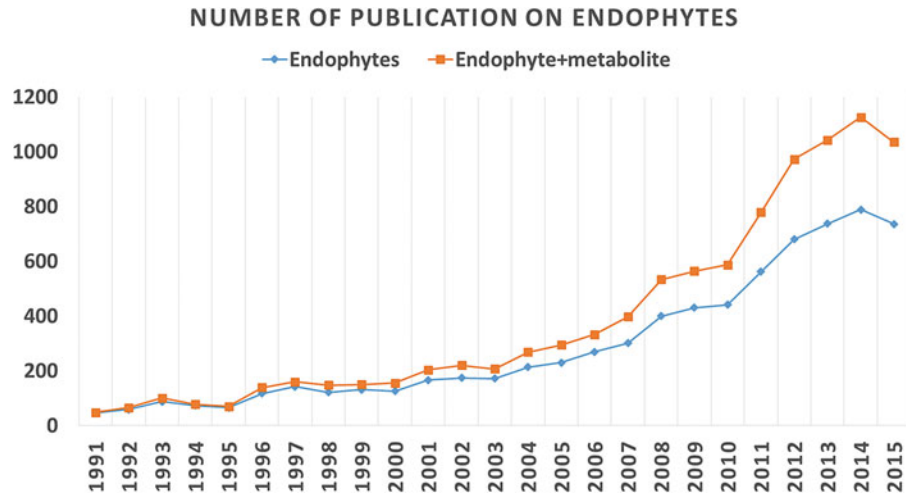


Fig. 3 Number of publications on endophytes from 1991 to 2015 (data used from Scopus with a keyword endophyte; endophyte + metabolite). From the past 25 years, research on endophytes has outnumbered many other areas of research with >6200 publications

from endophytes. Perhaps in the future, we can see more products from endophytes in the markets, as the sequencing costs are getting reasonably cheaper, which may provide some ways to develop new products faster, discussed below.

VI. What Can Be Expected in the Future?

Endophytic fungi dwell in complex ecosystems, and the evolution of secondary metabolites over hundreds of million years has enabled them to defend, thrive, and survive in those environments (Yim et al. 2007; Brakhage and Schroeckh 2011). The function of genes and genomes in biotic and abiotic interactions between the microorganism and its environment is an essential prerequisite for drug discovery (Ungerer et al. 2008). Comparative genomics, proteomics, and transcriptomics are techniques used today for studying fungal genomes, proteomes, and transcriptomes. These techniques can lead to identification of small molecules that provide a competitive or adaptive advantage to the organism in specific environmental niches (Walsh and Fischbach 2010). Genomics, genetics, and gene-guided discovery of microbial secondary metabolites are transforming our understanding of gene-to-molecule relationships and bridging the gaps to find gene function (Challis 2008; Walsh and Fischbach 2010).

Genome sequencing offers previously unseen sights for understanding the life of slow-growing, invisible fungal colonizers of plant tissue. For example, the genome of the horizontally transmitted endophyte of rubber tree, *Xylona heveae*, revealed only few plant cell wall-degrading enzymes, which may indicate that this endophyte strictly depends on the host for survival and acquires nutrients available in the apoplast (Gazis et al. 2016). The sequenced genome of the endophyte *Pestalotiopsis fici* W106-1/CGMCC3.15140, isolated from tea (*Camellia sinensis*), exhibits an exceptionally high number of genes involved in secondary metabolism (Wang et al. 2015a), giving an example of the high potential of biochemical synthesis of a wide range of compounds in endophytic fungi. The genome of the endophyte *Penicillium aurantiogriseum* NRRL 62431 (*P. expansum*) from hazel (*Corylus avellana* L.) was reported with a biosynthesis pathway for the anticancer drug paclitaxel, providing important knowledge on potential biosynthesis of this drug compound and its exploitation at industrial level (Yang et al. 2014). Another example of potential exploitation of endophyte genomes

for industry is *Ascocoryne sarcoides*, a fungus isolated from ulmo (*Eucryphia cordifolia*) that produces C8 volatile metabolites, exploitable as biofuel, when grown on a cellulose-based medium (Gianoulis et al. 2012). *A. sarcoides* genome was identified with genes for cellulose degradation and putative biofuel production pathways within ~80 biosynthetic clusters (Gianoulis et al. 2012). These few reports reflect, though still modest in number, the dimensions that lie in the sequencing and identification efforts of various biosynthesis pathways in the endophyte genomes for exploitation by industry. Regardless of the slow growth and low accumulation of biomass by endophytic fungi, the most important pathways can be expressed by means of synthetic biology in heterologous hosts.

Transfer of pathway genes from the producer organism to another foreign host and heterologous expression can yield high quantities of rare compounds (Wenzel and Müller 2005). The most typical host used for heterologous expression of complex metabolic pathways, such as those of echinomycin and triostin A, is *Escherichia coli* (Pfeifer et al. 2001; Watanabe 2008). *E. coli* is often used for production of fungal proteins and secondary metabolites even today (Ongley et al. 2013; Hatakeyama et al. 2016). The production of taxadiene, an intermediate of paclitaxel, was synthesized in *E. coli* already in 2001 (Huang et al. 2001), and taxadiene and taxadien-5a-ol have been produced in yeast (Engels et al. 2008). Recent studies have shown that heterologous expression of entire biosynthetic pathways of yew species in yeast is possible (Venugopalan and Srivastava 2015). In general, yeast is another well-favored organism for heterologous production of both fungal proteins and secondary metabolites (Tsunematsu et al. 2013a). Compounds, or their intermediates, such as Aspyridone A from the filamentous fungus *Aspergillus nidulans* (Xu et al. 2010), spirotryprostatins from *A. fumigatus* (Tsunematsu et al. 2013b), and radicicol from *Pochonia chlamydosporia* (Zhou et al. 2010), have been successfully produced in yeast. However, normally, a similar host for heterologous expression yields better results, and Aspergilli have increasingly been employed for secondary metabolite expression of filamentous fungi (Anyaogu and Mortensen 2015). For example, *Aspergillus nidulans* has been used for the heterologous production of asperfuranone (Chiang et al. 2013), *A. niger* for production of enniatins (Richter et al. 2014), and the fungal antibiotic citrinin has been produced in *A. oryzae* (Sakai et al. 2008).

Other means of gaining the full potential of horizontally transmitted endophytic fungi that are slow-growing and difficult to culture is enhancement of secondary metabolism. For example, cultivation in conditions simulating the natural environment of the fungus can yield the desired natural products (Fischer et al. 2016). As endophytes are influenced by the host metabolites throughout the endophytic phase, cultivation on plant-based media may trigger secondary metabolism. Cocultivation with a bacterial or a fungal partner, such as a phytopathogen, has been shown to induce secondary metabolism in fungi (Schroeckh et al. 2014; Bertrand et al. 2013; Cueto et al. 2001) and is already widely applied on endophytes (Combès et al. 2012; Chagas et al. 2013; Ola et al. 2013; Wang et al. 2015b; Ebrahim et al. 2016). The endophytic fungus *Alternaria tenuissima* produces several polyketides, such as the antifungal stemphyperylenol (Fig. 2), active against the pathogen *Nigrospora sphaerica* in mixed cultures. Even application of bacterial lipopolysaccharides can trigger secondary metabolism in a fungus to a desired level (Svahn et al. 2014). If none of these approaches are successful in inducing the endophyte for production of the target compounds, deletion, overexpression, or inhibition of histone-modifying enzymes can be helpful (Chujo and Scott 2014), as these methods have enabled isolation of new compounds from fungi (Nützmann et al. 2011; Chung et al. 2013). Manipulation of histone acetylation can lead to activation of silent gene clusters, although such approach is not targeted toward secondary metabolism, specifically (Grunstein 1997).

Besides genome mining, new endophytic secondary metabolites can be discovered through metabolomics (Kusari et al. 2009;

Bhagobathy and Joshi 2011; Combès et al. 2012). For example, metabolomic tools enabled identification of 3-oxo-9,11-octadecadienoic acid (Fig. 2) from the endophyte *Paraconiothyrium variabile* of *Cephalotaxus harringtonia*, as a negative modulator of beauvericin biosynthesis in the phytopathogen *Fusarium oxysporum* (Combès et al. 2012), However, true high-throughput, omics-level analysis of fungal secondary metabolites requires considerable tool development, as the current methods used for metabolomic studies do not greatly differ from the traditional, activity-based fractioning.

A field where high throughput is routine is metabarcoding, which aims to analyze communities of fungal endophytes. Increasing numbers of reports describe the community structures of endophytic fungi in various host species, already discussed above. Due to the competition and fast progress in these techniques, the costs of NGS will likely continue to come down, increasing the use and applications of metabarcoding. In the future, we will probably see more studies on community structure changes of endophytic fungi in the host plant in response to, e.g., plant metabolite production. Such study was done by Saunders and Kohn already in 2009 by traditional methods, by isolating and comparing community structures of endophytic fungi in maize plants producing an antifungal compound and in nontoxic mutant plants. Other comparisons of endophytic communities to be studied by metabarcoding could include plants under environmental change, biotic stress, or time-lapse studies or studies of endophytic communities within a plant community.

What we are lacking at the moment are good metagenomic tools to allow screening of unculturable endophytes for secondary metabolites. Whereas endophyte genomes are sequenced at an increasing speed and allow discovery of new secondary metabolite biosynthesis clusters, heterologous expression of compounds seems to advance rather slowly. Therefore, the slow development of synthetic biology tools may become a bottleneck for bringing new endophyte-based drug compounds to the markets.

References

Álvarez-Loayza P, White JF Jr, Torres MS, Balslev H, Kristiansen T, Svenning J-C et al (2011) Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. PLoS One 6(1): e16386

Anyaogu DC, Mortensen UH (2015) Heterologous production of fungal secondary metabolites in Aspergilli. Front Microbiol 6:77

Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: process, challenges, and frontiers. Fungal Biol Rev 21:51–66

Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88:541–549

Arnold AE, Meija LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci USA 100:15649–15654

Bacon CW, Glenn AE, Yates IE (2008) *Fusarium verticillioides*: managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Rev 27:411–446

Bae H, Roberts DP, Lim H-S, Strem MD, Park S-C, Ryu C-M, Melnick RL, Bailey BA (2011) Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. Mol Plant Microbe Interact 24:336–351

Bálint M, Tiffin P, Hallström B, O'Hara RB, Olson MS, Fankhauser JD et al (2013) Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). PLoS One 8(1):e53987

Bearden SW, Perry RD (1999) The Yfe system of *Yersinia pestis* transports iron and manganese and is required for full virulence of plague. Mol Microbiol 32:403–414

Bertrand S, Schumpp O, Bohni N, Monod M, Gindro K, Wolfender J-L (2013) De novo production of metabolites by fungal co-culture of *Trichophyton rubrum* and *Bionectria ochroleuca*. J Nat Prod 76:1157–1165

Bhagobathy RK, Joshi SR (2011) Metabolite profiling of endophytic fungal isolates of five ethnopharmacologically important plants of Meghalaya, India. J Metabolomics Syst Biol 2:20–31

Blodgett JT, Eyles A, Bonello P (2007) Organ-dependent induction of systemic resistance and systemic susceptibility in *Pinus nigra* inoculated with *Sphaeropsis sapinea* and *Diplodia scrobiculata*. Tree Physiol 27:511–517

Bomke C, Tudzynski B (2009) Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry 70(15–16):1876–1893. https://doi.org/10.1016/j.phytochem.2009.05.020

Brakhage AA (2012) Regulation of fungal secondary metabolism. Nat Rev Microbiol 11(1):21–32. https://doi.org/10.1038/nrmicro2916

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet Biol 48(1):15–22. https://doi.org/10.1016/j.fgb.2010.04.004

Bullington LS, Larkin BG (2015) Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines. Fungal Ecol 17:170–178

Bush LP, Cornelius PL, Buckner RC, Varney DR, Chapman RA, Burriss PB II, Kennedy CW, Jones TA, Saunders MJ (1982) Association of *N*-acetyl loline and *N*-formyl loline with *Epichloe typhina* in tall fescue. Crop Sci 22:941–943

Campanile G, Ruscelli A, Luisi N (2007) Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by in vitro and in planta tests. Eur J Plant Pathol 117:237–246

Caporaso JG, Lauber CL, Walters WA et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6(8):1621–1624. https://doi.org/10.1038/ismej.2012.8

Carroll GC (1988) Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69:2–9

Carroll GC (1991) Fungal associates of woody plants as insect antagonists in leaves and stems. In: Barbosa P, Krischik VA, Jones CG (eds) Microbial mediation of plant-herbivore interactions. Wiley, New York, pp 253–271

Caruso M, Colombo AL, Crespi-Perellino N et al (2000) Studies on a strain of *Kitasatospora* sp. paclitaxel producer. Ann Microbiol 50(2):89–102

Chagas FO, Dias LG, Pupo MT (2013) A mixed culture of endophytic fungi increases production of antifungal polyketides. J Chem Ecol 39:1335–1342

Challis GL (2008) Mining microbial genomes for new natural products and biosynthetic pathways. Microbiology 154(Pt 6):1555–1569. https://doi.org/10.1099/mic.0.2008/018523-0

Chiang YM, Oakley CE, Ahuja M, Entwistle R, Schultz A, Chang SL, Sung CT, Wang CC, Oakley BR (2013) An efficient system for heterologous expression of secondary metabolite genes in *Aspergillus nidulans*. J Am Chem Soc 135 (20):7720–7731

Chujo T, Scott B (2014) Histone H3K9 and H3K27 methylation regulates fungal alkaloid biosynthesis in a fungal endophyte–plant symbiosis. Mol Microbiol 92:413–434

Chung YM, El-Shazly M, Chuang DW, Hwang TL, Asai T, Oshima Y, Ashour ML, Wu YC, Chang FR (2013) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, induces the production of anti-inflammatory cyclodepsipeptides from *Beauveria felina*. J Nat Prod 76:1260–1266

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69:10–16

Clay K (2004) Fungi and the food of gods. Nature 427:401–402

Cleary M, Nguyen D, Marčiulynienė D, Berlin A, Vasaitis R, Stenlid J (2016) Friend or foe? Biological and ecological traits of the European ash dieback pathogen *Hymenoscyphus fraxineus* in its native environment. Sci Rep 6:21895

Combès A, Ndoye I, Bance C, Bruzaud J, Djediat C, Dupont J et al (2012) Chemical communication between the endophytic fungus *Paraconiothyrium variabile* and the phytopathogen *Fusarium oxysporum*. PLoS One 7:e47313

Crosasso P, Ceruti M, Brusa P et al. (2000) Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J Control Release 63(1–2):19–30. doi:S0168-3659(99)00166-2

Cueto M, Jensen PR, Kauffman C, Fenical W, Lobkovsky E, Clardy J (2001) Pestalone, a new antibiotic produced by a marine fungus in response to bacterial challenge. J Nat Prod 64:1444–1446

Danielsen S, Jensen DF (1999) Fungal endophytes from stalks of tropical maize and grasses: isolation, identification, and screening for antagonism against *Fusarium verticillioides* in maize stalks. Biocontrol Sci Tech 9:545–553

Delaye L, García-Guzmán G, Heil M (2013) Endophytes versus biotrophic and necrotrophic pathogens are fungal lifestyles evolutionarily stable traits? Fungal Divers 60:125e135

Ebrahim W, El-Neketi M, Lewald L-I, Orfali RS, Lin W, Rehberg N, Kalscheuer R, Daletos G, Proksch P (2016) Metabolites from the fungal endophyte *Aspergillus austroafricanus* in axenic culture and in fungal–bacterial mixed cultures. J Nat Prod 79:914–922

Engels B, Dahm P, Jennewein S (2008) Metabolic engineering of taxadiene biosynthesis in yeast as a first step towards Taxol (Paclitaxel) production. Metab Eng 10:201–206

Felicetti B, Cane DE (2004) Aristolochene synthase: mechanistic analysis of active site residues by site-directed mutagenesis. J Am Chem Soc 126 (23):7212–7221. https://doi.org/10.1021/ja0499593

Fischer J, Schroeckh V, Brakhage AA (2016) Awakening of fungal secondary metabolite gene clusters. In: Schmoll M, Dattenböck C (eds) Gene expression systems in fungi: advancements and applications. fungal biology. Springer, Heidelberg, pp 253–273

Fisher PJ, Petrini O, Sutton BC (1993) A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England. Sydowia 45:338–345

Fletcher LR, Harvey IC (1981) An association of a *Lolium* endophyte with ryegrass staggers. NZ Vet J 29:185–186

Flores-Bustamante ZR, Rivera-Orduña FN, Martínez-Cárdenas A et al (2010) Microbial paclitaxel:

advances and perspectives. J Antibiot 63(8):460–467. https://doi.org/10.1038/ja.2010.83

Gallagher RT, Hawkes AD, Steyn PS, Vieggaar R (1984) Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: structure elucidation of lolitrem B. J Chem Soc Chem Commun 1984:614–616

Ganley RJ, Brunsfeld SJ, Newcombe G, Margulis L (2004) A community of unknown endophytic fungi in western white pine. Proc Natl Acad Sci USA 101(27):10107–10112

Gazis R, Kuo A, Riley R, LaButti K, Lipzen A, Lin J, Amirebrahimi M, Hesse CN, Spatafora JW, Henrissat B, Hainaut M, Grigoriev IV, Hibbett DS (2016) The genome of *Xylona heveae* provides a window into fungal endophytism. Fungal Biol 120 (1):26–42

Gianoulis TA, Griffin MA, Spakowicz DJ, Dunican BF, Alpha CJ et al (2012) Genomic analysis of the hydrocarbon-producing, cellulolytic, endophytic fungus *Ascocoryne sarcoides*. PLoS Genet 8(3): e1002558

Glenn TC (2011) Field guide to next-generation DNA sequencers. Mol Ecol Resour 11:759–769

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389:349–352

Gunatilaka L (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69:509–526

Gurudatt PS, Priti V, Shweta S et al (2010) Attenuation of camptothecin production and negative relation between hyphal biomass and camptothecin content in endophytic fungal strains isolated from *Nothapodytes nimmoniana* Grahm (Icacinaceae). Curr Sci 98:1006–1010. http://www.cabdirect.org/abstracts/20103140806.html;jsessionid=B012DC2FB1C24A1016097716892C2F2D

Hantke K, Braun V (2000) The art of keeping low and high iron concentrations in balance. In: Storz G, Hengge-Aronis R (eds) Bacterial stress responses. ASM Press, Washington, DC, pp 275–288

Hardoim P, van Overbeek L, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320

Hartley SE, Eschen R, Horwood JM, Gange AC, Hill EM (2015) Infection by a foliar endophyte elicits novel arabidopside-based plant defence reactions in its host, *Cirsium arvense*. New Phytol 205:816–827

Hatakeyama M, Kitaoka T, Ichinose H (2016) Heterologous expression of fungal cytochromes P450 (CYP5136A1 and CYP5136A3) from the white-rot basidiomycete *Phanerochaete chrysosporium*: functionalization with cytochrome b5 in *Escherichia coli*. Enzym Microb Technol 89:7–14

Heinig U, Scholz S, Jennewein S (2013) Getting to the bottom of Taxol biosynthesis by fungi. Fungal Divers 60(1):161–170. https://doi.org/10.1007/s13225-013-0228-7

Herre EA, Bael SAV, Maynard Z, Robbins N, Bischoff J, Arnold AE, Rojas E, Mejía LC, Cordero RA, Woodward C, Kyllo DA (2005) Tropical plants as chimera: some implications of foliar endophytic fungi for the study of host plant defense, physiology, and genetics. In: Burslem DFRP, Pinard MA, Hartley SE (eds) Biotic interactions in the tropics. Cambridge University Press, Cambridge, pp 226–240

Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chem Int Ed 48(26):4688–4716. https://doi.org/10.1002/anie.200806121

Hibbett DS, Ohman A, Glotzer D et al (2011) Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biol Rev 25 (1):38–47. https://doi.org/10.1016/j.fbr.2011.01.001

Higginbotham SJ, Arnold AE, Ibañez A, Spadafora C, Coley PD, Kursar TA (2013) Bioactivity of fungal endophytes as a function of endophyte taxonomy and the taxonomy and distribution of their host plants. PLoS One 8:e73192

Huang QL, Roessner CA, Croteau R, Scott AI (2001) Engineering *Escherichia coli* for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. Bioorg Med Chem 9:2237–2242

Huse SM, Welch DM, Morrison HG et al (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol 12 (7):1889–1898. https://doi.org/10.1111/j.1462-2920.2010.02193.x

Jaber LR, Vidal S (2009) Interactions between an endophytic fungus, aphids, and extrafloral nectaries: do endophytes induce extrafloral-mediated defences in *Vicia faba*? Funct Ecol 23:707–714

Jallow MFA, Dugassa-Gobena D, Vidal S (2008) Influence of an endophytic fungus on host plant selection by a polyphagous moth via volatile spectrum changes. Arthropod Plant Interact 2:53–62

Jennewein S, Rithner CD, Williams RM et al (2001) Taxol biosynthesis: taxane 13 alpha-hydroxylase is a cytochrome P450-dependent monooxygenase. Proc Natl Acad Sci USA 98(24):13595–13600. https://doi.org/10.1073/pnas.251539398

Jennewein S, Wildung MR, Chau M et al (2004) Random sequencing of an induced *Taxus* cell cDNA library for identification of clones involved in Taxol biosynthesis. Proc Natl Acad Sci USA 101 (24):9149–9154. https://doi.org/10.1073/pnas.0403009101

Johnson LJ, Koulman A, Christensen M, Lane GA, Fraser K, Forester N, Johnson RD, Bryan GT, Rasmussen S (2013) An extracellular siderophore is required to maintain the mutualistic interaction of *Epichloë festucae* with *Lolium perenne*. PLoS Pathog 9:e1003332

Jumpponen A (2001) Dark septate endophytes – are they mycorrhizal? Mycorrhiza 11:207–211

Jumpponen A, Jones KL (2009) Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytol 184(2):438–448. https://doi.org/10.1111/j.1469-8137.2009.02990.x

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root colonizing fungi. New Phytol 140:295–310

Kajula M, Tejesvi M, Kolehmainen S, Mäkinen A, Hokkanen J, Mattila S, Pirttilä AM (2010) The siderophore ferricrocin produced by specific foliar endophytic fungi in vitro. Fungal Biol 114:248–254

Katsir L, Schilmiller AL, Staswick PE et al (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci USA 105(19):7100–7105. https://doi.org/10.1073/pnas.0802332105

Keller NP (2015) Translating biosynthetic gene clusters into fungal armor and weaponry. Nat Chem Biol 11(9):671–677. https://doi.org/10.1038/nchembio.1897

Kemler M, Garnas J, Wingfield MJ et al (2013) Ion Torrent PGM as tool for fungal community analysis: a case study of endophytes in *Eucalyptus grandis* reveals high taxonomic diversity. PLoS One 8(12):e81718. https://doi.org/10.1371/journal.pone.0081718

Khan AL, Hamayun M, Kang S-M, Kim Y-H, Jung H-Y, Lee J-H, Lee I-J (2012) Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. BMC Microbiol 12:3

Knappe TA, Linne U, Zirah S et al (2008) Isolation and structural characterization of capistruin, a lasso peptide predicted from the genome sequence of *Burkholderia thailandensis* E264. J Am Chem Soc 130(34):11446–11454. https://doi.org/10.1021/ja802966g

Kogel KH, Franken P, Hückelhoven R (2006) Endophyte or parasite – what decides? Curr Opin Plant Biol 9:358e363

Koskimäki JJ, Hokkanen J, Jaakola L, Suorsa M, Tolonen A, Mattila S, Pirttilä AM, Hohtola A (2009) Flavonoid biosynthesis and degradation play a role in early defence responses of bilberry (*Vaccinium myrtillus*) against biotic stress. Eur J Plant Pathol 125:629–640

Kunin V, Engelbrektson A, Ochman H et al (2010) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol 12(1):118–123. https://doi.org/10.1111/j.1462-2920.2009.02051.x

Kusari S, Zühlke S, Kosuth J, Cellárová E, Spiteller M (2009) Light-independent metabolomics of endophytic *Thielavia subthermophila* provides insight into microbial hypericin biosynthesis. J Nat Prod 72:1825–1835

Larkin BG, Hunt LS, Ramsey PW (2012) Foliar nutrients shape fungal endophyte communities in western white pine (*Pinus monticola*) with implications for white-tailed deer herbivory. Fungal Ecol 5:252–260

Lodge DJ, Fisher PJ, Sutton BC (1996) Endophytic fungi of *Manilkara bidentata* leaves in Puerto Rico. Mycologia 88:733–738

Lopez-Llorca LV, Jansson H-B, Vicente JGM, Salinas J (2006) Nematophagous fungi as root endophytes. In: Schulz B, Boyle C, Sieber T (eds) Microbial root endophytes. Springer, Berlin, Germany, pp 191–206

Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp. and endophytic fungus in *Artemisia annua*. Plant Sci 151:67–73

Mardis ER (2008) Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 9:387–402. https://doi.org/10.1146/annurev.genom.9.081307.164359

Martins F, Pereira JA, Bota P, Bento A, Baptista P (2016) Fungal endophyte communities in above- and belowground olive tree organs and the effect of season and geographic location on their structures. Fungal Ecol 20:193–201

Miller JD, Cherid H, Sumarah MW, Adams GW (2009) Horizontal transmission of the *Picea glauca* foliar endophyte *Phialocephala scopiformis* CBS 120377. Fungal Ecol 2:98–104

Monk KA, Samuels GJ (1990) Mycophagy in grasshoppers (Orthoptera: Acrididae) in Indo-Malayan rain forests. Biotropica 22:16–21

Nagy LG, Petkovits T, Kovács GM, Voigt K, Vágvölgyi C, Papp T (2011) Where is the unseen fungal diversity hidden? A study of *Mortierella* reveals a large contribution of reference collections to the identification of fungal environmental sequences. New Phytol 191:789–794

Narisawa K, Kawamata H, Currah RS, Hashiba T (2002) Suppression of Verticillium wilt in eggplant by some fungal root endophytes. Eur J Plant Pathol 108:103–109

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436–439

Navarro-Meléndez AL, Heil M (2014) Symptomless endophytic fungi suppress endogenous levels of salicylic acid and interact with the jasmonate-dependent indirect defense traits of their host, Lima bean (*Phaseolus lunatus*). J Chem Ecol 40:816–825

Nützmann HW, Reyes-Dominguez Y, Scherlach K, Schroeckh V, Horn F, Gacek A, Schümann J, Hertweck C, Strauss J, Brakhage AA (2011) Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation. Proc Natl Acad Sci USA 108:14282–14287

Ola ARB, Thomy D, Lai D, Brötz-Oesterhelt H, Proksch P (2013) Inducing secondary metabolite production by the endophytic fungus *Fusarium tricinctum* through coculture with *Bacillus subtilis*. J Nat Prod 76:2094–2099

Ongley SE, Bian X, Neilan BA, Müller R (2013) Recent advances in the heterologous expression of microbial natural product biosynthetic pathways. Nat Prod Rep 30:1121–1138

Onrubia M, Moyano E, Bonfill M et al (2011) The relationship between TXS, DBAT, BAPT and DBTNBT gene expression and taxane production during the development of *Taxus baccata* plantlets. Plant Sci 181(3):282–287. https://doi.org/10.1016/j.plantsci.2011.06.006

Page M, Landry N (1996) Patent US5561055 – bacterial mass production of taxanes with *Erwinia*. http://www.google.com/patents/US5561055

Page M, Landry N, Boissinot M et al. (2000) Patent US6030818 – Bacterial mass production of taxanes and paclitaxel. http://www.google.com/patents/US6030818

Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, Van Den Huevel J (eds) Microbiology of the phyllosphere. Cambridge University Press, Cambridge, pp 175–187

Pfeifer BA, Admiraal SJ, Gramajo H, Admiraal SJ, Cane DE, Khosla C (2001) Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*. Science 291:1790–1792

Porras-Alfaro A, Bayman P (2011) Hidden fungi, emergent properties: endophytes and microbiomes. Annu Rev Phytopathol 49:291–315. https://doi.org/10.1146/annurev-phyto-080508-081831

Powell PE, Cline GR, Reid CPP, Szaniszlo PJ (1980) Occurrence of hydroxamate siderophore iron chelators in soils. Nature 287:833–834

Priti V, Ramesha BT, Singh S et al. (2009) How promising are endophytic fungi as alternative sources of plant secondary metabolites? http://www.cabdirect.org/abstracts/20093253127.html

Ramirez-Estrada K, Osuna L, Moyano E et al (2015) Changes in gene transcription and taxane production in elicited cell cultures of *Taxus* x *media* and *Taxus globosa*. Phytochemistry 117:174–184. https://doi.org/10.1016/j.phytochem.2015.06.013

Redman RS, Ranson JC, Rodriguez RJ (1999) Conversion of the pathogenic fungus *Colletotrichum magna* to a nonpathogenic, endophytic mutualist by gene disruption. Mol Plant-Microbe Interact 12:969–975

Richter L, Wanka F, Boecker S, Storm D, Kurt T, Vural Ö, Süßmuth R, Meyer V (2014) Engineering of *Aspergillus niger* for the production of secondary metabolites. Fungal Biol Biotechnol 1:4

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. Int Soc Microb Ecol 2:404–416

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Rodriguez Estrada AE, Jonkers W, Kistler HC, May G (2012) Interactions between *Fusarium verticillioides, Ustilago maydis*, and *Zea mays*: an endophyte, a pathogen, and their shared plant host. Fungal Genet Biol 49:578–587

Rosconi F, Davyt D, Martínez V, Martínez M, Abin-Carriquiry JA, Zane H, Butler A, de Souza EM, Fabiano E (2013) Identification and structural characterization of serobactins, a suite of lipopeptide siderophores produced by the grass endophyte *Herbaspirillum seropedicae*. Environ Microbiol 15:916–927

Sachin N, Manjunatha BL, Mohana Kumara P et al. (2013) Do endophytic fungi possess pathway genes for plant secondary metabolites?. http://connection.ebscohost.com/c/articles/89388446/do-endophytic-fungi-possess-pathway-genes-plant-secondary-metabolites

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Annu Rev Ecol Syst 29:319–343

Saikkonen K, Ion D, Gyllenberg M (2002) The persistence of vertically transmitted fungi in grass metapopulations. Proc R Soc B Biol Sci 269:1397–1403

Sakai K, Kinoshita H, Shimizu T, Nihira T (2008) Construction of a citrinin gene cluster expression system in heterologous *Aspergillus oryzae*. J Biosci Bioeng 106:466–472

Saunders M, Kohn LM (2009) Evidence for alteration of fungal endophyte community assembly by host defense compounds. New Phytol 182:229–238

Schippers B, Bakker AW, Bakker PA (1987) Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practises. Annu Rev Phytopathol 25:339–358

Schrettl M, Bignell E, Kragl C, Joechl C, Rogers T, Arst HN Jr, Haynes K, Haas H (2004) Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. J Exp Med 200:1213–1219

Schroeckh V, Scherlach K, Nutzmann HW et al (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. Proc Natl Acad Sci USA 106(34):14558–14563. https://doi.org/10.1073/pnas.0901870106

Schroeckh V, Nützmann H-W, Brakhage AA (2014) Fungal-actinomycete interactions—wakening of silent fungal secondary metabolism gene clusters via interorganismic interactions. In: Osbourn A, Goss RJ, Carter GT (eds) Natural products: discourse, diversity, and design. John Wiley & Sons, Inc, Hoboken, NJ, pp 147–158

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schulz B, Rommert AK, Dammann U, Aust HJ, Strack D (1999) The endophyte-host interaction: a balanced antagonism? Mycol Res 10:1275–1283

Seemann M, Zhai G, de Kraker JW et al. (2002) Pentalenene synthase. Analysis of active site residues by site-directed mutagenesis. J Am Chem Soc 124 (26):7681–7689. doi:ja026058q

Sharma A, Straubinger RM (1994) Novel taxol formulations: preparation and characterization of taxol-containing liposomes. Pharm Res 11(6):889–896

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26(10):1135–1145. https://doi.org/10.1038/nbt1486

Siegel MC, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Bacon CW, Johnson MC (1990) Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. J Chem Ecol 16:3301–3315

Smith H, Wingfield MJ, Petrini O (1996) Botryosphaeria dothidea endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. For Ecol Manag 89:189–195

Solis MJL, Dela Cruz TE, Schnittler M, Unterseher M (2016) The diverse community of leaf-inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina*, *F. elastica* and *F. religiosa*. Mycoscience 57:96–106

Staniek A, Woerdenbag HJ, Kayser O (2009) *Taxomyces andreanae*: a presumed paclitaxel producer demystified? Planta Med 75(15):1561–1566. https://doi.org/10.1055/s-0029-1186181

Stone JK, Polishook JD, White JF Jr (2004) Endophytic fungi. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, New York, USA, pp 241–270

Strobel G, Stierle A, Stierle D et al (1993) *Taxomyces andreanae*, a proposed new taxon for a bulbilliferous hyphomycete associated with Pacific Yew (*Taxus brevifolia*). Mycotaxon 47:71–80. http://www.mycobank.org/BioloMICS.aspx?TableKey=14682616000000061&Rec=6371&Fields=All

Sumarah MW, Adams GW, Berghout J, Slack GJ, Wilson AM, Miller JD (2008) Spread and persistence of a rugulosin-producing endophyte in *Picea glauca* seedlings. Mycol Res 112:731–736

Suzuki S, He Y, Oyaizu H (2003) Indole-3-Acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. Curr Microbiol 47:138–143

Svahn KS, Göransson U, Chryssanthou E, Olsen B, Sjölin J, Strömstedt AA (2014) Induction of gliotoxin secretion in *Aspergillus fumigatus* by bacteria-associated molecules. PLoS One 9:e93685

Tanaka A, Christensen MJ, Takemoto D, Park P, Scott B (2006) Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. Plant Cell 18:1052–1066

Tejesvi MV, Ruotsalainen AL, Markkola AM, Pirttilä AM (2010) Root endophytes along a primary succession gradient in northern Finland. Fungal Divers 41:125–134

Tejesvi MV, Kajula M, Mattila S, Pirttilä AM (2011) Bioactivity and genetic diversity of endophytic fungi in *Rhododendron tomentosum* Harmaja. Fungal Divers 47:97–107

Tejesvi MV, Segura DR, Schnorr KM, Sandvang D, Mattila S, Olsen PB, Neve S, Kruse T, Kristensen HH, Pirttilä AM (2013) An antimicrobial peptide from endophytic *Fusarium tricinctum* of *Rhododendron tomentosum* Harmaja. Fungal Divers 60:153–159

Tian Y, Amand S, Buisson D, Kunz C, Hachette F, Dupont J, Nay B, Prado S (2014) The fungal leaf endophyte *Paraconiothyrium variabile* specifically metabolizes the host-plant metabolome for its own benefit. Phytochemistry 108:95–101

Tsunematsu Y, Ishiuchi K, Hotta K, Watanabe K (2013a) Yeast-based genome mining, production and mechanistic studies of the biosynthesis of fungal polyketide and peptide natural products. Nat Prod Rep 30:1139–1149

Tsunematsu Y, Ishikawa N, Wakana D, Goda Y, Noguchi H, Moriya H, Hotta K, Watanabe K (2013b) Distinct mechanisms for spiro-carbon formation reveal biosynthetic pathway crosstalk. Nat Chem Biol 9:818–825

Tudzynski B, Sharon A (2002) Biosynthesis, biological role and application of fungal phyto-hormones. In: Osiewacz HD (ed) The Mycota X. Industrial applications. Springer, Berlin, Germany, pp 183–212

Ul-Hassan SR, Strobel GA, Booth E et al (2012) Modulation of volatile organic compound formation in the Mycodiesel-producing endophyte *Hypoxylon* sp. CI-4. Microbiology 158(Pt 2):465–473. https://doi.org/10.1099/mic.0.054643-0

Ungerer MC, Johnson LC, Herman MA (2008) Ecological genomics: understanding gene and genome function in the natural environment. Heredity 100(2):178–183. https://doi.org/10.1038/sj.hdy.6800992

Unterseher M, Siddique AB, Brachmann A, Peršoh D (2016) Diversity and composition of the leaf mycobiome of beech (*Fagus sylvatica*) are affected by local habitat conditions and leaf biochemistry. PLoS One 11(4):e0152878

Vadassery I, Ritter C, Venus Y, Camehl I, Varma A, Shahollari B, Novák O, Strnad M, Ludwig-Müller J, Oelmüller R (2008) The role of auxins and cytokinins in the mutualistic interaction between arabidopsis and *Piriformospora indica*. Mol Plant-Microbe Interact 21:1371–1383

van Loon LC, Bakker PA, van der Heijdt WH, Wendehenne D, Pugin A (2008) Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. Mol Plant-Microbe Interact 21:1609–1621

Venugopalan A, Srivastava S (2015) Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol Adv 33(6 Pt 1):873–887. https://doi.org/10.1016/j.biotechadv.2015.07.004

Vu T, Hauschild R, Sikora RA (2006) *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. Nematology 8:847–852

Walsh CT, Fischbach MA (2010) Natural products version 2.0: connecting genes to molecules. J Am Chem Soc 132(8):2469–2493. https://doi.org/10.1021/ja909118a

Wang X, Zhang X, Liu L, Xiang M, Wang W, Sun X, Che Y, Guo L, Liu G, Guo L, Wang C, Yin WB, Stadler M, Zhang X, Liu X (2015a) Genomic and transcriptomic analysis of the endophytic fungus *Pestalotiopsis fici* reveals its lifestyle and high potential for synthesis of natural products. BMC Genomics 16:28

Wang W-X, Kusari S, Sezgin S, Lamshöft M, Kusari P, Kayser O, Spiteller M (2015b) Hexacyclopeptides secreted by an endophytic fungus *Fusarium solani* N06 act as crosstalk molecules in *Narcissus tazetta*. Appl Microbiol Biotechnol 99:7651–7662

Watanabe K (2008) Exploring the biosynthesis of natural products and their inherent suitability for the rational design of desirable compounds through genetic engineering. Biosci Biotechnol Biochem 72:2491–2506

Weber D (2009) Endophytic fungi, occurrence and metabolites. In: Anke T, Weber D (eds) The Mycota, vol 5. Springer, Berlin, Germany, pp 153–195

Wenzel SC, Müller R (2005) Recent developments towards the heterologous expression of complex bacterial natural product biosynthetic pathways. Curr Opin Biotechnol 16:594–606

Wildung MR, Croteau R (1996) A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis. J Biol Chem 271(16):9201–9204

Wille PA, Aeschbacher RA, Boller T (1999) Distribution of fungal endophyte genotypes in doubly infected host grasses. Plant J 18:349–358

Wilson D (1995) Endophyte – the evolution of a term, and clarification of its use and definition. Oikos 73:274–276

Wooley JC, Godzik A, Friedberg I (2010) A primer on metagenomics. PLoS Comput Biol 6(2):e1000667. https://doi.org/10.1371/journal.pcbi.1000667

Xu W, Cai X, Jung ME, Tang Y (2010) Analysis of intact and dissected fungal polyketide synthase-nonribosomal peptide synthetase in vitro and in *Saccharomyces cerevisiae*. J Am Chem Soc 132:13604–13607

Xu XH, Su ZZ, Wang C, Kubicek CP, Feng XX, Mao LJ, Wang JY, Chen C, Lin FC et al (2014) The rice endophyte *Harpophora oryzae* genome reveals evolution from a pathogen to a mutualistic endophyte. Sci Rep 4:5783

Yang Y, Zhao H, Barrero RA, Zhang B, Sun G, Wilson IW, Xie F, Walker KD, Parks JW, Bruce R, Guo G, Chen L, Zhang Y, Huang X, Tang Q, Liu H, Bellgard MI, Qiu D, Lai J, Hoffman A (2014) Genome sequencing and analysis of the paclitaxel-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431. BMC Genomics 15:69

Yim G, Wang HH, Davies J (2007) Antibiotics as signalling molecules. Philos Trans R Soc Lond Ser B Biol Sci 362(1483):1195–1200. doi:4Q62642631X855V0

Zhang P, Zhou PP, LJ Y (2009) An endophytic taxol-producing fungus from *Taxus media*, *Cladosporium cladosporioides* MD2. Curr Microbiol 59(3):227–232. https://doi.org/10.1007/s00284-008-9270-1

Zhou H, Qiao K, Gao Z, Vederas JC, Tang Y (2010) Insights into radicicol biosynthesis via heterologous synthesis of intermediates and analogs. J Biol Chem 285(53):41412–41421

Secondary Metabolites of Basidiomycetes

Anja Schüffler[1]

CONTENTS

[1] Institut für Biotechnologie und Wirkstoff-Forschung gGmbH, Kaiserslautern, Germany; e-mail: schueffler@ibwf.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

I. Introduction

Basidiomycetes, a major class of higher fungi adapted to many different climates, habitats, and substrates, have developed a rich and very diverse secondary metabolism. Its products differ in biogenetic origin and structure remarkably from the metabolites of ascomycetes or other prolific producers of secondary metabolites like actinomycetes or myxobacteria. There are, however, some similarities to the products of plants, especially with regard to some polyketides, acetylenes, and sesquiterpenoids. The first systematic investigations of basidiomycete metabolites originated after the discovery and introduction into clinical practice of penicillin, the first antibiotic not derived by chemical synthesis. Its great success initiated a real "gold rush" in the search for new antibiotics and prospective producers. From 1940 to the early 1950s, mycelial cultures or fruiting bodies of more than 2000 basidiomycetes were screened for the production of antibiotics by the pioneering groups of M. Anchel, A. Hervey, F. Kavanagh, W. J. Robbins, and W. H. Wilkins (for reviews, see Wilkins and Harris 1943; Florey et al. 1949). Their investigations resulted in the discovery of pleuromutilin, the lead compound for the semisynthetic tiamulin used in veterinary practice and recently also in humans (Kavanagh et al. 1951; Högenauer 1979; Daum et al. 2007). This systematic search came to an end after Waksman's discovery of the streptomycetes as the most prolific producers of antibiotic metabolites. These soil bacteria are easily obtained and can be grown in simple media in large fermenters which greatly facilitates the discovery and production process. Meanwhile the basidiomycetes and mainly their fruiting bodies attracted the interest of natural product chemists as a source of toxins (Bresinsky and Besl 1985), hallucinogens (Schultes and Hofmann 1980), and pigments (Gill and Steglich 1987; Gill 1999; Zhou and Liu 2010). A systematic screening of mycelial cultures only regained interest when it became increasingly difficult to discover new chemical entities among the metabolites of actinomycetes and other bacterial sources. This was furthered by the recent progress in cultivation techniques and the development of fast methods for the isolation and structural determination of natural compounds. The following chapter mainly deals with compounds described from 2008 on and focuses on biologically active metabolites. For earlier reviews on bioactive natural products of basidiomycetes and secondary metabolites of fungi, the reader is referred to Zhong and Xiao (2009), Pei et al. (2010), Zhou and Liu (2010), De Silva et al. (2013), Schüffler and Anke (2014), and Stadler and Hoffmeister (2015).

II. Secondary Metabolites and Their Biological Activities

Basidiomycetes like other saprophytic or soil-inhabiting microorganisms are prolific producers of secondary metabolites. Their mycelia and fruiting bodies are exposed to a number of predators or competitors, which in some cases can explain the production of antibiotics, insecticides, or feeding deterrents. The antibiotics, e.g., α-methylene lactones and ketones, have a very broad and unspecific action against prokaryotes and eukaryotes, while others are highly selective with regard to their biochemical targets and organisms. As demonstrated for the producers of antifungal strobilurins, antibiotic production can be stimulated severalfold in the presence of other, possibly competing, fungi (Kettering et al. 2004). In many cases, however, a possible benefit for the producing fungus is not obvious. This is especially true for secondary metabolites for which up to now no antibiotic or other biological activities have been detected. According to Zähner et al. (1983), these could be part of a still ongoing "evolutionary playground" providing new chemical solutions for an improved fitness of the producing organisms. This edition covers basidiomycetal natural products and their biological activities published since 2008 to the beginning of 2017. Fungi (genus) and secondary metabolites, which were subjects of the

last edition, were reviewed and revised. New ones were added. If there were no new studies published on items covered from 1998 to 2008, the reader is referred to the previous edition. The compounds are arranged by their presumed biogenetic origin and the producing fungi. Synthetic approaches and biosynthetic studies are included for selected natural products. Additionally all names were revised, and the actual taxonomic names were used according to Index Fungorum (http://www.indexfungorum.org). If names in publications are not identical to the currently accepted name, then the current name will be given in parentheses.

A. Terpenoids

Terpenoids are among the prominent metabolites of basidiomycetes. Most of these have structures not encountered elsewhere in nature, with the notable exception that some sesquiterpenes have ring structures also found in higher plants, e.g., caryophyllanes and acoranes (Lorenzen and Anke 1998; Abraham 2001). Geosmin, with its characteristic musty-earthy odor and first described as a typical streptomycete metabolite, has now been identified as responsible for the characteristic odor of *Cortinarius herculeus* (Cortinariaceae), *Cystoderma amianthinum* (Agaricaceae), and *Cystoderma carcharias* (Breheret et al. 1999).

The biosynthesis of basidiomycete terpenoids is not yet exhaustively studied. Up-to-date metabolic pathways of ascomycetes are much more exhaustibly described. But considering the continuous progress in sequencing and comparative annotating of genomes, as well as homo- or heterologous expression of genes, all this will increase knowledge about biosynthetic pathways in basidiomycetes. Several reviews which shed light on terpenoid biosynthesis and the biocatalytic portfolio of basidiomycetes were published by the group of Schmidt-Dannert (Wawrzyn et al. 2012a; Quin et al. 2013a, 2014; Schmidt-Dannert 2014). Optimization strategies and synthetic biology approaches toward fungal terpenoids were summarized by Xiao and Zhong (2016).

1. Sesquiterpenoids

Basidiomycetes are prolific producers of sesquiterpenoids. Many of these are readily detected and isolated in different screenings because of their unusually high antibiotic and cytotoxic activities. These are often due to a high chemical reactivity which, however, makes these compounds less desirable as possible lead structures. Reviews covering all sesquiterpenes are regularly published by Fraga (2009, 2010, 2011, 2012, 2013).

In the last edition, sesquiterpenoids of *Resupinatus leightonii* (current name: *Hohenbuehelia leightonii*, Pleurotaceae), *Ripartites tricholoma* and *R. metrodii* (Tricholomataceae), *Radulomyces confluens* (Pterulaceae), *Gloeophyllum* sp. (Gloephyllaceae), *Bovista* sp. (Lycoperdaceae), *Macrocystidia cucumis* (Polyporaceae), *Creolophus cirrhatus* (Hericiaceae), *Hericium* sp. (Hericiaceae), *Dacrymyces* sp. (Dacrymycetaceae), and *Limacella illinita* (Amanitaceae) were described. In the current literature, no new secondary metabolites or recent scientific research on the metabolites included in the last edition were published.

a) *Pholiota* spp. (Strophariaceae)
From the culture broth of *Pholiota adiposa*, a spiroxane sesquiterpene named 15-hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene (Fig. 1) was isolated (Liu et al. 2008a). Unfortunately, no data on its bioactivity were presented. In 2011 five new cadinane sesquiterpenes were isolated from a *Pholiota* sp. named pholiotins A–E (Fig. 1) and tested against *Aspergillus flavus*, *Fusarium nivale* (current name: *Microdochium nivale*), and *Pyricularia oryzae*. Only pholiotin D was able to inhibit the growth of *A. flavus* with an IC_{50} value of 25.1 μM, whereas all others did not show any activity up to 200 μg/ml (Lin et al. 2016).

b) *Stereum* spp. (Stereaceae)
The genus *Stereum* is intensively investigated. Between 2008 and 2017, about 200 publications dealt with this genus. In the following section, certain sesquiterpenes of this genus are presented.

The antifungal sterelactones A–D (Fig. 1) were isolated from a *Stereum* sp. (IBWF 01060; Opatz et al. 2008). The tetracyclic sesquiterpenes

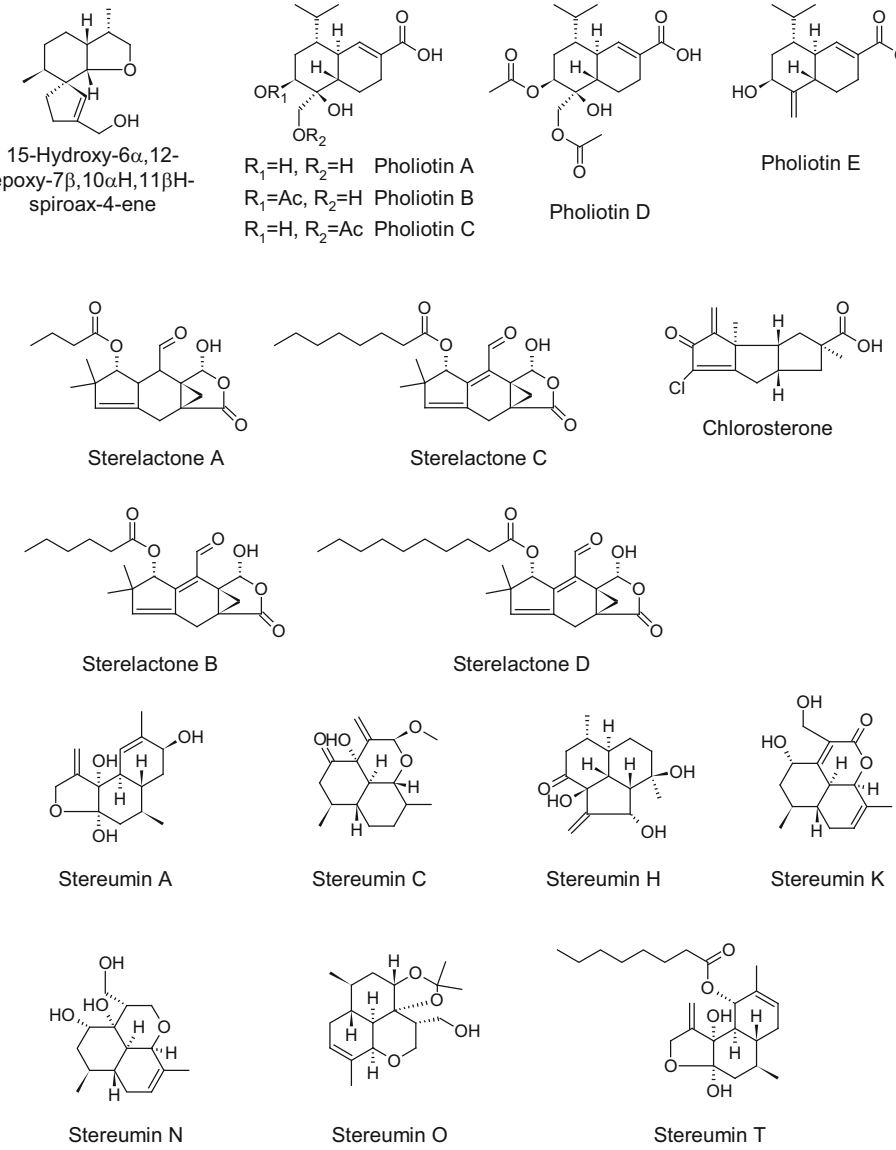


Fig. 1 Sesquiterpenes from *Pholiota* spp. and *Stereum* spp.

with an isolactarane skeleton showed antifungal and antibacterial activity. They inhibited conidia germination of *Magnaporthe oryzae* (current name: *P. oryzae*), *Fusarium graminearum* (current name: *Gibberella zeae*), and *Phytophthora infestans* with IC_{100} values between 1.0 and 5.0 μg/ml and exhibited cytotoxic effects on HeLaS3 (human adenocarcinoma) and HepG2 (human hepatocellular carcinoma) cells at higher concentrations. The authors argue that the dialdehyde structure and the unsaturated aldehyde moiety are responsible for the bioactivity (Opatz et al. 2008).

The first example of a chlorinated triquinane from a terrestrial fungus named chlorostereone (Fig. 1) was isolated from *Stereum* sp. (IBWF01082). This compound exhibited antifungal and antibacterial activity in agar diffusion assays against *Nematospora coryli* (current name: *Eremothecium coryli*), *Mucor miehei* (current name: *Rhizomucor miehei*), *Bacillus brevis*, and *B. subtilis* and showed cytotoxicity

against Jurkat cells (human T-cell leukemia). The exocyclic enone moiety easily forms adducts with thiol groups and seems to be responsible for the activity (Liermann et al. 2010).

The stereumins A–E (stereumins A, C: Fig. 1) are cadinane sesquiterpenoids, described from the culture broth of *Stereum* sp. (strain CCTCC AF 207024; Li et al. 2008). They showed toxicity toward the free-living nematode *Panagrellus redivivus* at high concentrations (Li et al. 2008). No data on the bioactivity of the stereumins F and G derived from the same strain were given (Liu et al. 2010). Novel compounds with a stereumane-type backbone named stereumins H–J (stereumin J: Fig. 1) were later isolated from the same strain (Li et al. 2011). No activity against *Panagrellus redivivus*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and different cell lines was observed (Li et al. 2011). The stereumins K to P (stereumins K, O: Fig. 1) were described from a different strain (*Stereum* sp. CCTCC AF 2012007). However, stereumin O was shown to be an artifact maybe due to the reaction of stereumin N with acetone. No bioactivities were given by Zheng et al. (2013). Later the stereumins Q to U were described from *Stereum* cf. *sanguinolentum* (BCC 22926). One of them (stereumin T: Fig. 1) was active against *B. cereus* and moderately cytotoxic, whereas all others were inactive (Bunyapaiboonsri et al. 2014).

The sterostreins A–C, three dimeric sesquiterpenes and sterostreins D and E, and two illudalanes (sterostreins A, B, D, E: Fig. 2) were isolated from *Stereum ostrea* (BCC22955; Isaka et al. 2012). Sterostrein A exhibits modest cytotoxic activities against cancer cell lines and activity against *Plasmodium falciparum* (malaria parasite) but was inactive against *Mycobacterium tuberculosis* (up to 50 μg/ml). Sterostreins D and E were inactive in the aforementioned assays, and sterostrein D was

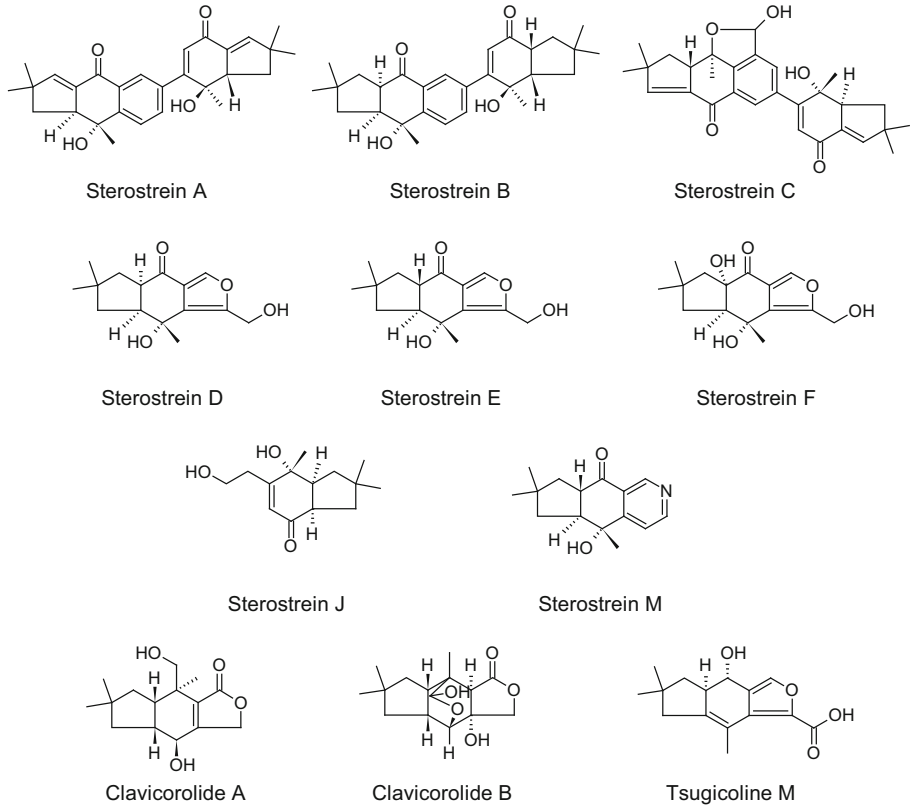


Fig. 2 Sesquiterpenes from *Stereum* spp. and *Clavicorona pyxidata*

slightly cytotoxic. Later on furan-illudalanes (sterostreins F–H), one illudane (sterostrein I), norilludalanes (sterostreins J–L), and three pyridine-containing tricyclic illudalanes (sterostreins M–O) were isolated from the same strain when cultured under different conditions (sterostreins F, J, M: Fig. 2; Isaka et al. 2012). One of the last additions to the sesquiterpene library described from *Stereum* is the sterostreins R, S, and T (related to sterostrein C) isolated from *Stereum* sp. (YMF1.1686; Tian et al. 2016). Unfortunately, no bioactivity data were given for the sterostreins F to T.

c) *Clavicorona pyxidata* (current name: *Artomyces pyxidatus*, Auriscalpiaceae)
Solid cultures of *Clavicorona pyxidata* (current name: *Artomyces pyxidatus*, an edible mushroom, used in traditional medicine in China) were extracted, and two sesquiterpenoids of the clavicoronane-type, clavicorolides A and B, and one new protoilludane-type sesquiterpenoid, named tsugicoline M, were isolated (clavicorolides A, B, tsugicoline M: Fig. 2). They did neither exhibit antimicrobial (*E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*) nor cytotoxic activities against HeLa cells (Zheng and Shen 2009).

d) *Pleurotus* spp. (Pleurotaceae)
In recent years, *Pleurotus* species were subject of several studies. In 2013 the pleurospiroketals A–E (pleurospiroketals A, B, C: Fig. 3) with an up to now not described benzannulated 5,5-spiroketal skeleton were isolated from *Pleurotus cornucopiae*. The compounds were tested for inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophages, and the pleurospiroketals A to C were active at 6.8, 12.6, and 20.8 μM (IC_{50}), respectively (Wang et al. 2013b).

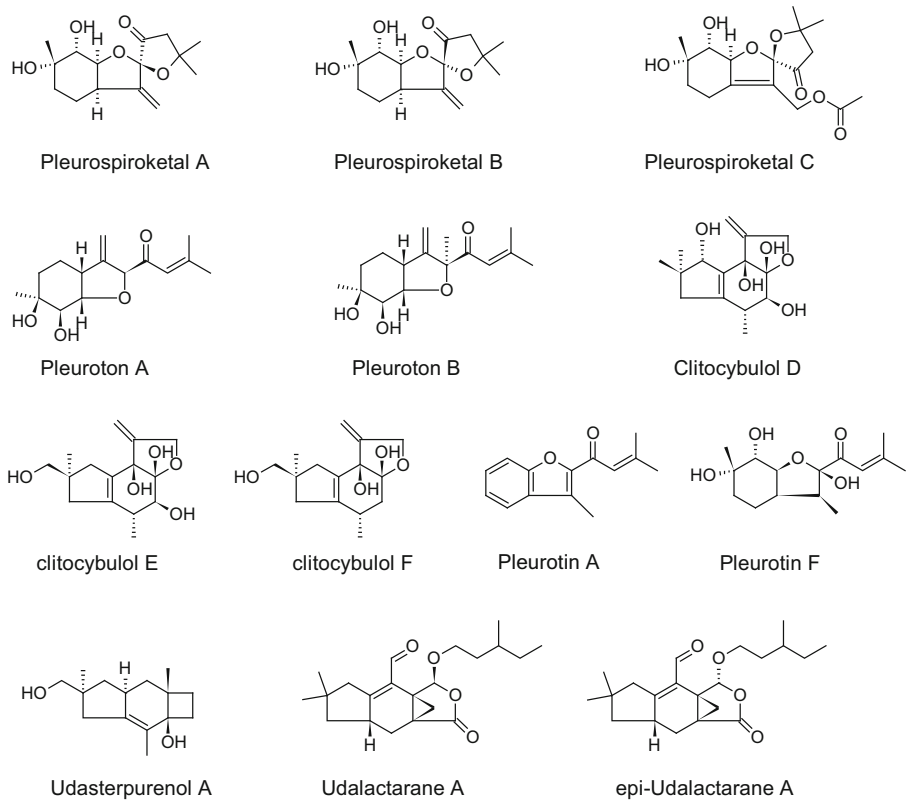


Fig. 3 Sesquiterpenes from *Pleurotus* spp. and *Phlebia uda*

From a different species, *Pleurotus cystidiosus*, pleurotons A and B (bisabolane-type sesquiterpenoids, Fig. 3), and clitocybulols D, E, and F (clitocybulol derivatives, Fig. 3) were isolated from mycelial cultures (Zheng et al. 2015). The clitocybulols A, B, and C had been isolated previously from *Clitocybula oculus* by Ayer et al. in 1998.

Cytotoxicity was assessed with human prostate cancer cell lines DU-145 and C42B, and IC_{50} values were very low for pleuroton B (DU-145, 28 nM; C42B, 52 nM), whereas the cytotoxic activities for all the other compounds were less pronounced. Zheng et al. (2014) filed a patent claiming that clitocybulols D, E, and F are strongly active against prostate cancer cells DU-145, C42B, and LNCaP. Further studies revealed that pleuroton B triggers apoptosis in DU-145 cells. The differences in cytotoxicity between pleuroton A and B are significant although the structural differences are marginal (one additional hydroxyl at C-8) and both have an α,β-unsaturated carbonyl moiety and an exomethylene (Zheng et al. 2015).

From a different strain (CBS 100129) of *Pleurotus cystidiosus*, nine new clitocybulol derivatives named clitocybulols G–O were described together with clitocybulols C–E (Tao et al. 2016b). All compounds were tested for inhibition of protein tyrosine phosphatase 1B (PTP1B) which is a target for type 2 diabetes mellitus and obesity. Clitocybulols G, L, and C exhibited modest activity. In addition, the inhibition of α-glucosidase, sucrase, and maltase was tested, but no activity was observed (Tao et al. 2016b).

By the same authors of pleurospiroketal F and pleurotins A–F (pleurotins A, F: Fig. 3), new metabolites from *Pleurotus citrinopileatus* were described and tested for inhibition of PTP1B (Tao et al. 2016c). Unfortunately, the first metabolite named pleurotin is a quinone isolated from different *Pleurotus* species (Robbins et al. 1947).

Pleurotin A and E inhibited PTP1B with IC_{50} values of 32.1 and 30.5 μM, respectively. Further studies revealed that pleurotin A is a noncompetitive inhibitor of PTP1B. All new compounds were claimed to be not cytotoxic. Interestingly pleuroton B was produced by this species too but was not evaluated against PTP1B (Tao et al. 2016c).

e) *Phlebia uda* (current name: *Mycoacia uda*, Meruliaceae)

Udasterpurenol A (Fig. 3) and udalactaranes A (Fig. 3) and B were isolated from *Phlebia uda* (IBWF07065; current name: *Mycoacia uda*, Schüffler et al. 2012). They are the first natural products described from this species. The udalactaranes, isolated as mixtures with the corresponding epimers, inhibited spore germination of *F. graminearum* (current name: *Gibberella zeae*) and *Ph. infestans*. Only udalactarane A was able to inhibit germination of *M. oryzae* (current name: *P. oryzae*) spores at 10 μg/ml ($IC_{75–100}$). Udasterpurenol A was inactive in these assays, and all compounds had no cytotoxic effects on HepG2 cells.

f) *Flammulina velutipes* (Physalacriaceae)

The focus of a study on *Flammulina velutipes* cultured on solid rice by Wang et al. (2012b) was to analyze what kind of bioactive compounds is produced in "functional food." Six cuparene-type sesquiterpenes, named enokipodins E–J (enokipodin F: Fig. 4); two sterpurane-type sesquiterpenes, sterpurol A and B; and the known sesquiterpenes 2,5-cuparadiene-1,4-dione, enokipodins B and D, as well as sterpuric acid, were isolated. The compounds were tested for cytotoxic, antibiotic (*E. coli*, *C. albicans*, *B. subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *A. fumigatus*), and antioxidant activity in a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay. Enokipodin J, 2,5-cuparadiene-1,4-dione, and enokipodins B and D were moderately cytotoxic and showed antioxidant activity. Enokipodin F and the aforementioned secondary metabolites were able to weakly inhibit the growth of *B. subtilis*, and enokipodins F, G, and J weakly inhibited *A. fumigatus*. No inhibition was observed for the other microorganisms. The enokipodins A and B, both active against *Cladosporium herbarum*, *B. subtilis*, and *S. aureus* (Ishikawa et al. 2000, 2001), and the enokipodins C and D, active against *B. subtilis* and *S. aureus* (Ishikawa et al. 2001), had been previously isolated from fermentations in malt peptone broth of *Flammulina velutipes*. From a culture of *Flammulina velutipes* on solid rice,

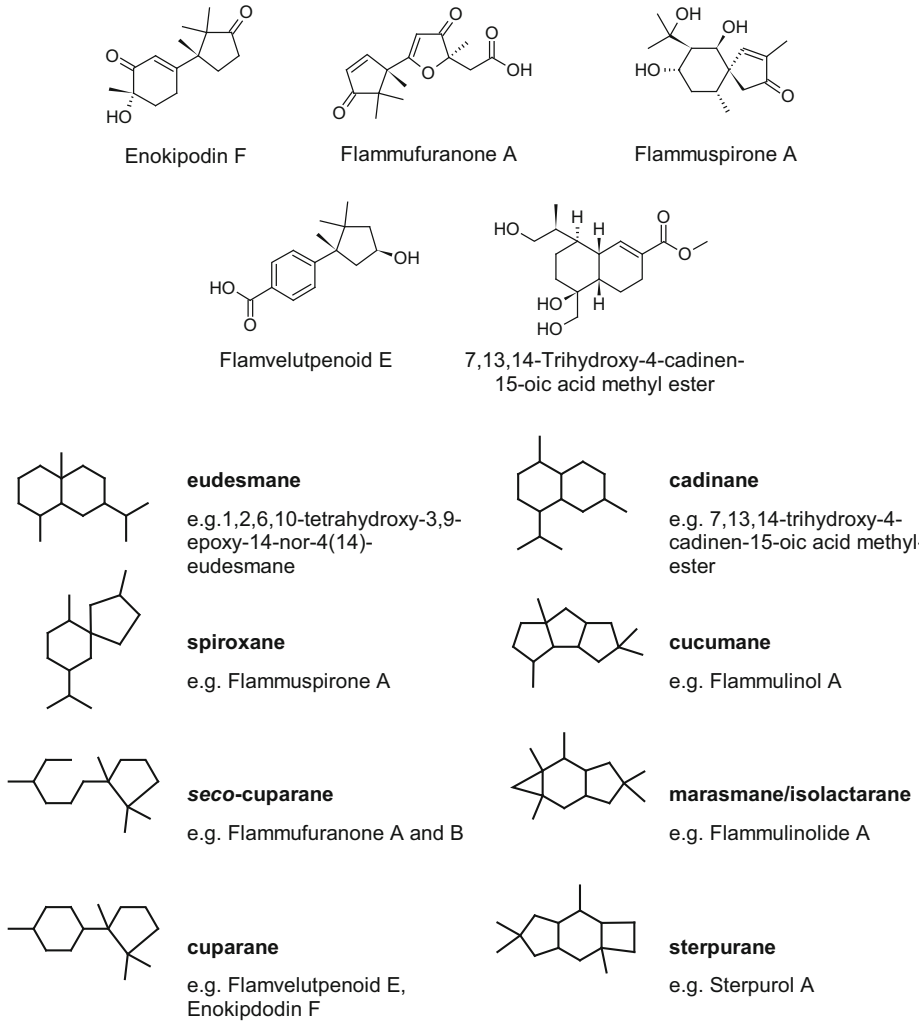


Fig. 4 Sesquiterpenes from *Flammulina velutipes* (single structures and overview of sesquiterpene diversity)

Tao et al. (2016a) isolated flammufuranones A (Fig. 4) and B (*seco*-cuparane sesquiterpenes) and 13 new sesquiterpenes (flammuspirones A–J ; flammuspirone A: Fig. 4; 7,13,14-trihydroxy-4-cadinen-15-oic acid methyl ester; Fig. 4; 1,2,6,10-tetrahydroxy-3,9-epoxy-14-nor-5(15)-eudesmane, flamvelutpenoid E ; Fig. 4; flamvelutpenoid F).

The compounds were tested for 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, dipeptidyl peptidase 4 (DPP-4), and aldose reductase inhibition. Inhibitors of these enzymes are thought to ameliorate hyperglycemic and hyperlipidemic diseases. The flammuspirones A and C, 7,13,14-trihydroxy-4-cadinen-15-oic acid methyl ester, and 1,2,6,10-tetrahydroxy-3,9-epoxy-14-nor-5(15)-eudesmane inhibited HMG-CoA reductase, and the flammuspirones C, D, E, and H, 7,13,14-trihydroxy-4-cadinen-15-oic acid methyl ester, and 1,2,6,10-tetrahydroxy-3,9-epoxy-14-nor-5(15)-eudesmane were able to inhibit DPP-4 at rather high concentrations. Aldose-reductase was not inhibited, and no cytotoxicity against various cell lines was observed.

A genome sequence analysis revealed that *Flammulina velutipes* possesses 12 putative sesquiterpene synthases which are responsible for the diversity if sesquiterpenes known from *Flammulina velutipes* (overview of known backbones, Fig. 4). A prediction was made on

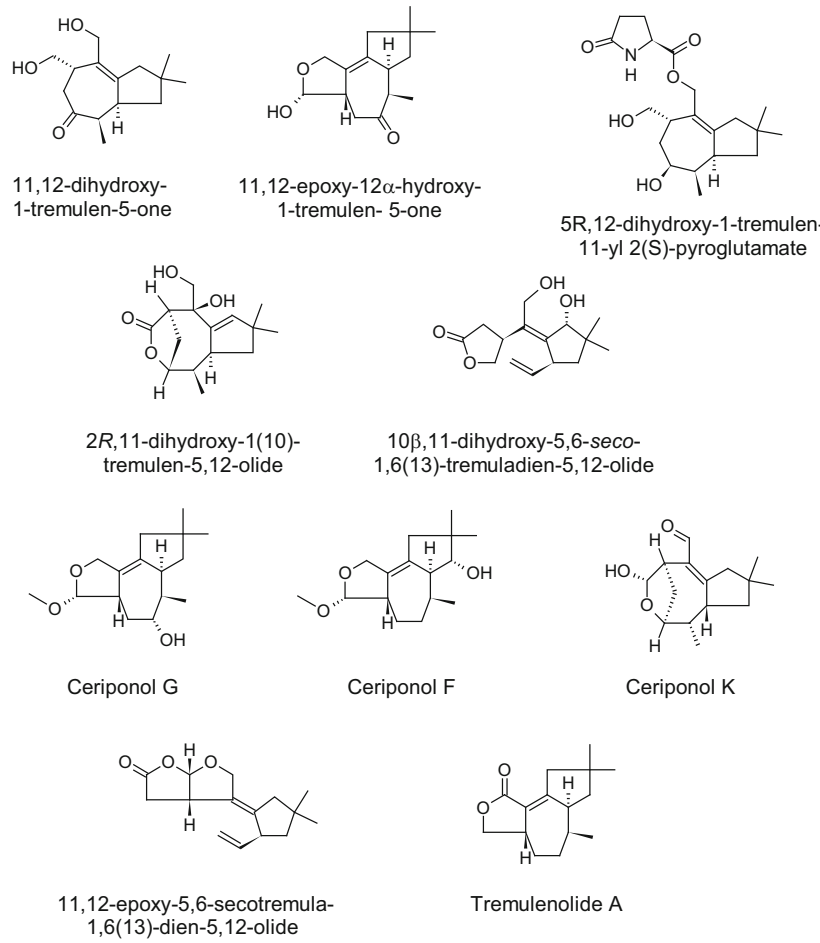


Fig. 5 Sesquiterpenes from *Conocybe siliginea*, *Ceriporia* spp., and *Flavodon flavus*

what biogenetic network is responsible for the biosynthesis of the observed sesquiterpenes (Tao et al. 2016a).

g) *Conocybe siliginea* (Bolbitiaceae)
Six tremulane sesquiterpenes, conocenols A–D and conocenolides A and B, have been isolated from cultures of a Chinese collection of *Conocybe siliginea* (Liu et al. 2007). From the same cultivar, five new tremulane sesquiterpenes were described from a scale-up culture in 2008 (Zhou et al. 2008) and named 11,12-dihydroxy-1-tremulen-5-one, 11,12-epoxy-12β-hydroxy-1-tremulen-5-one, 5α,12-dihydroxy-1-tremulen-11-yl 2(*S*)-pyroglutamate, 2α,11-dihydroxy-1(10)-tremulen-5,12-olide, and 10β,11-dihydroxy-5,6-*seco*-1,6(13)-tremuladien-5,12-olide (Fig. 5). In 2014 three new sesquiterpenoids 5α,11,12,14-tetrahydroxy-1-tremulene; 4α,11,12,14-tetrahydroxy-1-tremulene; and 5-*O*-acetyl-7,14-dihydroxy-protoilludanol were described again from the same isolate by Yang et al. (2014b) together with the known tremulenediol A, dankasterone, conocenol B, and tremulene-6,11,12-triol. The three new compounds were tested against a set of five human cancer cell lines, but none of them showed cytotoxic activity.

h) *Ceriporia* spp. (Phanerochaetaceae)
From an endophytic *Ceriporia lacerata*, the ceriponols A–K (ceriponols F, G, K: Fig. 5)

together with three known tremulane sesquiterpenoids; tremulenediol A, 11,12-dihydroxy-1-tremulen-5-one (Fig. 5); and conocenol B, previously described from *Conocybe siliginea* (Liu et al. 2007), were isolated (Ying et al. 2013). Ceriponols F, K, and G showed moderate cytotoxic activity against human cancer cell lines. *Ceriporia alachuana* produced two new tremulane-type sesquiterpenoids named tremulenolide D and muurolane-10β,15-diol, together with the known secondary metabolites tremulenediol A, 2β-hydroxy-α-candinol, epicubenol, and 3β-hydroxy-δ-candinol (Liu et al. 2013). So far, not much is known about the biological activities of the mentioned tremulane sesquiterpenes. The first tremulanes have been reported from the wood-rotting-*Phellinus tremulae* (Hymenochaetaceae) by Ayer and Cruz (1993).

i) *Flavodon flavus* (Meruliaceae)
In cultures of *Flavodon flavus* BCC17421, an isolate collected in Thailand, one new *seco*-tremulane named 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide (Fig. 5) was isolated together with the conocenolides A and B, tremulenediol A, tremulenolide A (Fig. 5), and two lanostane triterpenoids, trametenolic acid B, and pinicolic acid A (Isaka et al. 2016c).

Several bioassays were performed with all of the isolated compounds, and 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide and pinicolic acid A had modest cytotoxic activity. Except for tremulenolide A, all others had no antiplasmodial activity against *Pl. falciparum*. Only pinicolic acid A (IC_{50} 15 μg/ml) inhibited the propagation of herpes simplex virus type 1 (Isaka et al. 2016c).

From a different *Flavodon flavus* strain isolated from a mangrove in Thailand, tremulenolide A was isolated and tested against *S. aureus* and *Cryptococcus neoformans* and was found to possess very weak antibiotic activities (Klaiklay et al. 2012). Interestingly 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide was described in 2016 from a solid fermentation on rice of an endophytic *Colletotrichum capsici* (isolated from *Siegesbeckia pubescens*, Asteraceae; Wang et al. 2016a).

j) *Granulobasidium vellereum* (Cyphellaceae)
In recent years the saprotrophic wood-decomposing fungus *Granulobasidium vellereum* was subject of intensive research. All together around 40 illudoid sesquiterpene compounds were isolated from cultures. A series of four reports of new and known secondary metabolites of this species was published from one cultivar isolated from *Fraxinus excelsior*. Jülich et al. (2013) isolated three new sesquiterpenes, 2-hydroxycoprinolone, 8-deoxy-4α-hydroxytsugicoline, 8-deoxydihydrotsugicoline (Fig. 6), together with the radulones A (Fig. 6), and B, and coprinolone ketodiol.

All compounds were tested against fungi (*Penicillium canescens*, *F. oxysporum*, *Heterobasidion occidentale*, *Coniothyrium sporulosum* [current name: *Paraphaeosphaeria sporulosa*], *Coniophora puteana*, *Bjerkandera adusta*, *Phlebiopsis gigantea*) which share the same natural habitat with *Granulobasidium vellereum*. Only radulone A inhibited the growth of *Phl. gigantea*, *Co. puteana*, and *H. occidentale* (Jülich et al. 2013). The antimicrobial as well as cytotoxic activity of radulone A had been described earlier by Fabian et al. (1998).

Another 12 sesquiterpenoids were isolated from the same cultivar from *Fraxinus excelsior* (Nord et al. 2014b). Ten of them were new and named 2α-hydroxycoprinolone (Fig. 6), 3-hydroxycoprinolone, coprinolone diol B, granulodiene A, granulodiene B, granulone A (Fig. 6), 8-deoxy-4α-hydroxytsugicoline B, granulone B (all protoilludanes), demethylgranulone (Fig. 6), and cerapicolene (Fig. 6; cerapicane skeleton). Two metabolites were known compounds (radudiol, D6-coprinolone).

The new compounds were tested against a set of fungi (*Pe. canescens*, *F. oxysporum*, *H. occidentale*), but none showed antifungal activity. However, at 100 μM granulone A was able to promote the growth of lettuce seedlings by 34% compared to controls (Nord et al. 2014b).

Granuloindens A and B and dihydrogranuloinden (Fig. 6) together with radulactone (Fabian et al. 1998), pterosin M (Hasegawa et al. 1974), echinolactones A (Suzuki et al. 2005) and D (Hasegawa et al. 1974) were isolated by Nord et al. (2014a). Granuloinden B was shown to possess cytotoxic activities,

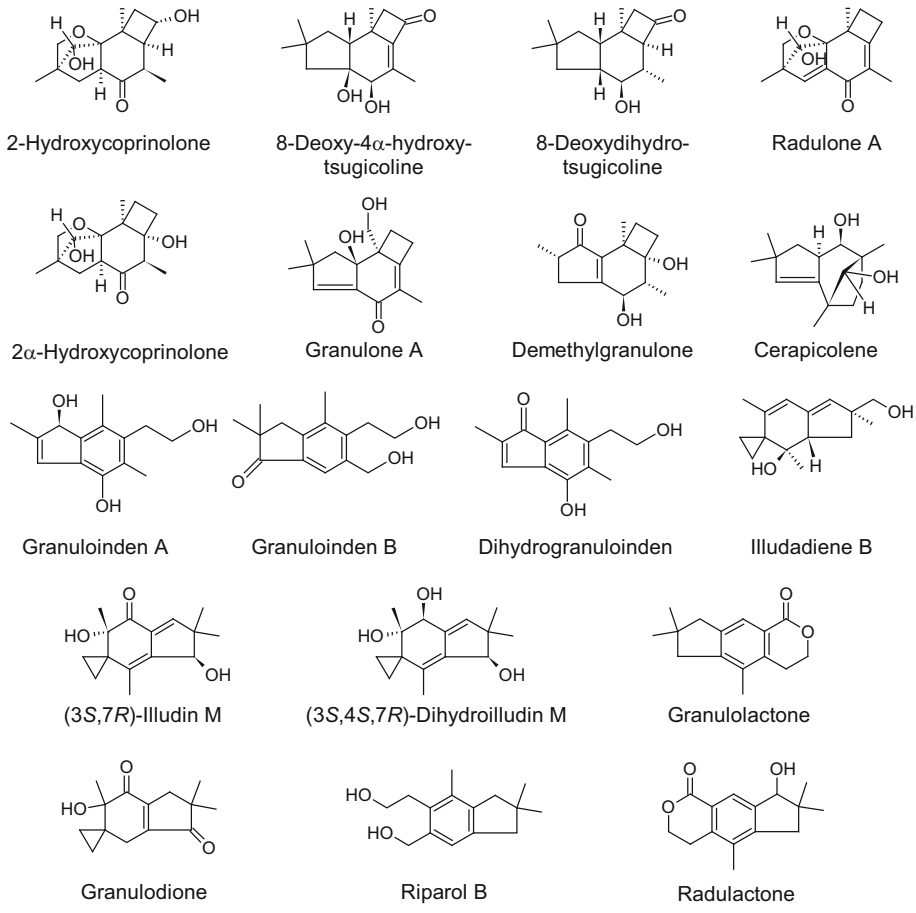


Fig. 6 Sesquiterpenes from *Granulobasidium vellereum*

while granuloinden A and dihydrogranuloinden had none or only moderate cytotoxic activity.

In 2015 the enantiomers of the known compounds illudin M ((3*S*,7*R*)-illudin M; Fig. 6) and dihydroilludin M ((3*S*,4*S*,7*R*)-dihydroilludin M; Fig. 6), the diastereomers of illudin M ((3*S*,7*S*)-illudin M) and illudin S ((3*S*,6*S*,7*R*)-illudin S), and two new illudanes, illudadienes A and B (Fig. 6), were isolated from the same strain of *Granulobasidium vellereum* (Nord et al. 2015).

They turned their attention to the cytotoxicity of these compounds since illudins M and S, originally isolated from *Omphalotus olearius* (syn. *Clitocybe illudens*; McMorris and Anchel 1965), are known for their high cytotoxicity. (3*S*,7*S*)-Illudin M and (3*S*,6*S*,7*R*)-illudin S showed CC_{50} values (concentration resulting in 50% cell viability compared to the controls) of 0.38 μM and 0.098 μM for hepatocyte-derived carcinoma cell line Huh7 and 0.38 μM and 0.023 μM for the human CD4+ lymphocyte-derived MT4 cell line, respectively. Unexpectedly (3*S*,7*R*)-illudin M, the diastereomer of (3*S*,7*S*)-illudin M, was less cytotoxic. (3*S*,4*S*,7*R*)-Dihydroilludin M and illudadiene B showed lower cytotoxic effects which might be due to the lack of a Michael acceptor (Nord et al. 2015). Illudadiene A was not tested.

A different cultivar of *Granulobasidium vellereum* isolated from a fruiting body on a fallen log of *Ulmus* sp. produced the illudane granulolactone (Fig. 6) and the 15-norilludane granulodione (Fig. 6), together with seven known sesquiterpenoids: radulactone (Fig. 6), echinolactone A, alcyopterosin N (Palermo et al. 2000), riparol B (Fig. 6), radulone B, coprinolone ketodiol, and illudadiene B (Fig. 6; Kokubun et al. 2016).

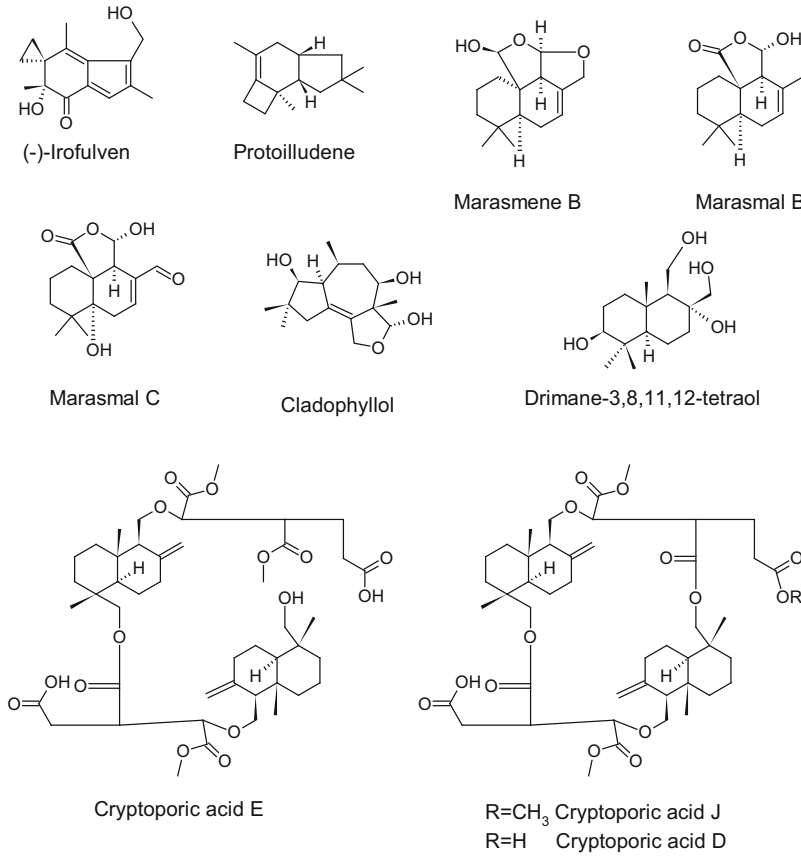


Fig. 7 Sesquiterpenes from *Omphalotus* spp. and *Marasmius* spp.

In vitro assays with *Tetranychus urticae* (two-spotted spider mite) and *Heliothrips haemorrhoidalis* (glasshouse thrips) were performed. Granulodione caused very high mortality to *Tetranychus urticae* 2 h after exposure. Not all compounds were tested against *Heliothrips haemorrhoidalis*, but riparol B (100% mortality after 72 h) and radulactone (83% mortality after 72 h) showed significant activity (Kokubun et al. 2016).

k) *Omphalotus olearius* (current name: *Omphalotus illudens*, Omphalotaceae)

Omphalotus olearius (current name: *O. illudens*, syn. *Clitocybe illudens*) is a rich source of sesquiterpenoids with carbon skeletons named after the original producer (e.g., illudane, protoilludane, illudalane). A synthetic analogue of illudin S, (−)-irofulven (Fig. 7; McMorris et al. 2004), has entered phase II clinical trials and demonstrated activity in ovarian, gastrointestinal, and non-small cell lung cancer. As compared to the natural product, irofulven has a much better therapeutic index and pharmacological profile (Paci et al. 2006). Its development reached phase III clinical trials with Eisai Co., Ltd. but was stopped due to lack of efficacy in 2012 (Williams 2013).

In 2012 the draft genome of *O. olearius* VT-653.13 was published (Wawrzyn et al. 2012b). The so-called sesquiterpenome was evaluated by analyzing its genome, and 11 putative sesquiterpene synthases (STS) named Omp1–10 (with 5a and 5b, both located on the same scaffold) were identified and characterized individually by heterologous expression in *E. coli*.

Omp6, which was crystallized in 2013 (Quin et al. 2013b), and Omp7 are D-6 protoilludene synthases. Except for Omp2, which was not functional, Omp1, Omp3, Omp4, Omp5a, Omp5b, Omp8, and Omp9 are responsible for

the diversification of sesquiterpene products in *O. olearius*.

Meanwhile it was possible by combinatorial engineering to use *E. coli* as a cell factory for the production of protoilludene (Fig. 7; Yang et al. 2016). By optimizing the mevalonate pathway to get a balanced production of farnesyl diphosphate and the utilization of Omp7, a production of 1199 mg/l of protoilludene was possible (initial titer 1.14 mg/l).

l) *Marasmius* spp. (Marasmiaceae)

Three new drimane skeleton sesquiterpenoids, named marasmene B, marasmal B, and marasmal C (Fig. 7), were isolated from *Marasmius* sp. (IBWF 96046; Liermann et al. 2012) together with *epi*-marasmal C. They were tested for antifungal activity against the germination of *M. oryzae* (current name: *P. oryzae*), *F. graminearum* (current name: *Gibberella zeae*), *Ph. infestans*, and *Botrytis cinerea*. Marasmene B was active at 5 μg/ml (IC_{100}) and marasmal B at 50 μg/ml (IC_{100}) against *M. oryzae*, *F. graminearum*, and *Ph. infestans*, while marasmal C-*epi*-marasmal C-mixture was only active against *M. oryzae* at 100 μg/ml. None of the compounds was able to inhibit *Bo. cinerea*.

The tremulane cladophyllol (Fig. 7), drimane-3,8,11,12-tetraol (Fig. 7), and two dimeric drimanes named cryptoporic acid J (Fig. 7) and cryptoporic acid K were isolated from solid-state fermentations of *Marasmius cladophyllus* (Meng et al. 2011). In addition, the known dimeric drimane sesquiterpenes cryptoporic acid D (Fig. 7), cryptoporic acid E (Fig. 7), and 15-hydroxyl cryptoporic acid H were found. The new compounds were tested against *E. coli*, *B. subtilis*, and *Mycobacterium smegmatis* in an agar diffusion assays, but only cryptoporic acid J showed very weak activity against *B. subtilis*.

Interestingly the dimeric drimane sesquiterpene derivatives of cryptoporic acid are well known. The first derivatives, cryptoporic acids A and B and later on C–G, were isolated from fruiting bodies of *Cryptoporus volvatus* (Polyporaceae) in 1987–1992, respectively (e.g., Hashimoto et al. 1987, 1989; Asakawa et al. 1992). They are described as the bitter principles of this fungus. Several derivatives were described from *Cryptoporus volvatus* cultures (cryptoporic acids H/I: Hirotani et al. 1991; Wu et al. 2011a; Wang et al. 2016b; Zhou et al. 2016) as well as from miscellaneous other fungi like *Ganoderma neojaponicum* (Ganodermataceae; Hirotani et al. 1991), *Polyporus arcularius* (current name: *Lentinus arcularius*, Polyporaceae) and *Polyporus ciliatus* (current name: *Lentinus substrictus*, Polyporaceae; Cabrera et al. 2002), *Cryptoporus sinensis* (Polyporaceae; Wu et al. 2011a, b), *Fomitella fraxinea* (current name: *Perenniporia fraxinea*, Polyporaceae; Yoshikawa et al. 2013), as well as *Poria albocincta* (current name: *Porogramme albocincta*, Polyporaceae; Isaka et al. 2014a). They possess various biological activities, e.g., inhibition of the release of superoxide anions (Hashimoto et al. 1989; Asakawa and Hashimoto 1998), antitumor promotion activities in colon cancer (tested in mice; Asakawa and Hashimoto 1998; Narisawa et al. 1992) and skin cancer (tested in mice; Matsunaga et al. 1991), as well as inhibition of influenza virus replication in vitro (Gao et al. 2017).

m) *Marasmiellus* spp. (Omphalotaceae)

Three unusual tricyclic cis-caryophyllane sesquiterpenes named 2*S*,3*R*-dihydroxy-carophyllan-[5,8]-6,7-olide; 2*S*-hydroxy-3-oxo-carophyllan-[5,8]-6,7-olide; and 2*S*,3*R*,7*S*-trihydroxy-carophyllan-[4,7]-6,8-oxide (Fig. 8) were isolated from *Marasmiellus troyanus* (Evans et al. 2010). Unfortunately no data are available on their bioactivities.

Two new eudesmane-type sesquiterpenoids 14(10→1)abeo-eudesmane-13-hydroxyl-11-ene and 14(10→1)abeo-eudesmane-11,13-diol (Fig. 8) were isolated from cultures of *Marasmiellus ramealis* found in China (Yang et al. 2015). The inhibition of acetylcholinesterase (AChE) was determined for the two new compounds. At a concentration of 100 μM, both showed moderate inhibitory activity of 29% and 41%, respectively. Surprisingly, other compounds typically found in ascomycete species like cytochalasins C and D, 13,14-epoxycytochalasin D, stachyline C, as well as mellein derivatives were also isolated from this *Marasmiellus ramealis* (Yang et al. 2015).

Two hirsutane sesquiterpenes, the marasmiellins A and B (Fig. 8), were isolated from *Marasmiellus* sp. BCC 22389 from Thailand (Isaka et al. 2016b). Both compounds were tested in bioassays against several cell lines, *Mycobacterium tuberculosis*, and *Pl. falciparum* but found inactive.

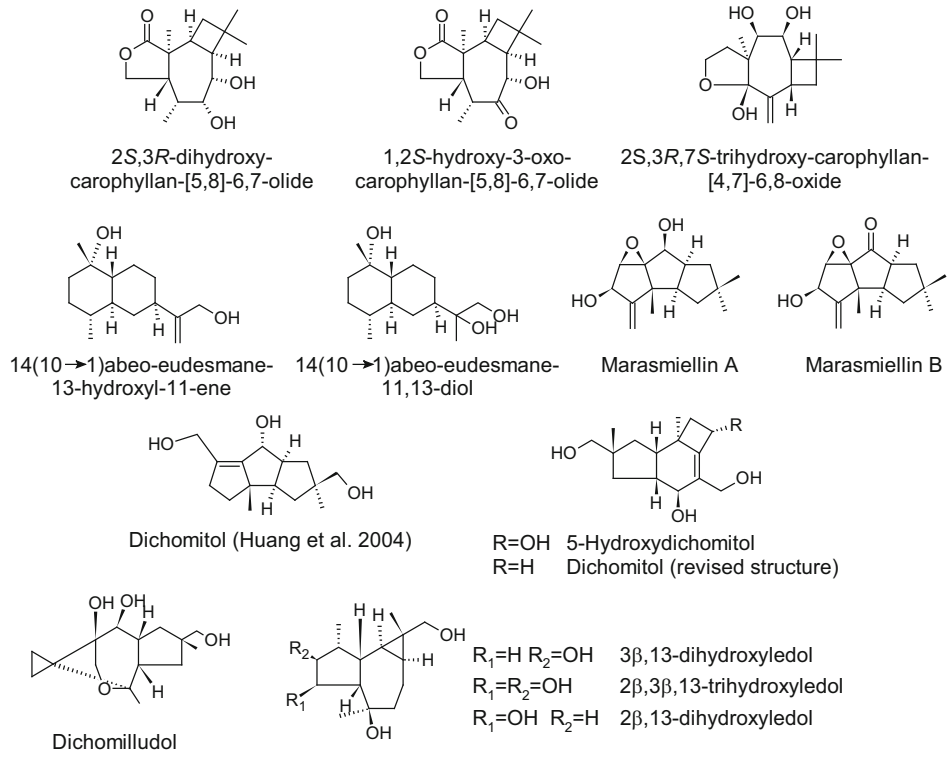


Fig. 8 Sesquiterpenes from *Marasmiellus* spp. and *Dichomitus squalens*

n) *Dichomitus squalens* (Polyporaceae)
From mycelial cultures of a Chinese collection of *Dichomitus squalens*, the three new sesquiterpenes dichomitol (Fig. 8; dichomitin A), 2β,13-dihydroxyledol (dichomitin B), and dichomitone (dichomitin C) were described by Huang et al. (2004). The structure of dichomitol is interesting because C-15 is attached to C-6 instead of C-4 as in other hirsutane sesquiterpenes. Dichomitol is the first example of 1,10-*seco*-2,3-*seco*-aromandendrane sesquiterpenes. The nomenclature of the compounds is confusing because in the same paper the three compounds were given two different names each (above in brackets). Of the three compounds, 2β,13-dihydroxyledol exhibited nematicidal activity against *Bursaphelenchus xylophilus*, a *Pinus* pathogen with a LC_{50} of 35.6 μg/ml. In 2006 the total synthesis of dichomitol (revised structure, Fig. 8) was published (Mehta and Pallavi 2006), and the authors found significant spectral differences in the synthetic product and the natural product dichomitol published by Huang et al. (2004). The revised structure of dichomitol was published together with the new structures 5-hydroxydichomitol, dichomilludol, 3β,13-dihydroxyledol, and 2β,3β,13-trihydroxyledol, as well as the known 2β,13-dihydroxyledol (Fig. 8; Xie et al. 2011). The new compounds were evaluated for their cytotoxicity and activity against *Bursaphelenchus xylophilus* (pine wood nematode) but were found to be inactive (Xie et al. 2011).

o) *Coprinus* spp. (Agaricaceae) and *Coprinopsis* spp. (Psathyrellaceae)
The illudins C_2 and C_3 (Fig. 9) were isolated from *Coprinus atramentarius* (current name: *Coprinopsis atramentaria*) due to their antimicrobial activity against *S. aureus* (Lee et al. 1996). Recently they were found to modulate lipolysis in differentiated 3T3-L1 adipocytes

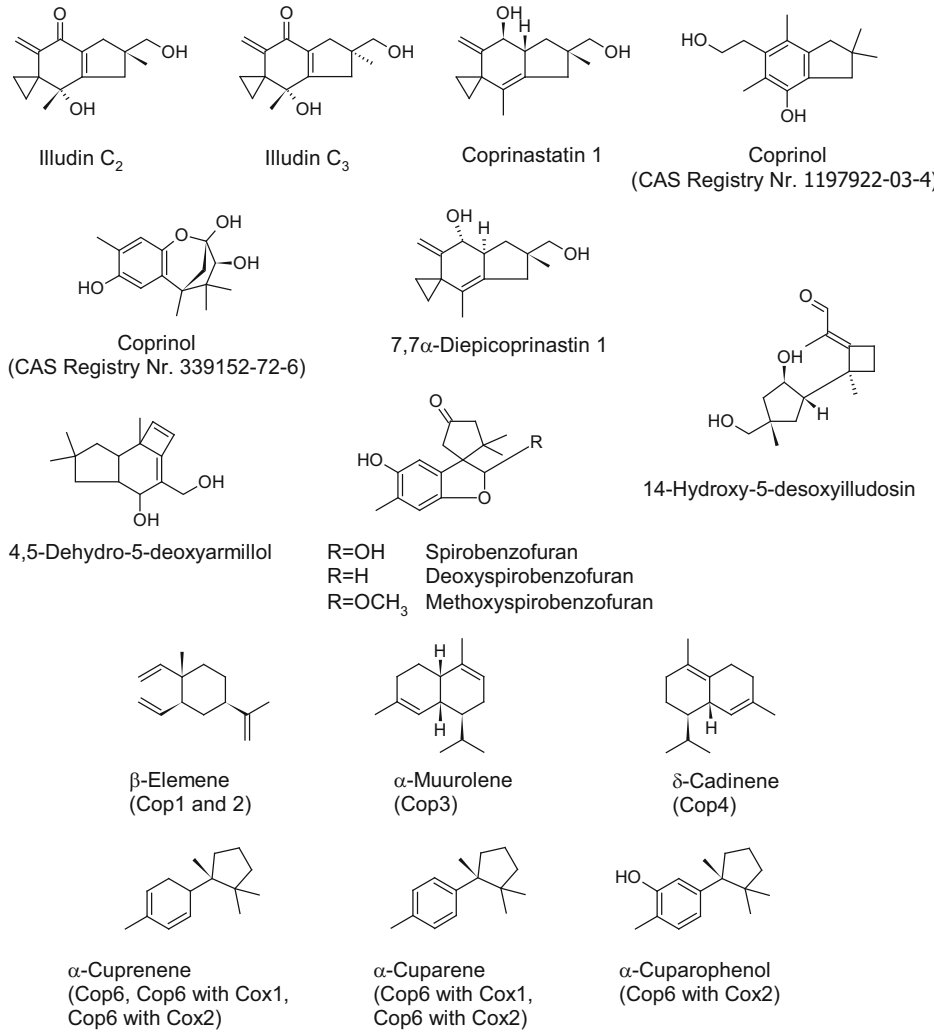


Fig. 9 Sesquiterpenes from *Coprinus* spp. and *Coprinopsis* spp.

(mouse cell line; Kim et al. 2014). Such modulators are of interest for the treatment of obesity.

Extracts of *Coprinus cinereus* (current name: *Coprinopsis cinerea*) inhibited the growth of the murine lymphocytic leukemia cell line P388, and by bioassay-guided separation, coprinastatin 1 (Fig. 9) and "coprinol" (Fig. 9; CAS Registry Number 1197922-03-4; this name was already used for a different cuparane sesquiterpene from *Coprinus* sp. described by Johansson et al. (2001) with CAS Registry Number 339152-72-6; Fig. 9) were isolated. Only coprinastatin 1 was able to inhibit the growth of P388 lymphocytic leukemia mouse cell line with an ED_{50} (effective dose) of 5.3 μg/ml. It also inhibited the growth of the pathogen *Neisseria gonorrhoeae* (Pettit et al. 2010b). Just recently this "coprinol" (CAS Registry Number 1197922-03-4) was synthesized (Suresh et al. 2016).

From the same cultivar above, three novel sesquiterpenes named 7,7a-diepicoprinastatin 1, 14-hydroxy-5-desoxy-2*S*,3*S*,9*R*-illudosin, and 4,5-dehydro-5-deoxyarmillol (Fig. 9) and the known armillol were isolated (Pettit et al. 2010a). 14-Hydroxy-5-desoxy-2*S*,3*S*,9*R*-illudosin was cytotoxic (P388 cells), and all other compounds were inactive. Except for 4,5-dehy-

dro-5-deoxyarmillol, all compounds were tested against a panel of bacteria and yeasts but were shown inactive (Pettit et al. 2010a).

The culture broth of *Coprinus echinosporus* (current name: *Coprinopsis echinospora*) showed antioxidative activity, and the known spirobenzofuran together with the new deoxyspirobenzofuran and methoxyspirobenzofuran (Fig. 9) were isolated. For spirobenzofuran and methoxyspirobenzofuran, a potent antioxidant activity could be determined (Ki et al. 2015).

Coprinopsis cinerea is one of the basidiomycetes which was sequenced quite early (in 2003; JGI 2016). Meanwhile *Coprinopsis marcescibilis* (former name: *Psathyrella macrescibilis*), *Coprinellus micaceus* (former name: *Coprinus micaceus*), *Coprinellus pellucidus* (former name: *Coprinus pellucidus*), and *Coprinopsis sclerotiger* genomes are also available (JGI 2016). In *Coprinus cinerea* 9/55 (current name: *Coprinopsis cinerea*), six sesquiterpene synthase homologues named Cop1 to Cop6 were identified and transformed into *E. coli* (Agger et al. 2009). Cop6 seemed to be part of a biosynthetic gene cluster being flanked by two putative cytochrome P450 monooxygenases named Cox1 and Cox2. Only Cop5 was not functional, but all other sesquiterpene synthase homologues yielded specific terpenes. The main product of Cop1 and Cop2 was β-elemene (Fig. 9), of Cop3 α-muurolene (Fig. 9), and of Cop4 δ-cadinene (Fig. 9). Cop6, a trichodiene synthase homologue, produced α-cuprenene (α-cuprenene synthase, Fig. 9). Coexpression of Cop6 with Cox1 and/or Cox2 in *Saccharomyces cerevisiae* gave several products; Cop6 with Cox1 α-cuprenene and three additional compounds (α-cuparene, Fig. 9; and two unknown were detected) Cop6 with Cox2 yielded α-cuprenene, α-cuparene together with α-cuparophenol (Fig. 9), and one unknown compound; when Cop6 with Cox1 and Cox2 was coexpressed, all aforementioned compounds were detected together with one unknown α-cuprenene derivative (Agger et al. 2009).

As for *O. olearius*, the availability of whole genomes makes it easier to identify natural products by heterologous expression and to characterize single enzymes for their substrate specificity, kinetic properties, as well as reaction conditions (e.g., Lopez-Gallego et al. 2010) and last but not least opens up possibilities of engineering biosynthetic pathways to obtain valuable natural products.

p) *Boletus calopus* (current name: *Caloboletus calopus*) and *Boletus edulis* (Boletaceae)

From fresh fruiting bodies of *Boletus calopus* (current name: *Caloboletus calopus*), cyclopinol

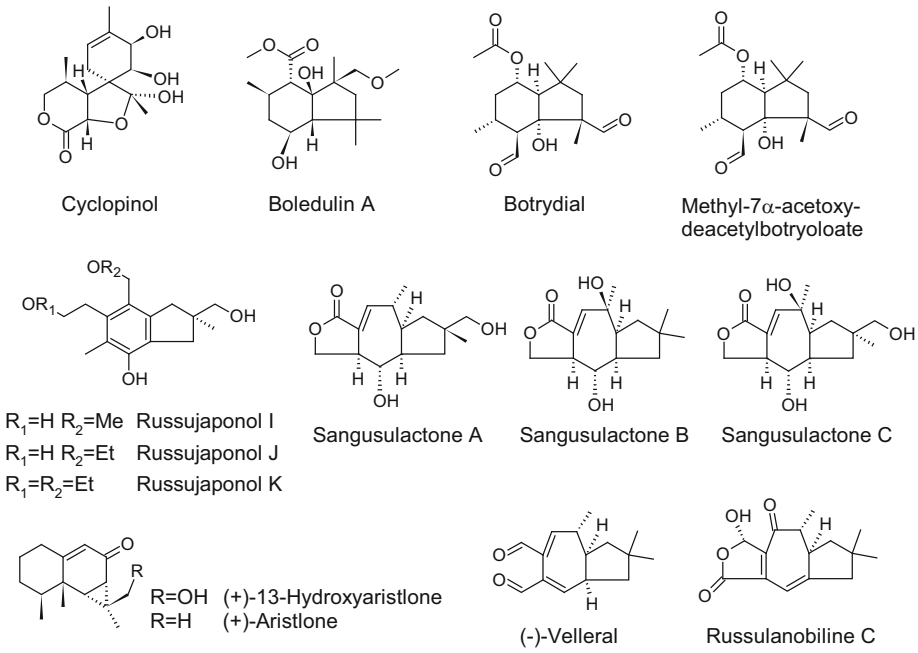


Fig. 10. Sesquiterpenes from *Boletus* spp. and *Russula* spp.

(Fig. 10) together with two related known compounds, cyclocalopin A and *O*-Acetylcyclocalopin A, identified before as the bitter principles of this species, was isolated (Hellwig et al. 2002). No further characteristics of this new compound were given (Liu et al. 2008b).

Three new botryane sesquiterpenoids, boledulins A–C (boledulin A: Fig. 10), were isolated from a culture of *Boletus edulis* collected in China (Feng et al. 2011). Only boledulin A showed cytotoxic activity against a panel of human cell lines, whereas the other two boledulins were not active. Closely related structures like botrydial (Fig. 10) or 12-hydroxydehydrobotrydienol are produced by *Bo. cinerea* by a characterized sesquiterpene synthase which was published in 2008 (Pinedo et al. 2008). Six related structures were found in the ascomycete *Daldinia concentrica*; one of them is methyl-7α-acetoxydeacetylbotryoloate (Fig. 10; Qin et al. 2008).

q) *Russula* spp. (Russulaceae)

New sesquiterpenoids were isolated in recent years from several *Russula* species. From *Russula japonica*, the illudoid sesquiterpenes russujaponols G–L (russujaponols I, J, K: Fig. 10) were isolated (Yoshikawa et al. 2009), which expands the diversity of the sesquiterpenoids isolated from this cultivar. In 2006 the russujaponols A–F were isolated from fruiting bodies (Yoshikawa et al. 2006), and their neurite outgrowth-promoting activity was published. The new russujaponols I, J, and K can promote neurite outgrowth in cultured rat cortical neurons in an neurotrophic bioassay (Yoshikawa et al. 2009).

Sangusulactones A–C (Fig. 10) and the known blennin A and 15-hydroxyblennin A were isolated from fruiting bodies of *Russula sanguinea*. Unfortunately no bioactivity data were given (Yaoita et al. 2012).

Russula lepida (current name: *Russula rosea*) was studied by Lee et al. (2016) in a search for protein tyrosine phosphate 1B (PTP1B) inhibitors. Such inhibitors could be used as medication for type 2 diabetes and obesity. In addition to two triterpenes which were active in the PTP1B, assay (+)-1,2-didehydro-9-hydroxy-aristlone and (+)-13-hydroxy-aristlone (Fig. 10) together with the known (+)-aristlone (Fig. 10) were isolated. Both new compounds were not able to inhibit PTP1B and showed no cytotoxicity against two human cancer cell lines (Lee et al. 2016).

Fruiting bodies of *Russula nobilis* were the subject of a comprehensive study by Malagòn et al. (2014). They extracted intact and damaged fruiting bodies and compared their secondary metabolite profiles. With other *Russula* species, these experiments were done by Clericuzio and Sterner (1997) and Stadler and Sterner (1998). In intact fruiting bodies, Malagòn et al. (2014) found velutinal stearate, (+)-velutinal, and a mixture of fatty acid ethyl esters. Then they ground fruiting bodies, left them at the air, and analyzed them at different time points after the damage. They observed a change in metabolite patterns in the first 30 min. The sesquiterpene dialdehyde (−)-velleral (Fig. 10, cause of the pungency of *Russula* species) was only observed in the first minute after damage together with velutinal stearate which was detectable until 20 min. Then more polar compounds were detected. Beside seven known compounds, three new sesquiterpenes, named russulanobilines A–C (russulanobiline C: Fig. 10), were isolated and characterized. It is indeed interesting that an enzymatic ad hoc reaction is able to convert the tasteless fatty acid ester velutinal stearate to the bitter (−)-velleral as a chemical defense system. In addition the new compounds were tested against the human lung cancer cell line H460 and showed modest cytotoxicity (Malagòn et al. 2014).

2. Diterpenoids and Sesterpenes

Diterpenoids and sesterpenes are less frequently encountered among the basidiomycete metabolites. However, some of them have interesting ring systems so far not found elsewhere. Semisynthetic pleuromutilins have even become antibiotics used in veterinary (valnemulin and tiamulin; Stipkovits et al. 2005; Long et al. 2006) and human medicine (retapamulin; (Jones et al. 2006).

The diterpenoids of *Mycena tintinnabulum* (Tricholomataceae) were described in the previous edition.

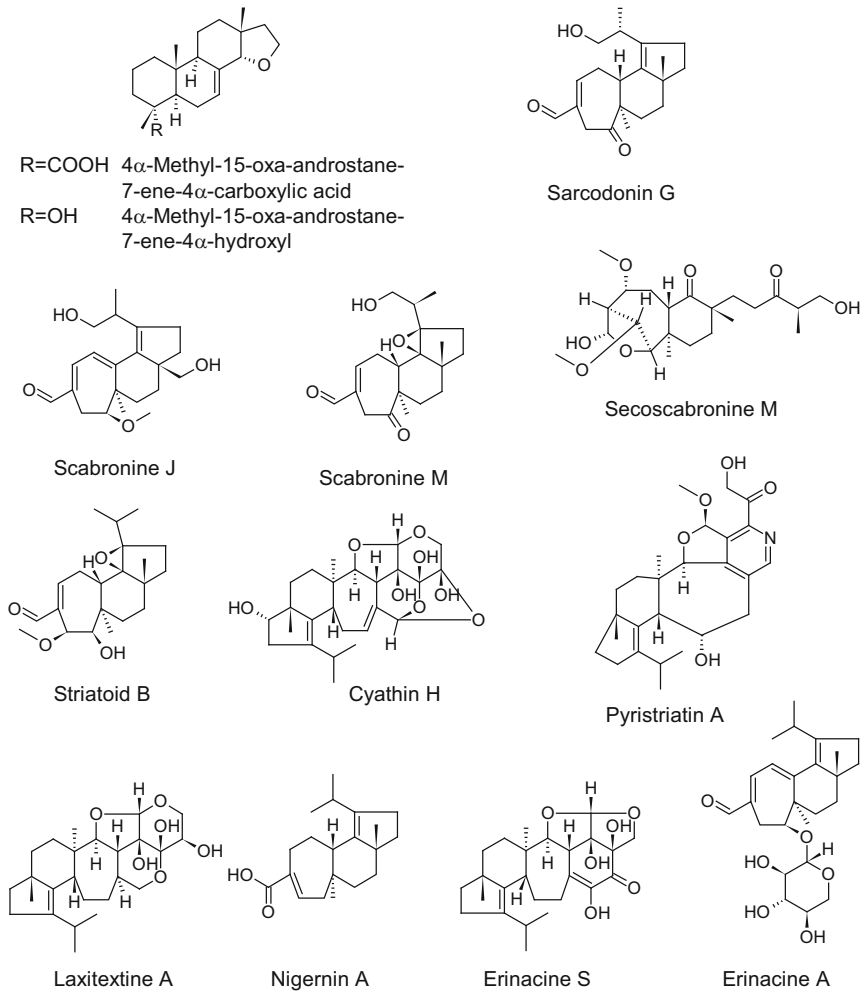


Fig. 11 Diterpenoids and sesterpenes

a) *Marasmiellus ramealis* (Omphalotaceae)
One new 4β-methyl-15-oxa-14β-androstane-7-ene-4α-carboxylic acid (Fig. 11) and one known androstane derivative (4β-methyl-15-oxa-14β-androstane-7-ene-4α-hydroxyl; Fig. 11) belonging to the type of isopimaric diterpenes were isolated from a culture of *Marasmiellus ramealis* (Yang et al. 2014a).

Both compounds were tested for AChE inhibition, and 4β-methyl-15-oxa-14β-androstane-7-ene-4α-carboxylic acid weakly inhibited AChE, but 4β-methyl-15-oxa-14β-androstane-7-ene-4α-hydroxyl showed no activity (Yang et al. 2014a). The only difference between both compounds is the carboxyl group at C-4, which seems to play a crucial role for this activity.

b) Cyathane Diterpenoids from *Sarcodon scabrosus* (Bankeraceae) and Other Fungi
The cyathins and striatins are prominent products of *Cyathus* spp. Some of them have very high cytotoxic activities. Interesting additions to this class of compounds are the sarcodonins (Shibata et al. 1998) and the scabronines (Kita et al. 1998; Ohta et al. 1998; Ma et al. 2004) from *Sarcodon scabrosus*. The sarcodonins have a bitter taste and anti-inflammatory and antibacterial activities (Shibata et al. 1998; Hirota et al. 2002; Kamo et al. 2004). Sarcodonin G (Fig. 11), showing a proliferation inhibition of HeLa cells, was able to induce apoptosis in a dose-dependent manner as well as caspase3

and caspase9 activation (Dong et al. 2009). Interesting pharmacological activities have been reported for scabronines.

Scabronines A and G have been shown to promote the secretion of neurotrophic factors, including nerve growth factor from 1321N1 (human astrocytoma) cells, causing the enhancement of differentiation (neurite outgrowth) of PC-12 (pheochromocytoma of rat adrenal medulla) cells (Obara et al. 1999). Obara et al. (2001) suggested that scabronine G and its methyl ester would enhance the secretion of neurotrophic factors from human brain astrocytoma cell line 1321N1 by activation of protein kinase C-ζ. Recently, the cyathane diterpenoids are subject of intensive research. One new scabronine J (Fig. 11) was isolated from fruiting bodies of *Sarcodon scabrosus* (Ma and Ruan 2008). The scabronines K and L were isolated together with the known sarcodonins G, A, and M and scabronine H. All were evaluated for nerve growth factor-mediated neurite outgrowth with rat pheochromocytoma cell line PC12. Only sarcodonins A and G were able to promote neurite outgrowth at 25 μM (Shi et al. 2011b). Scabronine M (Fig. 11) isolated later is also able to inhibit nerve growth factor-mediated neurite outgrowth in PC12; starting at concentrations of 1 μM, it slightly induced neurite outgrowth. It was shown that scabronine M leads to a decrease of the phosphorylation of ERK1/2 (extracellular signal-regulated kinases) and Trk A (tropomyosin receptor kinase A) in PC12 cells, and it was postulated that the epoxy moiety at C-3 and C-4 is responsible for the observed activity (Liu et al. 2012). A 3,4-*seco*-cyathane diterpenoid derivative named secoscabronine M (Fig. 11) was tested in the same assay but was not able to promote neurite outgrowth. The intact tricycle motif seems therefore necessary for this activity (Shi et al. 2012).

From *Cyathus striatus* (Agaricaceae) cultures, striatoids A–F (striatoid B: Fig. 11) and cyathane diterpenoids with a 15,4′-ether ring system were isolated and tested for nerve growth factor-mediated neurite outgrowth in PC12. They all were able to increase neurite outgrowth at 10–40 μM (Bai et al. 2015).

Solid cultures of *Cyathus africanus* (Agaricaceae) are the source for new cyathane diterpenes named cyathins D–H (cyathin H: Fig. 11; Han et al. 2013b) and cyathins W, V, and T (Han et al. 2015). These compounds were tested for reduction of NO production from LPS-activated mouse macrophage RAW 264.7 cells. None of the compounds had cytotoxic effects, but cyathins F and H were strong inhibitors of NO production. In recent years, cyathins were isolated and characterized especially in two directions: inhibition of NO production and induction of apoptosis (cyathin I from *Cyathus hookeri*, Xu et al. 2013; cyathins J–P from *Cyathus gansuensis*, Wang et al. 2014a; cyathins Q and R from *Cyathus africanus*, He et al. 2016; Huang et al. 2015).

In 2016 pyridino-cyathane diterpenoids named pyristriatins A (Fig. 11) and B were isolated from *Cyathus* cf. *striatus* (Richter et al. 2016). Both compounds were able to inhibit the growth of gram-positive bacteria such as *S. aureus*, *Micrococcus luteus*, and *B. subtilis*, some fungi (*Mucor plumbeus*, *Rhodotorula glutinis*, *Schizosaccharomyces pombe*), and cancer cell lines.

Recently cyathanes were described from other fungi. The laxitextines A (Fig. 11) and B were isolated from *Laxitextum incrustatum* (Hericiaceae; Mudalungu et al. 2016). Antimicrobial activity was assayed and both compounds inhibited the growth of *B. subtilis*, *S. aureus*, and MRSA. In addition, the laxitextines are cytotoxic toward several cell lines. Fruiting bodies of *Phellodon niger* (Bankeraceae) contain several new cyathane-type diterpenoids named nigernins A (Fig. 11), B, and C to F. Unfortunately no data on their bioactivities are available (Fang et al. 2010, 2011).

Erinacine S (Fig. 11), a novel sesterpene, and the cyathane diterpene xyloside erinacine A (Fig. 11) were isolated from the mycelia from fermentations of *Hericium erinaceus* (Hericiaceae), the lion's mane mushroom (Chen et al. 2016a). This fungus is used in traditional Chinese medicine for various diseases.

The authors tested this compound in a mouse model of Alzheimer's disease. The mice were treated with erinacines S and A, and both compounds were able to reduce the plaque burden by 38 and 40%, respectively. The results suggest that these compounds may lead to increased Aβ degradation by elevating the insulin-degrading enzyme (Chen et al. 2016a). It needs to be verified if related cyathanes are active in these tests too. Since there are already several derivatives available, structure-activity studies might help to reveal the potential of this class of compounds.

Cyathane-type diterpenoids have therapeutic potential for the treatment of neurodegenerative affections like Alzheimer through

stimulation of nerve growth factor biosynthesis. In addition, they have anti-inflammatory properties as well as antimicrobial activities. For reviews on cyathane diterpenoids, please see Tang et al. (2015) and Ma et al. (2010), as well as Nakada (2014, for syntheses).

3. Triterpenoids

Basidiomycetes are prolific producers of triterpenoids, among them many with interesting biological activities. In recent years several reviews on triterpenes were published (e.g., Hill and Connolly 2015; Cazal et al. 2010; Ríos et al. 2012; Liu 2014; Chudzik et al. 2015).

The following genera or species, which were included in the last edition, are not included in this triterpenoid section: *Irpex* sp. (Steccherinaceae), *Favolaschia* spp. (Favolaschiaceae/today Mycenaceae is more likely), *Grifola frondosa* (Meripilaceae), *Leucopaxillus gentianeus* (Tricholomataceae), and *Clavariadelphus truncatus* (Clavariadelphaceae).

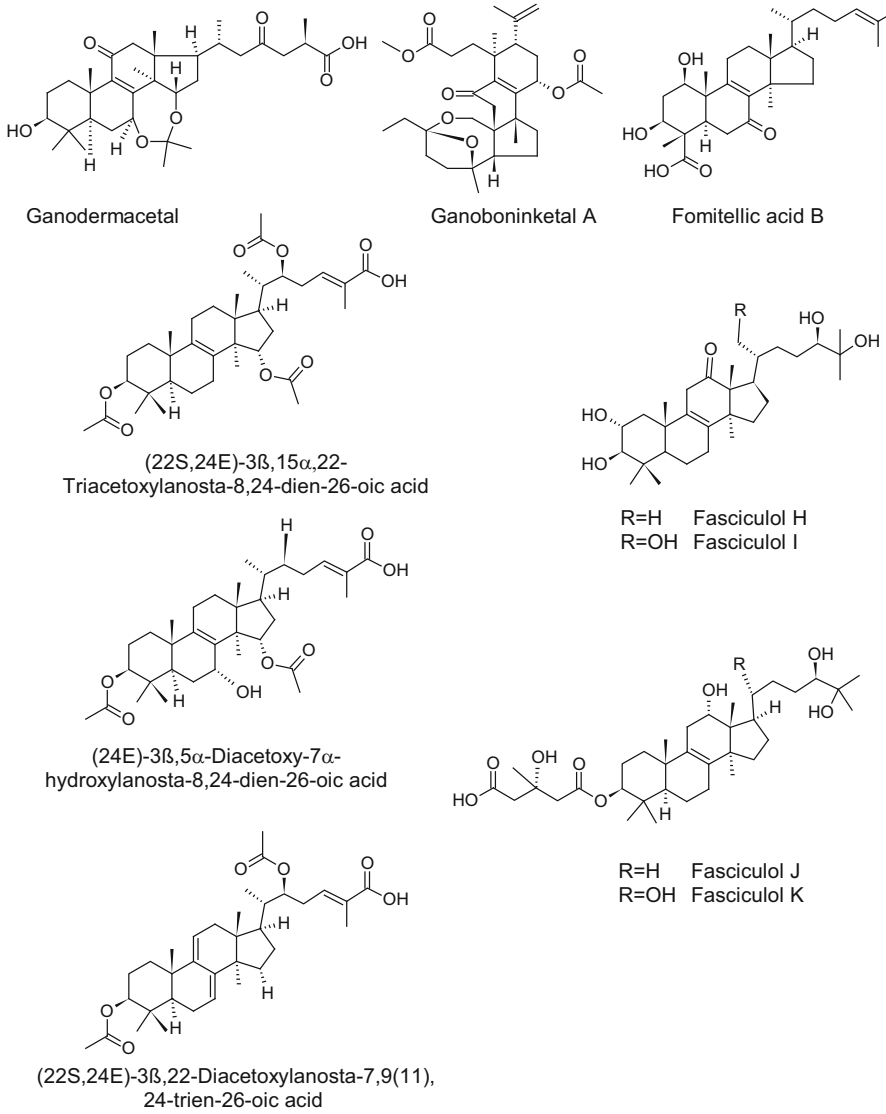


Fig. 12 Triterpenoids from *Ganoderma* spp., *Fomitella fraxinea*, and *Naematoloma fasciculare*

a) *Ganoderma* spp. (Ganodermataceae)
Ganoderma species are widely used in traditional medicine in Asia and are therefore preferentially studied. In recent years, several new triterpenes were described. Fruiting bodies of *Ganoderma amboinense* were investigated, and one new highly oxygenated lanostane triterpene named ganodermacetal (Fig. 12) was isolated together with 15 known compounds, like methyl ganoderate C, ganodermanontriol, and ganoderiol F (Yang et al. 2012). All of the aforementioned secondary metabolites were evaluated against brine shrimps (*Artemia salina*) and were found to be toxic at 10 μg/ml (Yang et al. 2012).

Three nortriterpenes with a rearranged 3,4-*seco*-27-norlanostane skeleton named ganoboninketals A–C (ganoboninketal A: Fig. 12) were isolated from *Ganoderma boninense* fruiting bodies (current name: *Ganoderma orbiforme*; Ma et al. 2014c). Several bioassays were conducted. The compounds had antiplasmodial activity (*Pl. falciparum*), were moderately cytotoxic, and were able to inhibit NO production in LPS-induced macrophages (Ma et al. 2014c).

Isaka et al. (2016a) isolated 42 different lanostane triterpenes from a static culture of *Ganoderma* sp. (BCC 16642) of which 16 were new. Several exhibited significant activity against *Mycobacterium tuberculosis* (MIC values for several compounds were between 0.78 and 6.25 μg/ml, e.g., (22S,24E)-3β,15α,22-triacetoxylanosta-8,24-dien-26-oic acid with 3.13 μg/ml; (24E)-3β,15α-diacetoxy-7α-hydroxylanosta-8,24-dien-26-oic acid with 1.56 μg/ml; and (22S,24E)-3β,22-diacetoxylanosta-7,9(11),24-trien-26-oic acid with 12.5 μg/ml; Fig. 12; Isaka et al. 2016a).

Recently a comprehensive review on triterpenoids of *Ganoderma* spp. covering structure elucidation and bioactivities was published by Xia et al. (2014).

b) *Fomitella fraxinea* (current name: *Perenniporia fraxinea*, Polyporaceae)
3*β*-Hydroxylanosta-8,24-dien-21-oic acid and fomitellic acids A–D (fomitellic acid B: Fig. 12), four new lanostanes, were isolated from the mycelia of *Fomitella fraxinea* (current name: *Perenniporia fraxinea*) in a search for new inhibitors of DNA polymerases (Tanaka et al. 1998). All five metabolites inhibited calf DNA polymerase α and rat DNA polymerase β at concentrations of 35–75 μM and 90–130 μM, respectively. Data on other biological activities are not given. Meanwhile studies toward the synthesis of fomitellic acids (Yamaoka et al. 2009a) and the total synthesis of fomitellic acid B (Yamaoka et al. 2009b) were realized. In a virtual screen for human E3 ubiquitin-protein ligase (Mdm2), inhibitory data for fomitellic acids A and B were calculated, but the results for both compounds were not outstanding contrary to other basidiomycete steroids like ganoderic acids F, X, and Y from *Ganoderma lucidum* (Froufe et al. 2013). Mdm2 is responsible for p53 proteasomal degradation. In some cancers, Mdm2 is overexpressed and inhibits the activity of p53, which is a crucial factor for apoptosis. Therefore, inhibition of Mdm2 is thought to be a target for the treatment of cancer.

c) *Naematoloma fasciculare* (current name: *Hypholoma fasciculare*, Hymenogastraceae)
Two new lanostane triterpenoids named fasciculols H and I (Fig. 12) were isolated from fruiting bodies of the poisonous mushroom *Naematoloma fasciculare* (current name: *Hypholoma fasciculare*) from China (Shi et al. 2011a). Fasciculol H weakly inhibited the human glioma cell line U87 and showed weak inhibitory activity against nuclear factor kappaB (NF-κB, involved in tumorigenesis). In 2013 Kim et al. published fasciculols J to M (fasciculols J, K: Fig. 12) together with several known compounds from fruiting bodies of *Naematoloma fasciculare* collected in Korea.

All secondary metabolites were tested for antiproliferative activities against human cancer cell lines and for inhibition of NO production in LPS-activated microglial cells. Fasciculols J and K had no cytotoxic effects against all cell lines, but fasciculols L and M were active with IC_{50} values between 3.99 and 8.53 μM. Only fasciculol L was able to inhibit NO formation with IC_{50} values of 49.9 μM (Kim et al. 2013).

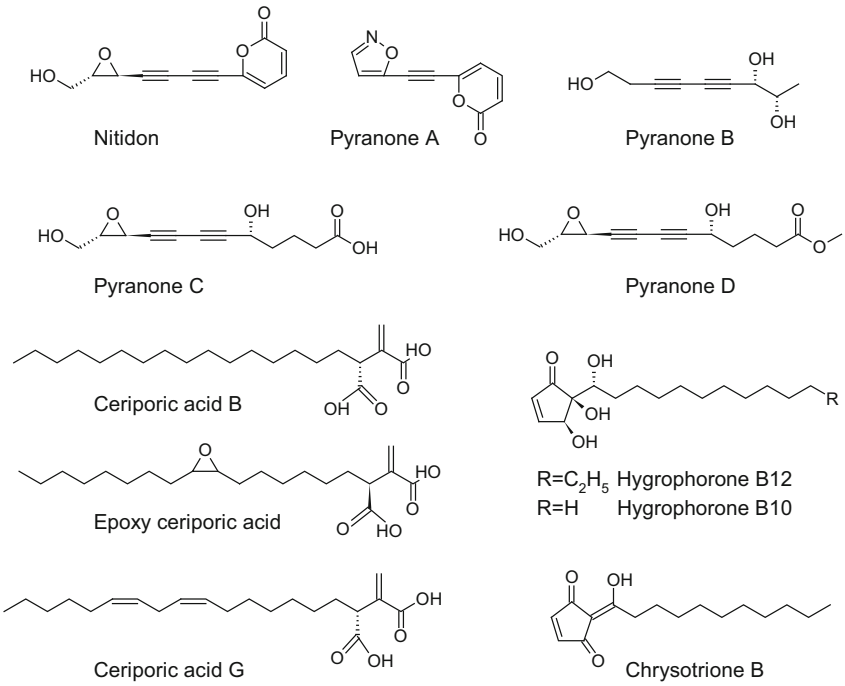


Fig. 13 Secondary metabolites of *Junghuhnia nitida*, *Ceriporia subvermispora*, and *Hygrophorus* spp.

B. Polyketides and Fatty Acid Derivatives

The following genera or species, which were included in the last edition, are not included in this edition: *Boreostereum vibrans* (Stereaceae), *Suillus luteus* (Suillaceae), *Trametes menziesii* (current name: *Leiotrametes menziesii*, Polyporaceae), *Gerronema* sp. (Tricholomataceae), imperfect basidiomycete strain 9624, and *Pterula* sp. (Pterulaceae). The terphenyls have been moved to "Amino Acid Derivatives and NRPS-Derived Compounds."

1. *Junghuhnia nitida* (Meruliaceae)

Many polyacetylenes have been described from basidiomycetes. Nitidon (Fig. 13), a highly oxidized pyranone derivative produced by *Junghuhnia nitida*, exhibits antibiotic and cytotoxic activities and induces morphological and physiological differentiation of tumor cells at nanomolar concentrations (Gehrt et al. 1998).

At a concentration of 100 ng/ml (0.46 μM), nitidon induces the differentiation of 25–30% of the HL-60 cells (human promyelocytic leukemia) into granulocyte-monocyte-like cells and a differentiation of 20% of U-937 cells (human histiocytic leukemia) into monocyte-like cells. The biological activity of nitidon is at least in part due to its high chemical reactivity. Addition of cysteine yielded adducts which were almost devoid of differentiation-inducing activity.

In 2010 the pyranones A–D (Fig. 13), four new polyacetylenes, were isolated together with nitidon from a culture of *Junghuhnia nitida* (Hu et al. 2014). Cytotoxicity was evaluated for the pyranones A–D with several cell lines, but only pyranone A inhibited cell growth with IC_{50} values between 4.13 and 11.65 μmol. The authors noted that pyranone D could be an artifact due to a treatment with methanol during isolation (Hu et al. 2014).

2. *Ceriporia subvermispora* (Phanerochaetaceae)

Ceriporia subvermispora is a white rot fungus which degrades lignin without substantially damaging the remaining cellulose. This bears implications, e.g., for the cellulose and paper industry. The reason for this selective

degradation of lignin was investigated by Watanabe's group (Ohashi et al. 2007). They found that alkylitaconic acids, e.g., ceriporic acid B (Fig. 13), suppress the production of hydroxyl radicals by the Fenton reaction (Rahmawati et al. 2005) even in the presence of reductants for Fe^{3+}. The alkyl side chain and the two carboxyl groups are essential for redox silencing and high stability against oxidative degradation by OH radicals. The absolute configuration of ceriporic acids A ((*R*)-3-tetradecylitaconic acid), B ((*R*)-3-hexadecylitaconic acid), and C ((*R*,*Z*)-2-(hexadec-7-enyl)-3-itaconic acid) was determined by Nishimura et al.(2009).

Meanwhile several new derivatives were described like ceriporic acid D (Nishimura et al. 2008), epoxy ceriporic acid ((*R*)-3-(7,8-epoxy-hexadecyl)-itaconic acid; Fig. 13; Nishimura et al. 2011), ceriporic acid E ((*R*)-3-[(*Z*)-tetradec-7-enyl]-itaconic acid), and ceriporic acid F ((*R*)-3-[(*Z*)-tetradec-5-enyl]-itaconic acid; (Nishimura et al. 2012a), ceriporic acid G (3-[(*Z*,*Z*)-hexadec-7,10-dienyl]-itaconic acid); and ceriporic acid H (3-[(*Z*)-octadec-9-enyl]-itaconic acid, Fig. 13; Nishimura et al. 2012b).

3. *Hygrophorus* spp. (Hygrophoraceae)

The hygrophorones, new acylpentenones, were isolated from fruiting bodies of *Hygrophorus latitabundus*, *Hy. olivaceoalbus*, *Hy. persoonii*, and *Hy. pustulatus* (Lübken et al. 2004, 2006; Lübken 2006). All hygrophorones exhibited modest antifungal activity against *Cladosporium cucumerinum*. In recent years, the hygrophorones were still in the focus of research. Several new hygrophorones were described from *Hy. abieticola*. These include the pseudohygrophorones A12 and B12 (Otto et al. 2016) as well as hygrophorone B12 (Fig. 13; Bette et al. 2015; including total synthesis). The three compounds were tested against *Bo. cinerea*, *Septoria tritici*, and *Ph. infestans*, and all exhibited modest to high antimicrobial activities (Otto et al. 2016).

The biosynthesis of hygrophorone B12 was studied in *Hy. abieticola* (Otto et al. 2015). Several ^{13}C-labeled precursors were injected into young fruiting bodies which were harvested 7 to 9 days later for analysis. It was demonstrated that hygrophorone B12 and presumably other hygrophorones are derived from a fatty acid-polyketide route. The presumed biosynthesis starts from 4-oxooctadec-2-enoic acid followed by an E/Z isomerization and hydroxylation followed by an intramolecular cyclization and oxidation to carbonyl group at C-6 which would form a chrysotrione B homologue, originally isolated from the fruiting bodies of the *Hy. chrysodon* (Fig. 13; Gilardoni et al. 2007). From this, hygrophorone B is formed by stereospecific carbonyl group reduction and hydroxylation (Otto et al. 2015).

Just recently four new derivatives were isolated from *Hy. abieticola* and named hygrophorone B10 (Fig. 13), E12, and H12 and 2,3-dihydrohygrophorone H12 (Otto et al. 2017). All of them were tested against the microorganisms mentioned above.

Hygrophorone B10 was as active as hygrophorone B12 against *Ph. infestans* (1.6 μM) but less active against fungi. Hygrophorones E12 and H12 showed modest antimicrobial activity, and 2,3-dihydrohygrophorone H12 was inactive. Several semisynthetic hygrophorone B12 derivatives were tested too, but all had weaker antimicrobial activities as compared to hygrophorone B12. These results give evidence that an α,β-unsatu-

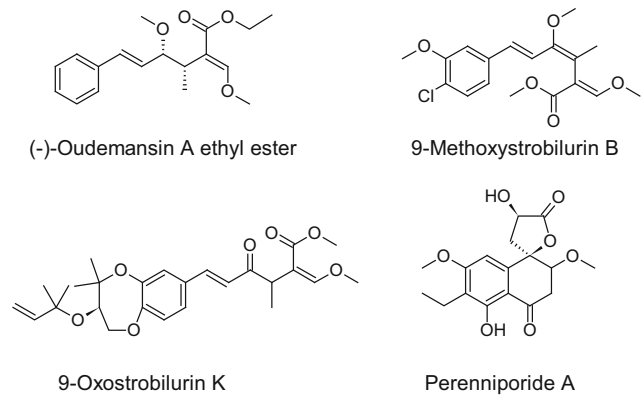


Fig. 14 Strobilurin and oudemansin derivatives from *Xerula* sp. and *Favolaschia* spp. and perenniporide A from *Perenniporia* sp.

rated carbonyl group (Michael acceptor) seems to be essential for the antimicrobial activity (Otto et al. 2017).

4. *Xerula* sp. (Physalacriaceae) and *Favolaschia* spp. (Mycenaceae)

The strobilurins have become lead structures for the synthesis of important agricultural fungicides. In recent years, several new examples of natural strobilurin and oudemansin derivatives were published.

Xerula sp. (strain BCC56836, Physalacriaceae) was investigated by Sadorn et al. (2016), and 12 (naturally) new compounds among them (−)-oudemansin A acid, (−)-oudemansin A ethyl ester (Fig. 14) and (−)-oudemansin X ethyl ester, (+)-oudemansin A lactone and (+)-oudemansin X lactone, (+)-dihydrooudemansinol, (−)-11-epidihydrooudemansinol, (+)-xeruhydrofuranol, (+)-9-epixeruhydrofuranol, and strobilurin A acid were isolated.

All of them were tested in various bioassays (multiresistant *Pl. falciparum*, *B. cereus*, *C. albicans*, *Alternaria brassicicola*, *Colletotrichum capsici*, and *Col. gloeosporioides*, cytotoxicity assays with several cell lines). No activity was detected for (−)-oudemansin A acid, (+)-oudemansin X lactone, (+)-dihydrooudemansinol, and (−)-11-epidihydrooudemansinol. (−)-Oudemansin A ethyl ester was able to inhibit the growth of *B. cereus* and *Col. capsici* at 25 μg/ml (MIC). (−)-Oudemansin X ethyl ester had antimalarial activity (IC_{50} 13.7 μM), *B. cereus* was inhibited at 25 μg/ml (MIC), and all filamentous fungi were inhibited between 12.5 and 50 μg/ml (MIC). (+)-Oudemansin A lactone like the two aforementioned compounds only exhibited weak cytotoxicity but no antifungal activity (Sadorn et al. 2016).

Two new strobilurins named 9-methoxystrobilurin B (Fig. 14) and 9-methoxystrobilurin G were isolated from *Favolaschia tonkinensis* (strain BCC 18689; Kornsakulkarn et al. 2010).

Both inhibited *Pl. falciparum* with IC_{50} values of 0.3 and 0.03 μg/ml, respectively. *C. albicans* was inhibited at 0.22 and 0.5 μg/ml (IC_{50}), and both inhibited the growth of cell lines with IC_{50} values between 0.36 and 23.98 μg/ml (Kornsakulkarn et al. 2010). Later on three new strobilurins, 9-oxostrobilurin G, 9-oxostrobilurin K, 9-oxostrobilurin I, and 9-oxostrobilurin A, previously described as a semisynthetic derivative by Engler-Lohr et al. (1999), all of them exhibiting a keto group at position 9, were described from *Favolaschia calocera* (Chepkirui et al. 2016). MIC values of these four compounds against *C. tenuis* were between 4.68 and 9.37 μg/ml and against *Mucor plumbeus* between 9.37 and 18.75 μg/ml. All strobilurin derivatives showed modest to high cytotoxic activities (Kornsakulkarn et al. 2010; Chepkirui et al. 2016).

5. *Perenniporia* sp. (Polyporaceae)

From larva of *Euops chinesis*, a phytophagous attelabid weevil with host specificity to *Fallopia japonica* and later from leaves of the medicinal plant itself *Perenniporia* sp. was isolated. From a solid media fermentation, four napthalenones named perenniporides A–D (perenniporide A: Fig. 14) were isolated.

Antifungal assays were conducted against *F. moniliforme* (current name: *Gibberella fujikuroi*), *Verticillium albo-atrum*, *Gibberella zeae*, *F. oxysporum*, and *Alternaria longipes*, and only perenniporide A was able to inhibit all of them between 10 and 20 μg/ml (MIC; Feng et al. 2012).

C. Compounds of Unclear Biogenetic Origin or Mixed Biosynthesis

The following genera or species which were included in the last edition are not included in here: *Tremella aurantialba* (current name: *Naematelia aurantialba*, Naemateliaceae), *Aporpium caryae* (current name: *Elmerina caryae*, Auriculariales), *Bondarzewia montana* (Bondarzewiaceae), *Cortinarius* sp. (Cortinariaceae), *Chamonixia pachydermis* (Boletaceae), and *Pholiota spumosa* (Strophariaceae).

1. *Boreostereum vibrans* (Gloeophyllaceae)

The vibralactones, unusual fused β-lactone-type metabolites, were isolated from cultures of *Boreostereum vibrans* (syn. *Stereum vibrans*; Liu et al. 2006; Jiang et al. 2008). Vibralactone (Fig. 15) is a potent inhibitor of pancreatic lipase with an IC_{50} value of 0.4 μg/ml. A structurally closely related metabolite, percyquinnin, had been isolated from fermentations of *Stereum complicatum* (Stereaceae) as a potent lipase inhibitor (Hoppmann et al. 2001). It later

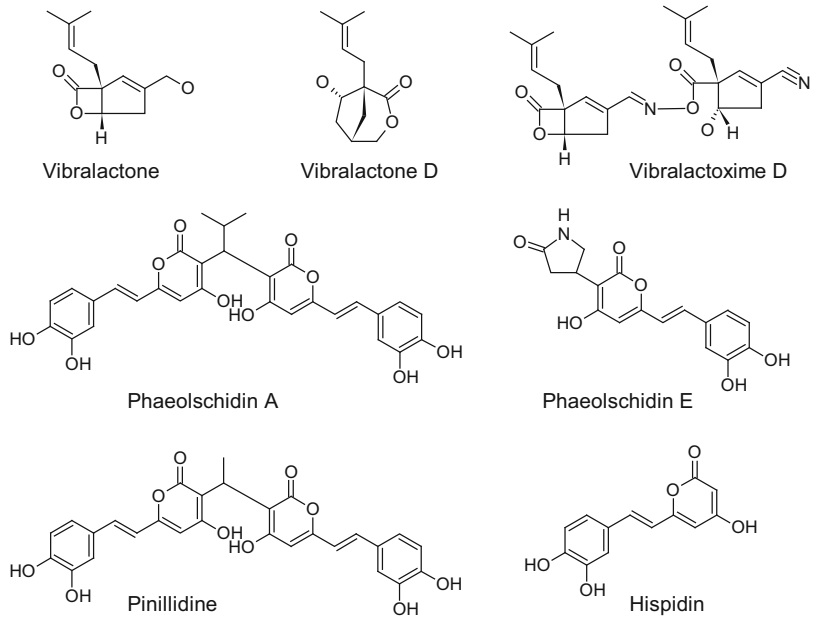


Fig. 15 Secondary metabolites of *Boreostereum vibrans* and *Phaeolus schweinitzii*

turned out to be identical with vibralactone. New related metabolites, 1,5-secovibralactone, vibralactone B, vibralactone C, acetylated vibralactone (Jiang et al. 2008), vibralactones D–F (vibralactone D: Fig. 15; Jiang et al. 2010), vibralactones G–J (Wang et al. 2012a), vibralactones K–M (Wang et al. 2013a), vibralactones N–Q, 10-lactyl vibralactone G, and (3*S**,4*R**)-6-acetoxymethyl-2,2-dimethyl-3,4-dihydro-2H-chromene-3,4-diol (Chen et al. 2014) have been described from the same fungus. Vibralactones *R* and *S* (together with vibralactone and vibralactone acetate) were isolated from an undescribed stereaceous basidiomycete (BY1; Schwenk et al. 2016). Recently 16 novel oximes and oxime esters related to vibralactone were isolated and named vibralactoximes A to P (vibralactoxime D: Fig. 15; Chen et al. 2016b) from *Boreostereum vibrans*. Meanwhile vibralactone syntheses were published by Zhou and Snider (2008a, b).

Bioactivities were reported for vibralactones D–F which weakly inhibited 11β-hydroxy-steroid dehydrogenases (Jiang et al. 2010). The vibralactoximes were tested for pancreatic lipase inhibitory activities, and vibralactoximes A, D, E, G, I, J, and K were active with IC_{50} values between 11.1 and 28.6 μmol. In addition, several vibralactoximes (D–L, N, O) were weakly cytotoxic (Chen et al. 2016b). Antifungal activity was tested by Schwenk et al. (2016), and vibralactone, vibralactone acetate, as well as vibralactone R showed no activity against *A. fumigatus* and modest activity against *Pe. notatum* and *Arthroderma benhamiae*. Vibralactone S was inactive.

In 2011 Zeiler et al. used vibralactone as a probe to study caseinolytic peptidase ClpP which is part of the Clp proteolytic complex. Some organisms like *Listeria monocytogenes* have two isoforms (ClpP1 and ClpP2), and first insights into the complex assembly (two homoheptameric ClpP1 and ClpP2 rings stacked on top of each other) of this class of bacterial enzymes were made possible by vibralactone which was able to bind to both isoforms (Zeiler et al. 2011).

Vibralactone was shown to be an inhibitor of acyl protein thioesterase (APT) 1 and 2 with IC_{50} values of 4.3 μM and 1.7 μM, respectively (List et al. 2014). A synthetic APT1 inhibitor is palmostatin B (IC_{50} value of 0.67 μM) inhibiting Ras depalmitoylation, thus interfering with Ras signaling activity (Ras: small GTPase regulating cell functions, dysregulation of Ras signaling is associated with cancer; Dekker et al. 2010).

Meanwhile the biosynthetic pathway of the vibralactones was elucidated. By ^{13}C-labeling and metabolite profiling, it was shown that the bicyclic lactone ring originates from the shikimate pathway (Zhao et al. 2013). The vibralactones are formed after ring rearrangement (intramolecular cyclization of a seven-membered ring intermediate) and prenylation, presumably catalyzed by VibPT, a prenyltransferase identified from *Boreostereum vibrans* (Zhao et al. 2013).

2. *Phaeolus schweinitzii* (Fomitopsidaceae)

Hispidin derivatives were isolated from fruiting bodies of *Phaeolus schweinitzii*—four of them are bishispidins named phaeolschidins A–D (phaeolschidin A: Fig. 15) and one hispidin derivate named phaeolschidin E (Fig. 15; Han et al. 2013a). Hispidin (Fig. 15) and the only to this date known bishispidin pinillidine (Fig. 15) were isolated by Wangun and Hertweck (2007) from *Phellinus pini* (current name: *Porodaedalea pini*, Hymenochaetaceae).

These (bis)styrylpyrones were evaluated in a DPPH scavenging assay, a total antioxidant capacity assay, and a lipid peroxidation assay. Phaeolschidins B–D and hispidin showed activity in the radical scavenging assay and in the antioxidant capacity assay. Phaeolschidins A and B as well as hispidin and pinillidine inhibited lipid peroxidation in a mice liver homogenate. All compounds had no cytotoxic effects. *Phaeolus schweinitzii* is a fungus which decays coniferous wood (Luana et al. 2015). Therefore, antioxidative compounds could be beneficial for the producer by quenching radical oxygen species produced by the plant as defense mechanism (Han et al. 2013a).

Hispidins do not only hold pharmacological activities but were recently shown to be responsible for fungal bioluminescence (Purtov et al. 2015). The isolated hispidin and its *cis* isomer via bioluminescence-based assays from fruiting bodies of *Pholiota squarrosa* showed that other bioluminescent fungi (*Mycena citricolor*, Mycenaceae; *Panellus stipticus*, Mycenaceae; *Armillaria borealis*, Physalacriaceae; *Neonothopanus nambi*, Omphalotaceae) do contain hispidin too. The prerequisites for fungal luminescence nevertheless are not only the production of hispidin but also the enzymes hispidin-3-hydroxylase and luciferase, which are responsible for light emission (Oliveira and Stevani 2009; Purtov et al. 2015).

3. *Hericium* spp. (Hericiaceae)

Hericium erinaceus is a medicinal fungus traditionally used in China and Japan. Several secondary metabolites were isolated from this fungus, i.e., hericenones and erinacines (to date more than 40 compounds are known). Several of the compounds isolated from this fungus are able to promote nerve growth factor (NGF) biosynthesis in rodent cultured astrocytes suggesting a benefit for the treatment or prevention of dementia (Kawagishi and Zhuang 2008; Ma et al. 2010). From fruiting bodies of *Hericium coralloides*, related compounds named corallocins A–C (Fig. 16) and hericin as well as an isoindolinone derivative were isolated.

Corralocin B showed cytotoxic activity and weak antifungal activity against *Mucor plumbeus* (Wittstein et al. 2016). Induction of neuronal cell differentiation was tested in PC12 cells, but corallocins A–C did not show activity. However, corallocins A and C increased NGF secretion from astrocytes (1321N1 cell line; Wittstein et al. 2016). This class of compounds bears potential to unravel NGF signaling pathways and the induction of NGF synthesis and might be of pharmacological interest for the treatment of neurodegenerative diseases (Ma et al. 2010).

4. *Stereum hirsutum* (Stereaceae)

In recent years, several publications dealing with *Stereum* species and their natural products were published especially from *Stereum hirsutum* which is a medicinal mushroom in China and Korea. In addition to new sesquiterpenoids, several compounds with unclear or mixed biosynthetic origin were described.

From submerged cultures of *Stereum* sp. YMF1.1684, phenostereum A (Fig. 16), a benzofuran, and the benzofuran-dimer phenostereum B (Fig. 16) were isolated (Sun et al. 2011) and tested in antibacterial and cytotoxicity assays but exhibited no activity.

One new dihydrobenzofuran was identified from a culture of *Stereum insigne* CGMCC5.57 (current name: *Xylobolus subpileatus*, Stereaceae; Tian et al. 2017). (*R*)-2,3-Dihydro-2-(1-methylethenyl)-5-((3-methylbut-2-en-1-yl)oxy)-benzofuran (Fig. 16) did not show any nematicidal or antibiotic activities.

Three new lactones were described from the culture filtrate of submerged cultures of *Stereum ostrea* (Kang and Kim 2016) and named ostalactones A–C (Fig. 16).

They were tested against human pancreatic lipase, and ostalactones A and B inhibited the lipase activity with IC_{50} values of 9.0 and 3.2 μM, respectively. Ostalactone C was not active. Some β-lactones are known lipase inhibitors, thus blocking the hydrolysis of triglycerides (Borgström 1988; Hadváry et al. 1988).

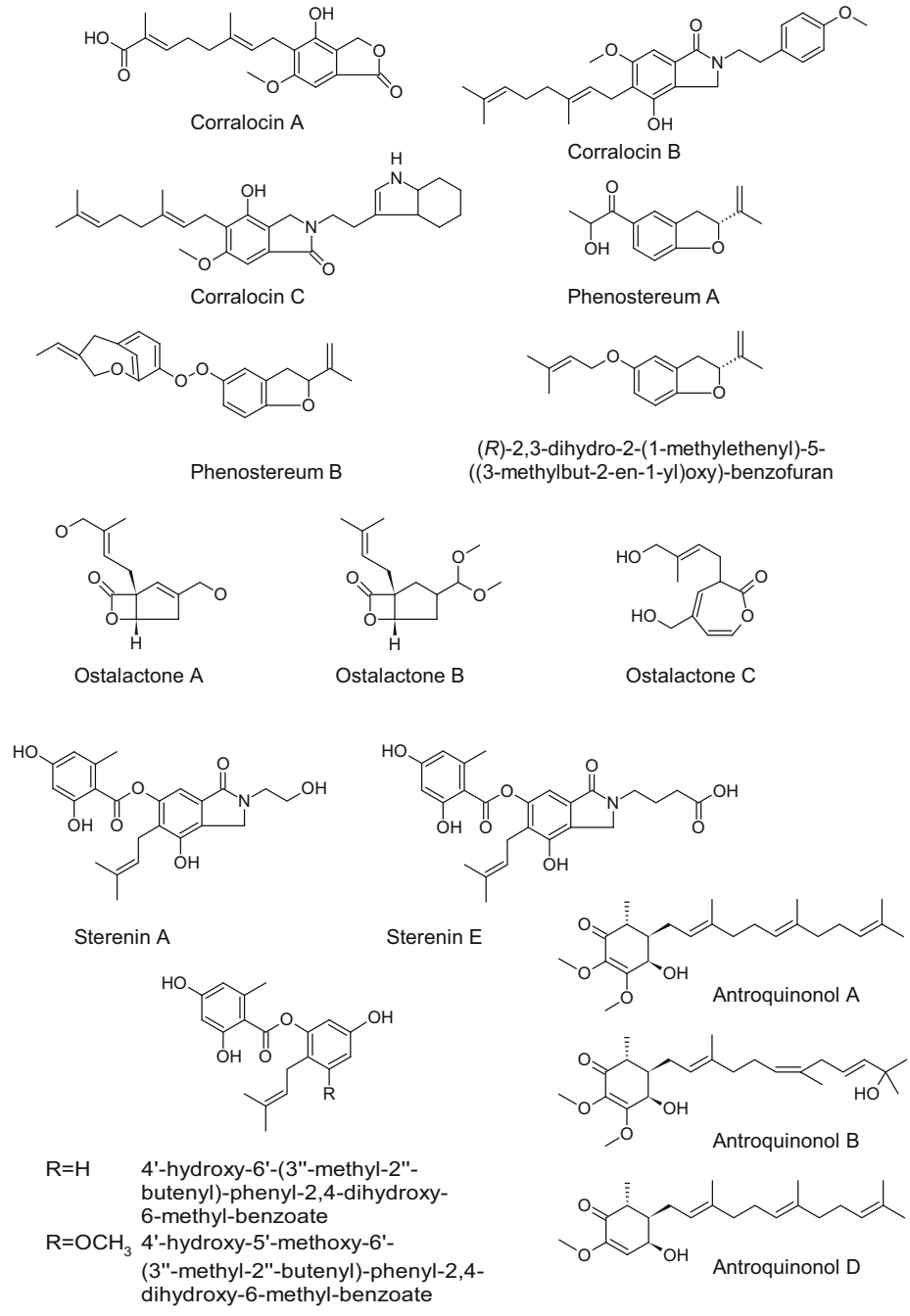


Fig. 16 Secondary metabolites from *Hericium coralloides*, *Stereum hirsutum*, and *Antrodia* spp.

In 2008 four 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) inhibitors were isolated from solid-state cultures of *Stereum* sp. SANK 21205 and were named sterenins A, B, C, and D (sterenin A: Fig. 16; Ito-Kobayashi et al. 2008). The IC_{50} values against human 11-β-HSD1 were between 230 nM (sterenin C), 240 nM (sterenin A), 2600 nM (sterenin D),

and 6600 nM (sterenin B). The activity against human 11-β-HSD1 seems to be isoform specific because no activity was observed against human 11-β-HSD2 (Ito-Kobayashi et al. 2008).

An extract of a solid culture of *Stereum hirsutum* (Wang et al. 2014b) was tested in vitro against yeast α-glucosidase (inhibitors thought to be useful for the development of hypoglycemic drugs) but showed inhibition only at very high concentrations. Further investigation of this extract leads to the isolation of sterenins E to M, and sterenins E–H (sterenin E: Fig. 16) and K–M were active against yeast α-glucosidase at concentrations between 3.06 and 36.64 μM (Wang et al. 2014b).

Related benzoate derivatives were isolated from solid-state fermentations on rice of *Stereum hirsutum* (Ma et al. 2014a), and 4′-hydroxy-5′-methoxy-6′-(3″-methyl-2″-butenyl)-phenyl-2,4-dihydroxy-6-methyl-benzoate and 4′-hydroxy-6′-(3″-methyl-2″-butenyl)-phenyl-2,4-dihydroxy-6-methyl-benzoate (Fig. 16) exhibited antibacterial activity against *B. subtilis* and methicillin-resistant and methicillin-sensitive *S. aureus*. 4′-Hydroxy-5′-methoxy-6′-(3″-methyl-2″-butenyl)-phenyl-2,4-dihydroxy-6-methyl-benzoate showed cytotoxic and potentially anti-inflammatory effects (Ma et al. 2014a).

5. *Antrodia* spp. (Fomitopsidaceae)

In 2007 a cytotoxic agent named antroquinonol (or antroquinonol A; Fig. 16) was isolated from *Antrodia camphorata* (current name: *Taiwanofungus camphoratus*; Lee et al. 2007) and from *Antrodia cinnamomea* (Lu et al. 2013). This fungus, endemic to Taiwan, grows only on *Cinnamomum kanehirai* (Lauraceae) and is worth 15,000–25,000$ per kg of fruiting body (Lu et al. 2013). Due to its use in traditional medicine especially in the treatment of cancer, immune-related diseases and its anti-inflammatory effects over 78 compounds were identified (for review, see Tzeng and Geethangili 2011). The ubiquinone derivative antroquinonol obtained from solid-state fermentations had strong cytotoxic effects against several human cell lines (Lee et al. 2007). In 2009 related compounds named antroquinonol B (Fig. 16); 4-acetyl-antroquinonol B, 2,3-(methylenedioxy)-6-methylbenzene-1,4-diol together with 2,4-dimethoxy-6-methylbenzene-1,3-diol; and the known alkaloid antrodin D were isolated.

These compounds were analyzed for their inhibition of nitric oxide synthase (iNOS) in LPS-activated murine macrophages and for their cytotoxic activity (Yang et al. 2009). Except for 2,3-(methylenedioxy)-6-methylbenzene-1,4-diol, all compounds inhibited the NO production. 2,4-Dimethoxy-6-methylbenzene-1,3-diol and antrodin D were not cytotoxic although all others showed activity (Yang et al. 2009).

Antroquinonol D (Fig. 16) was isolated as DNA methyltransferase 1 (DNMT1) inhibitor. DNMT1 catalyzes DNA methylation, and its overexpression is associated with cancer (Wang et al. 2014c).

Wang et al. (2014b) were able to demonstrate that antroquinonol D binds to the catalytic domain of DNMT1 and competes with the cofactor SAM for the binding site (IC_{50} lower than 5 μM). It is postulated that DNA demethylation is induced and the silencing of tumor suppressor genes is reversed leading to induced cancer cell death and inhibition of migration (Wang et al. 2014c).

In recent years, the synthesis of these compounds was addressed by different groups (e.g., (+)-antroquinonol and (+)-antroquinonol D, Sulake and Chen 2015; antroquinonol A, Villaume et al. 2015; (±)-antroquinonol, Hsu et al. 2015; (+)-antroquinonol, Sulake et al. 2015). In addition the literature on bioactivities is increasing (see Villaume et al. 2015 and references therein), and currently one clinical trial with antroquinonol (phase II) for non-small cell lung cancer stage IV was conducted (Ho et al. 2015; clinical trial information: https://clinicaltrials.gov/show/NCT02047344).

6. *Trametes speciosa* (Polyporaceae)

Eleven oxygenated cyclohexanoids named speciosins A–K (speciosin B: Fig. 17) and the known aporpinone A (Fig. 17, isolated from *Aporpium caryae*, Levy et al. 2003) were isolated from *Hexagonia speciosa* (current name: *Trametes speciosa*), a fungus which is native to the tropical and subtropical zones in China (Jiang et al. 2009). Unfortunately no

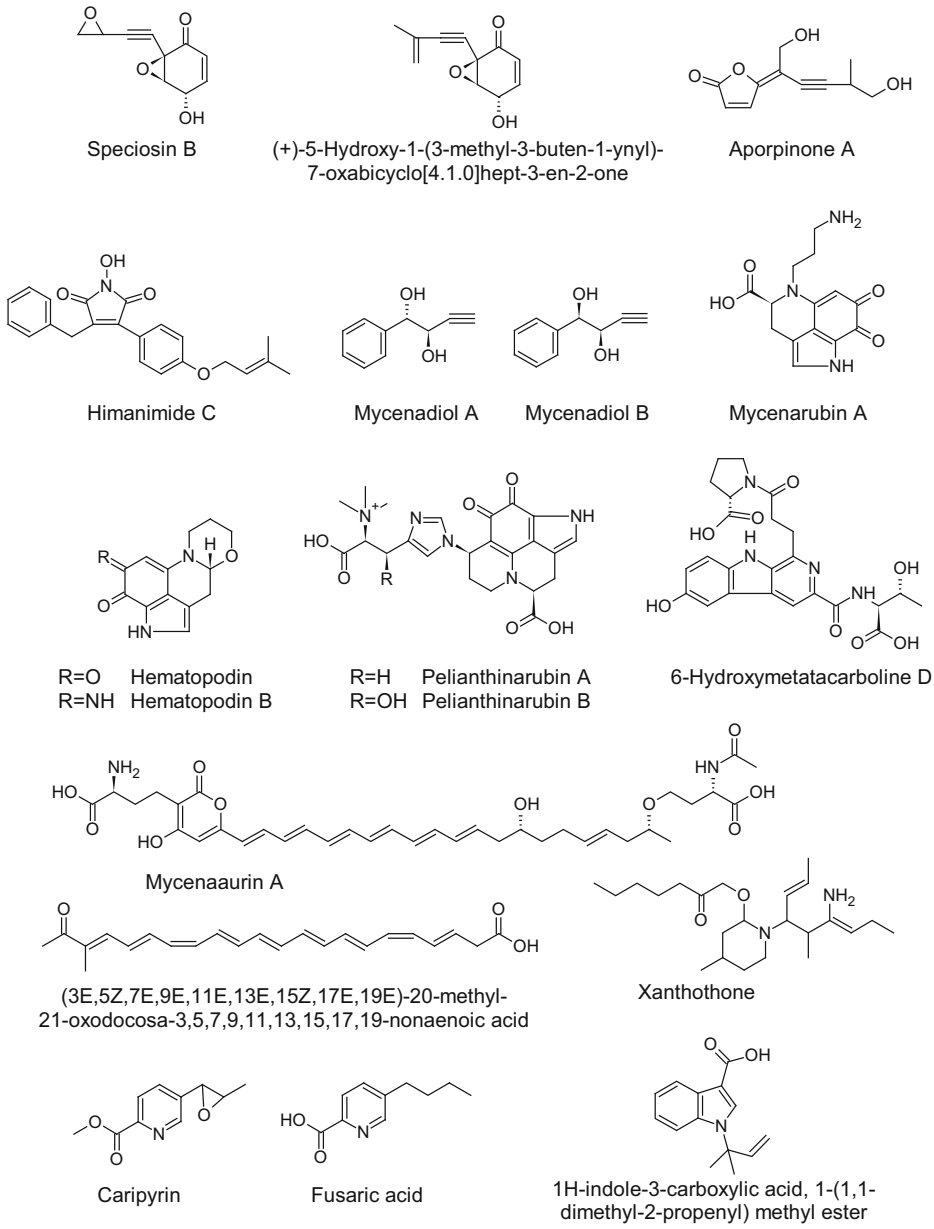


Fig. 17 Secondary metabolites of *Trametes speciosa*, *Serpula himantioides*, *Mycena* spp., *Coprinus xanthothrix*, *Caripia montagnei* (incl. fusaric acid), and *Aporpium caryae*

bioassays were conducted even though related compounds like (+)-5-hydroxy-1-(3-methyl-3-buten-1-ynyl)-7-oxabicyclo[4.1.0]-hept-3-en-2-one (Fig. 17) exhibit antifungal and plant growth-promoting activity (Kim et al. 2006). In 2011 speciosins L–T were described from the same fungus (Jiang et al. 2011). In this study, speciosins M, N, Q, B, D, E, F, I, and K were tested against human tumor cell lines. Only speciosin B was able to inhibit the growth of all cell lines with IC_{50} values between 0.23 μM and 3.3 μM (Jiang et al. 2011).

7. *Serpula himantioides* (Serpulaceae)

The himanimides were isolated from fermentations of a *Serpula himantioides* strain collected in Chile (Aqueveque et al. 2002). All four compounds are succinimide and maleimide derivatives, of which two are N-hydroxylated. Only himanimide C (Fig. 17) exhibits broad antibacterial, antifungal, and cytotoxic activity, suggesting a link to the N-hydroxylated maleimide moiety.

In 2005 himanimide C and unnatural analogues were synthesized and tested for antifungal activity against *Plasmopara viticola*, *Ph. infestans*, *Pyrenophora teres*, *Erysiphe graminis* (current name: *Blumeria graminis*), *Puccinia recondita*, and *Rhizoctonia solani* (current name: *Thanatephorus cucumeris*). No activity was detected for these plant pathogens in vitro and in vivo which seems to be due to a rapid metabolization (Selles 2005). In the first study by Aqueveque et al. (2002), other fungi were found sensitive. To date several synthetic approaches are published (Cheng et al. 2008; Basavaiah et al. 2010; Prateeptongkum et al. 2010).

8. *Mycena* spp. (Mycenaceae)

Mycenadiols A to D (mycenadiols A, B: Fig. 17) are phenylglycols and the first natural products from *Mycena pruinosoviscida*. They and synthetic diastereomers were tested in cytotoxicity, antimycobacterial, antiplasmodial, antifungal, and antibacterial assays but had no activity up to 50 μg/ml (Isaka et al. 2014b).

Several pigments from *Mycena* species were studied in recent years—they were isolated from fruiting bodies of the respective species.

Blue alkaloid pigments named sanguinones A and B, the degradation product decarboxydehydrosanguinone A, and the red pigment sanguinolentaquinone were isolated from *Mycena sanguinolenta* (Peters and Spiteller 2007a). Red pyrroloquinoline alkaloids and mycenarubins A (Fig. 17) and B were described from *Mycena rosea* (Peters and Spiteller 2007b). Related structures were isolated from the bleeding *Mycena*, *Mycena haematopus*. The stable degradation product haematopodin (Fig. 17) is known since 1993 (Baumann et al. 1993), but the pigments of intact fruiting bodies were not known. Peters et al. (2008) isolated the major red pigment (haematopodin B; Fig. 17) and minor new components, mycenarubins D, E, and F, as well as the known mycenarubin A and sanguinolentaquinone. Two red pyrroloquinoline alkaloids were isolated from fruiting bodies of *Mycena pelianthina* named pelianthinarubins A and B (Fig. 17). Although several pyrroloquinoline alkaloids are known for their bioactivities, both compounds exhibited no activity against several bacteria, fungi, garden cress, and springtail (Pulte et al. 2016).

With HR-MALDI-MS imaging, several β-carboline alkaloids were detected from fruiting bodies of *Mycena metata*, and the main compound, 6-hydroxymetatacarboline D (Fig. 17), was isolated and characterized in antimicrobial agar diffusion assays, but at 0.5 μmol no activity was observed. All minor compounds (6-hydroxymetatacarbolines A–C and E–I and metatacarbolines A–G) were determined from key ions found in LC-HR-ESIMS, LC-HR-ESIMS/MS, and LC-HR-ESIMS^3 (Jaeger et al. 2013).

Several polyene pigments are known from basidiomycetes like boletocrocins A and B (*Boletus* spp.; Kahner et al. 1998) and C (*Laetiporus sulphureus*; Davoli et al. 2005). An addition to this collection is the polyene pigment mycenaaurin A (Fig. 17), which was isolated from fruiting bodies of *Mycena aurantiomarginata*. In contrast to the aforementioned alkaloids for which no bioactivities could be detected, strong antibacterial activity against *B. pumilus* has been described for mycenaaurin A (Jaeger and Spiteller 2010).

Schwenk et al. (2014) found that a stereaceous basidiomycete upon injury of the mycelial layer on agar plates produces two yellow polyene pigments ((3Z,5E,7E,9E,11E,13Z,15E,17E)-18-methyl-19-oxoicosa-3,5,7,9,11,13,15,17-octaenoic acid and (3E,5Z,7E,9E,11E,13E,15Z,17E,19E)-20-methyl-21-oxodocosa-3,5,7,9,11,13,15,17,19-nonaenoic acid; Fig. 17).

They tested insecticidal effects and showed that *Drosophila melanogaster* exposed to the polyenes had less

pupating larvae after 7 days than untreated ones. These polyenes might be injury-induced repellents preventing the feeding or development of insects (Schwenk et al. 2014). The aforementioned polyenes are most likely derived from a polyketide backbone which was deduced from feeding experiments (Schwenk et al. 2014).

9. *Coprinus xanthothrix* (Psathyrellaceae)

Secondary metabolites from *Coprinus xanthothrix* were reported for the first time.

Liu et al. (2008c) isolated xanthothone (Fig. 17), which is a new N-containing secondary metabolite, together with the known 7,8,11-drimanetriol and 2-(1H-pyrrol-1-yl) ethanol. They were tested for nematicidal activity against *Panagrellus redivivus* and *Meloidogyne incognita*, but only xanthothone and 2-(1H-pyrrol-1-yl) ethanol were active with LD_{50} values of 250 and 125 μg/ml, respectively.

10. *Caripia montagnei* (current name: *Gymnopus montagnei*, Omphalotaceae)

Caripyrin (trans-5-(3-methyloxiranyl)pyridin-carboxylic acid methyl ester; Fig. 17; Rieger et al. 2010) was isolated from cultures of *Caripia montagnei* (current name: *Gymnopus montagnei*).

This novel pyridyloxirane was able to inhibit the germination of *M. oryzae*, but no activity was observed against *F. graminearum* and *Ph. infestans*. The structurally related fusaric acid (Fig. 17; mycotoxin with moderate toxicity known since 1934; Yabuta et al. 1934) was able to inhibit all three aforementioned organisms. In a plant protection assay where both compounds and spores of *M. oryzae* were sprayed on *Oryzae sativa*, caripyrin was able to reduce lesions more efficiently than fusaric acid. Additionally no cytotoxic or antibacterial activity was observed for caripyrin. Interestingly the bioactivity of caripyrin was not reduced upon incubation with cysteine. The full stereostructure and a short total synthesis were published by Andernach and Opatz (2014).

D. Amino Acid Derivatives and NRPS-Derived Compounds

Basidiomycetes produce a number of cyclopeptides with very interesting biological activities. These are being dealt within chapter "Cyclic Peptides and Depsipeptides from Fungi" by H. Anke and H. Laatsch.

1. *Aporpium caryae* (current name: *Elmerina caryae*, Auriculariales)

A culture of *Aporpium caryae* yielded 1H-indole-3-carboxylic acid, 1-(1,1-dimethyl-2-propenyl) methyl ester, and 1H-indole-3-carboxylic acid, 1-(2,3-dihydroxy-1,1-dimethylpropyl) methyl ester (Fig. 17), two metabolites of mixed biogenetic origin (Levy et al. 2000).

Both metabolites exhibited modest antifungal activities against the phytopathogenic *Cladosporium cucumerinum*. One total synthesis was described of 1H-indole-3-carboxylic acid, 1-(1,1-dimethyl-2-propenyl) methyl ester (Melkonyan et al. 2008) without any information on bioactivities. But 19 synthetic analogues of 1H-indole-3-carboxylic acid, 1-(2,3-dihydroxy-1,1-dimethylpropyl) methyl ester were tested against phytopathogenic fungi (*F. virguliforme*, current name: *Neocosmospora virguliforme*; *F. lateritium*, current name: *Gibberella baccata*; *Macrophomina phaseolina*; *Bo. cinerea*), and some of them were active against *F. virguliforme* (causal agent for sudden death syndrome of soybeans) even when applied as seed treatment (Bertinetti et al. 2011).

2. Terphenyls from *Thelephora* spp. (Thelephoraceae), *Hydnellum* spp. (Bankeraceae), *Sarcodon* spp., (Bankeraceae), *Tylopilus eximius* (current name: *Sutorius eximius*, Boletaceae) and *Paxillus curtisii* (current name: *Pseudomerulius curtisii*, Tapinellaceae)

Terphenyls are derived from the shikimate-chorismate pathway and are among the frequently encountered basidiomycete pigments (Gill and Steglich 1987; Gill 1999; Liu 2006). More recent additions are the thelephantins (e.g., A and H; Fig. 13) from *Thelephora aurantiotincta* and *Hydnellum caeruleum* (Quang et al. 2003a, b, 2004), terrestins from *T. terrestris* (Radulović et al. 2005), and curtisians from *Paxillus curtisii* (current name: *Pseudomerulius curtisii*) which has inhibitory activity against lipid peroxidation with IC_{50} values of 0.15, 0.17, 0.24, and 0.14 μg/ml (Yun et al. 2000). A

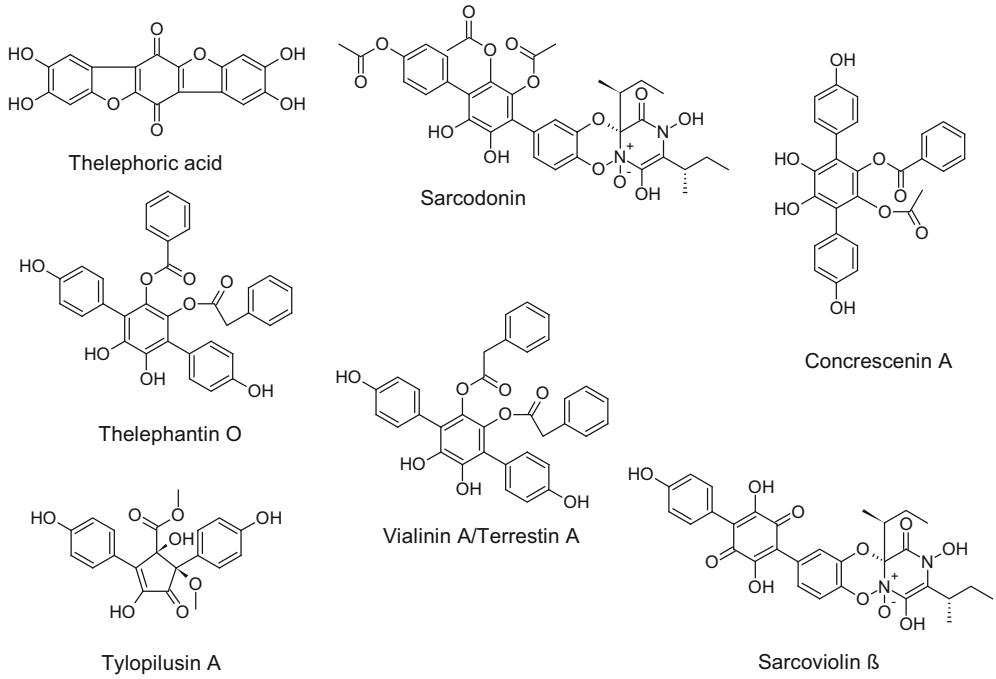


Fig. 18 Terphenyls from *Thelephora* spp., *Hydnellum* spp., *Sarcodon* spp., and *Tylopilus eximius*

number of biological activities have been ascribed to members of this group. Thelephoric acid (Fig. 18), the characteristic pigment of many Thelephoraceae, is claimed to be an antitumor (Kawai et al. 2005) and antiallergic agent (Tateishi et al. 2005). Sarcodonin (Fig. 18), a very interesting nitrogen-containing terphenyl derivative with a modified diketopiperazine attached to it, was isolated from fruiting bodies of *Sarcodon leucopus* (Geraci et al. 2000). *Sa. scabrosus* collected in China yielded sarcodonin δ (Ma and Liu 2005).

For *p*-terphenyls like atromentin from the homobasidiomycete *Tapinella panuoides* (AtrA; Schneider et al. 2008) and atromentic acid and other pulvinic acid derivatives produced by *Suillus grevillei* (GreA; Wackler et al. 2012), the biosynthesis was unraveled. Responsible are noncanonical NRPS-like enzymes lacking the ability to make peptide bonds. Standard NRPS modules are composed of an adenylation, a thiolation, and a condensation domain. The condensation domain catalyzes the amide bond. Condensation-domainless synthetases use aromatic α-keto acids as substrates. AtrA and GreA are quinone synthetases condensing 4-hydroxyphenylpyruvate monomers into atromentin which seems to be a universal precursor of terphenylquinones (for review, see Kalb et al. 2013).

In recent years, several new *p*-terphenyls were described, and novel biological activities were found for them. In the following paragraph, some outstanding examples are given.

Glucosidase inhibitory activity was the starting point to investigate extracts of fruiting bodies of *Hydnellum concrescens* collected in China (Wang et al. 2014d). Two new *p*-terphenyls named concrescenins A (Fig. 18) and B and six known compounds thelephantins L, I, J, and K, dihydroaurantiacin dibenzoate, and curtisian A were isolated.

Concrescenins A and B; thelephantins L, I, and K; dihydroaurantiacin dibenzoate; and curtisian A inhibited α-glucosidase with an IC_{50} of 0.99, 3.11, 4.53, 18.77, 2.98, 5.16, and 8.34 μM, respectively, whereas thelephantins J did not show inhibitory activity up to 50 μM. Inhibitors of α-glucosidase are thought to lower postprandial blood glucose and could be used as antidiabetic drugs (Wang et al. 2014d). Additionally the antioxidant effect tested via scavenging ability on DPPH was evaluated. Only concrescenin A and thelephantin I were active (Wang et al. 2014d).

Thelephantin O (Fig. 18) and the known vialinin A (or terrestin A, almost simultaneously described from *T. terrestris* by Radulović et al. (2005)) and *T. vialis* by Xie et al. (2005; Fig. 18) were isolated from fruiting bodies of *T. aurantiotincta* due to their cytotoxicity toward HepG2 and Caco2 (human epithelial colorectal adenocarcinoma) cells (Norikura et al. 2011). At 8 μM thelephantin O and vialinin A were able to inhibit the cell viability of HepG2 and Caco2 cells.

Interestingly the cell viability of non-cancerous human cryopreserved hepatocytes was almost not impaired by the treatment (Norikura et al. 2011). Later on the same group investigated the selective cytotoxicity and found that Fe^{2+} chelation reduced the activity (Norikura et al. 2013). Their study included the 2′,3′-dihydroxy-*p*-terphenyl derivatives thelephantin O, vialinin A, terrestrin B, and three synthetic new *p*-terphenyls (synthetic vialinin A derivatives 1–3), which had a vialinin A skeleton with partially or fully protected hydroxyl groups to study their influence on bioactivity. All except two of the synthetic products (synthetic vialinin A derivatives 2 and 3) were able to chelate iron and showed cytotoxic effects toward HepG2 cells and DPPH free-radical scavenging activity. In addition thelephantin O arrests the cell cycle in G1 phase which was suppressed by addition of $FeCl_2$. The authors assume that the *O*-dihydroxy substitution of the central benzene ring is the prerequisite for bioactivities (Norikura et al. 2013).

Fruiting bodies of *T. vialis* were extracted and vialinins A and B together with several other terphenyls were isolated (Xie et al. 2008). Almost all of them exhibited DPPH free-radical scavenging activity except for atromentin and cycloleucomelone. In addition the antiallergic potential was investigated.

All compounds were tested for inhibition of the release of β-hexosaminidase, a marker of degranulation in rat basophilic leukemia-2H3 cells (RBL-2H3), and the production of TNF-α (tumor necrosis factor α, inflammation cytokine). As standard, the clinical immunosuppressant FK506 (tacrolimus) was used. Only vialinins A and B were able to inhibit both assays significantly (IC_{50} P-hexosaminidase release: 500 nM of both compounds/control 0.03 nM and IC_{50} of TNF-α production 0.09 and 0.02 nM, respectively/control 0.25 nM), but they lose their activity when the Ca^{2+}-ATPase is inhibited and the protein kinase C (PKC) is activated (whereas FK506 is as active as before). These observations suggest that the inhibition occurs before the increase of calcium concentration and activation of PKC (Xie et al. 2008). Vialinin A was able to inhibit the production of inflammatory cytokines TNF-α, interleukin-4 (IL-4), and monocyte chemotactic protein-1 (MCP-1), the last two are responsible for immediate-type hypersensitivity (Onose et al. 2008). Inhibition of TNF-α was described for thelephantin G (Ye et al. 2009).

To investigate the mode of action of vialinin A and related compounds, several studies were conducted, e.g., on TNF-α release and production (Onose et al. 2012), inhibition assays with human ubiquitin-specific peptidase 5, other deubiquitinating enzymes and thiol proteases, as well as ligand-affinity studies where it was demonstrated that one target enzyme of vialinin A is the ubiquitin-specific peptidase 5/isopeptidase T (USP5/IsoT; Okada et al. 2013). Deubiquitinating enzymes are involved in pathogenesis of neurodegenerative disorders, viral infections, and cancer, and they could serve as target structures for the treatment of several human diseases (for review: Colland 2010; Hanpude et al. 2015). The latest study on vialinin A was published by Yoshioka et al. (2016). Small ubiquitin-related protein modifier (SUMO) family proteins are able to posttranslationally modify target proteins which plays important roles in dynamic response to growth conditions and cellular stresses like DNA damage control, regulation of transcription, chromatin remodeling, and control of cell cycle progress. SUMOs have the capability as potential pharmacological targets in cancer and Alzheimer's disease. SUMOylation and deSUMOylation are regulated by enzymes named SENP (SUMO-specific proteases) which are able to deconjugate SUMO from target proteins and cleave residues off the SUMO carboxyl terminus that exposes two glycines which are required for conjugation (Hendriks and Vertegaal 2016). To date several SENPs are known and most of them have isopeptidase and endopeptidase activity. Yoshioka et al. (2016) evaluated if p-terphenyl compounds inhibit the catalytic domain human SENP1 (cSENP1) and recombinant human full-length SENP1 (rfSENP1) by measuring the cleavage of the substrate SUMO-1-7-amino-4-methylcoumarin. Vialinin A (IC_{50} 1.89 μM/1.64 μM), thelephantin G (IC_{50} 1.52 μM/2.48 μM), DMT (IC_{50} 3.76 μM/2.71 μM), and atromentin (IC_{50} 6.1 μM/3.79 μM) inhibited cSENP1 and rfSENP1, respectively (Yoshioka et al. 2016).

A comprehensive review about the literature on bioactive metabolites of the genus *Sarcodon* summarizing results until 2010 was authored by Marcotullio (2011). Afterward, two new sarcoviolins (first sarcoviolins with cytotoxic activity at 100 μM were described by Cali et al. 2004), sarcoviolin β (Fig. 18) and episarcoviolin β, were isolated bioactivity

guided from *Sa. leucopus* fruiting bodies (Ma et al. 2014b). Compounds were tested for antioxidant activity in DPPH scavenging assay as well as for α-glucosidase inhibition. Sarcoviolin β and episarcoviolin β showed radical scavenging activity and α-glucosidase inhibition (Ma et al. 2014b).

Two racemic compounds named (±)-tylopilusin A (Fig. 18) and (±)-tylopilusin B (Fukuda et al. 2012) and later on tylopilusin C (Fukuda and Tomoda 2013) were isolated from fruiting bodies of *Tylopilus eximius* (current name: *Sutorius eximius*, Boletaceae). All of them inhibited the pigmentation (golden carotenoid staphyloxanthin) of methicillin-resistant *S. aureus* (MRSA) without effecting the growth of MRSA (Fukuda et al. 2012; Fukuda and Tomoda 2013).

The virulence factor staphyloxanthin is an antioxidant which might play a role in protecting MRSA to be susceptible to the host immune system (e.g., defense mechanism against ROS produced by neutrophils). Virulence factors are of interest in novel strategies to combat preferentially multiresistant bacteria. Atromentin which was isolated from the same culture might be the biosynthetic precursor of the aforementioned compounds (Fukuda and Tomoda 2013).

Currently there seems to be a major interest in characterizing the pharmacological properties of terphenyls. Many new derivatives were described in recent years, and only a fraction thereof is yet characterized.

III. Conclusions

As can be deduced from the numerous new structures, interest in basidiomycete secondary metabolism has gained momentum for various reasons. The availability of basidiomycete metabolites is facilitated by important progress in fermentation technologies and genetics, opening access to templates for chemical syntheses and providing new chemical approaches to yet unexplored biological targets.

The biological activities are interesting and may help to define new lead compounds offering structures not easily detected by the random screening of libraries derived from combinatorial chemical synthesis.

Most of the secondary metabolites are identified in specific assays or are evaluated selectively only in few bioassays which makes it hard to determine if they could serve as leads for novel pharmaceuticals or pesticides especially if no further research is published. Unfortunately for most of the aforementioned natural products, further characterization is hampered due to various reasons, e.g., nonavailability of sufficient quantities to test compounds in additional assays or the lack of a broad spectrum of assays. But nevertheless several promising examples can be found in this overview and in the future will show if they will provide lead structures, chemotaxonomic markers, or insights into ecological roles of secondary metabolites.

References

Abraham W-R (2001) Bioactive sesquiterpenes produced by fungi: are they useful for humans as well? Curr Med Chem 8:583–606. https://doi.org/10.2174/0929867013373147

Agger S, Lopez-Gallego F, Schmidt-Dannert C (2009) Diversity of sesquiterpene synthases in the basidiomycete *Coprinus cinereus*. Mol Microbiol 72:1181–1195. https://doi.org/10.1111/j.1365-2958.2009.06717.x

Andernach L, Opatz T (2014) Assignment of the absolute configuration and total synthesis of (+)-caripyrin. Eur J Org Chem 2014:4780–4784. https://doi.org/10.1002/ejoc.201402540

Aqueveque P, Anke T, Sterner O (2002) The himanimides, new bioactive compounds from *Serpula himantioides* (Fr.)Karst. Z Naturforsch C J Biosci 57:257–262. https://doi.org/10.1515/znc-2002-3-410

Asakawa Y, Hashimoto T (1998) Biologically active substances of Japanese inedible mushrooms. Heterocycles 47:1067. https://doi.org/10.3987/REV-97-SR(N)6

Asakawa Y, Hashimoto T, Mizuno Y et al (1992) Cryptoporic acids A-G, drimane-type sesquiterpenoid ethers of isocitric acid from the fungus *Cryptoporus volvatus*. Phytochemistry 31:579–592. https://doi.org/10.1016/0031-9422(92)90042-O

Ayer WA, Cruz ER (1993) The tremulanes, a new group of sesquiterpenes from the aspen rotting fungus *Phellinus tremulae*. J Org Chem 58(26):7529–7534

Ayer WA, Shan R, Trifonov LS, Hutchison LJ (1998) Sesquiterpenes from the nematicidal fungus *Clitocybula oculus* in honour of professor G. H. Neil

Towers 75th birthday. Phytochemistry 49:589–592. https://doi.org/10.1016/S0031-9422(98)00173-3

Bai R, Zhang CC, Yin X et al (2015) Striatoids A-F, cyathane diterpenoids with neurotrophic activity from cultures of the fungus *Cyathus striatus*. J Nat Prod 78:783–788. https://doi.org/10.1021/np501030r

Basavaiah D, Devendar B, Aravindu K, Veerendhar A (2010) A facile one-pot transformation of Baylis-Hillman adducts into unsymmetrical disubstituted maleimide and maleic anhydride frameworks: a facile synthesis of himanimide A. Chem Eur J 16 (2031–2035):S2031/1–S2031/59. https://doi.org/10.1002/chem.200902887

Baumann C, Bröckelmann M, Fugmann B et al (1993) Haematopodin, an unusual pyrroloquinoline derivative isolated from the fungus *Mycena haematopus*, agaricales. Angew Chem Int Ed Eng 32:1087–1089. https://doi.org/10.1002/anie.199310871

Bertinetti B, Scandiani M, Cabrera G (2011) Analogs of antifungal indoles isolated from *Aporpium caryae* with activity against sudden-death syndrome of soybean. Am J Plant Sci 2:245–254. https://doi.org/10.4236/ajps.2011.22026

Bette E, Otto A, Dräger T et al (2015) Isolation and asymmetric total synthesis of fungal secondary metabolite hygrophorone B^{12}. Eur J Org Chem 2015:2357–2365. https://doi.org/10.1002/ejoc.201403455

Borgström B (1988) Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. Biochim Biophys Acta 962:308–316

Breheret S, Talou T, Rapior S, Bessiere J-M (1999) Geosmin, a sesquiterpenoid compound responsible for the musty-earthy odor of *Cortinarius herculeus*, *Cystoderma amianthinum*, and *Cy. carcharias*. Mycologia 91:117. https://doi.org/10.2307/3761199

Bresinsky A, Besl H (1985) Giftpilze. Wissenschaftliche Verlagsgesellschaft, Stuttgart

Bunyapaiboonsri T, Yoiprommarat S, Nopgason R et al (2014) Cadinane sesquiterpenoids from the basidiomycete *Stereum* cf. *sanguinolentum* BCC 22926. Phytochemistry 105:123–128. https://doi.org/10.1016/j.phytochem.2014.06.006

Cabrera GM, Roberti MJ, Wright JE, Seldes AM (2002) Cryptoporic and isocryptoporic acids from the fungal cultures of *Polyporus arcularius* and *P. ciliatus*. Phytochemistry 61:189–193. https://doi.org/10.1016/S0031-9422(02)00221-2

Cali V, Spatafora C, Tringali C (2004) Sarcodonins and sarcoviolins, bioactive polyhydroxy-p-terphenyl pyrazinediol dioxide conjugates from fruiting bodies of the basidiomycete *Sarcodon leucopus*. Eur J Org Chem 2004(3):592–599. https://doi.org/10.1002/ejoc.200300407

Cazal CM, Choosang K, Severino VGP et al (2010) Evaluation of effect of triterpenes and limonoids on cell growth, cell cycle and apoptosis in human tumor cell lines. Anti Cancer Agents Med Chem 10:769–776

Chen H-P, Zhao Z-Z, Yin R-H et al (2014) Six new vibralactone derivatives from cultures of the fungus *Boreostereum vibrans*. Nat Prod Bioprospect 4:271–276. https://doi.org/10.1007/s13659-014-0029-z

Chen CC, Tzeng TT, Chen CC et al (2016a) Erinacine S, a rare sesterterpene from the mycelia of *Hericium erinaceus*. J Nat Prod 79:438–441. https://doi.org/10.1021/acs.jnatprod.5b00474

Chen H-P, Zhao Z-Z, Li Z-H et al (2016b) Novel natural oximes and oxime esters with a vibralactone backbone from the basidiomycete *Boreostereum vibrans*. ChemistryOpen 5:142–149. https://doi.org/10.1002/open.201500198

Cheng C-F, Lai Z-C, Lee Y-J (2008) Total synthesis of (±)-camphorataimides and (±)-himanimides by NaBH4/Ni(OAc)2 or Zn/AcOH stereoselective reduction. Tetrahedron 64:4347–4353. https://doi.org/10.1016/j.tet.2008.02.077

Chepkirui C, Richter C, Matasyoh JC, Stadler M (2016) Monochlorinated calocerins A-D and 9-oxostrobilurin derivatives from the basidiomycete *Favolaschia calocera*. Phytochemistry 132:95–101. https://doi.org/10.1016/j.phytochem.2016.10.001

Chudzik M, Korzonek-Szlacheta I, Król W (2015) Triterpenes as potentially cytotoxic compounds. Molecules 20:1610–1625. https://doi.org/10.3390/molecules20011610

Clericuzio M, Sterner O (1997) Conversion of velutinal esters in the fruit bodies of *Russula cuprea*. Phytochemistry 45:1569–1572. https://doi.org/10.1016/S0031-9422(97)00259-8

Colland F (2010) The therapeutic potential of deubiquitinating enzyme inhibitors. Biochem Soc Trans 38:137–143. https://doi.org/10.1042/BST0380137

Daum RS, Kar S, Kirkpatrick P (2007) Retapamulin. Nat Rev Drug Discov 6:865–866. https://doi.org/10.1038/nrd2442

Davoli P, Mucci A, Schenetti L, Weber RWS (2005) Laetiporic acids, a family of non-carotenoid polyene pigments from fruit-bodies and liquid cultures of *Laetiporus sulphureus* (Polyporales, Fungi). Phytochemistry 66:817–823. https://doi.org/10.1016/j.phytochem.2005.01.023

De Silva DD, Rapior S, Sudarman E et al (2013) Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Divers 62:1–40. https://doi.org/10.1007/s13225-013-0265-2

Dekker FJ, Rocks O, Vartak N et al (2010) Small-molecule inhibition of APT1 affects Ras localization and signaling. Nat Chem Biol 6:449–456. https://doi.org/10.1038/nchembio.362

Dong M, Chen S-P, Kita K et al (2009) Anti-proliferative and apoptosis-inducible activity of Sarcodonin G from *Sarcodon scabrosus* in HeLa cells. Int J Oncol 34:201–207. https://doi.org/10.3892/ijo_00000142

Engler-Lohr M, Ankea T, Hellwig V, Steglich W (1999) Noroudemansin A, a new antifungal antibiotic from *Pterula* species 82168 and three semisynthetic derivatives. Z Naturforsch C J Biosci 54:163–168

Evans L, Hedger J, O'Donnell G et al (2010) Structure elucidation of some highly unusual tricyclic cis-caryophyllane sesquiterpenes from *Marasmiellus troyanus*. Tetrahedron Lett 51:5493–5496. https://doi.org/10.1016/j.tetlet.2010.08.036

Fabian K, Lorenzen K, Anke T et al (1998) Five new bioactive sesquiterpenes from the fungus *Radulomyces confluens* (Fr.) Christ. Z Naturforsch C J Biosci 53:939–945

Fang S-T, Zhang L, Li Z-H et al (2010) Cyathane diterpenoids and nitrogenous terphenyl derivative from the fruiting bodies of basidiomycete *Phellodon niger*. Chem Pharm Bull 58:1176–1179. https://doi.org/10.1248/cpb.58.1176

Fang S-T, Feng T, Zhang L et al (2011) Cyathane diterpenoids from fruiting bodies of *Phellodon niger*. Nat Prod Bioprospect 1:37–40. https://doi.org/10.1007/s13659-011-0002-z

Feng T, Li Z-H, Dong Z-J et al (2011) Non-isoprenoid botryane sesquiterpenoids from basidiomycete *Boletus edulis* and their cytotoxic activity. Nat Prod Bioprospect 1(1):29–32. https://doi.org/10.1007/s13659-011-0005-9

Feng Y, Wang L, Niu S et al (2012) Naphthalenones from a *Perenniporia* sp. inhabiting the larva of a phytophagous weevil, *Euops chinesis*. J Nat Prod 75:1339–1345. https://doi.org/10.1021/np300263u

Florey H, Chain E, Heatley N et al (1949) Antibiotics. A survey of penicillin, streptomycin, and other antimicrobial substances from fungi, actinomycetes, bacteria, and plants. Oxford University Press, Oxford

Fraga BM (2009) Natural sesquiterpenoids. Nat Prod Rep 26:1125–1155. https://doi.org/10.1039/B908720F

Fraga BM (2010) Natural sesquiterpenoids. Nat Prod Rep 27:1681–1708. https://doi.org/10.1039/C0NP00007H

Fraga BM (2011) Natural sesquiterpenoids. Nat Prod Rep 30:1226. https://doi.org/10.1039/c3np70047j

Fraga BM (2012) Natural sesquiterpenoids. Nat Prod Rep 29:1334–1366. https://doi.org/10.1039/C2NP20074K

Fraga BM (2013) Natural sesquiterpenoids. Nat Prod Rep 30:1226. https://doi.org/10.1039/c3np70047j

Froufe HJC, Abreu RMV, Ferreira ICFR (2013) Virtual screening of low molecular weight mushrooms compounds as potential Mdm2 inhibitors. J Enzyme Inhib Med Chem 28:569–575. https://doi.org/10.3109/14756366.2012.658787

Fukuda T, Tomoda H (2013) Tylopilusin C, a new diphenolic compound from the fruiting bodies of *Tylopilus eximinus*. J Antibiot 66:355–357. https://doi.org/10.1038/ja.2013.23

Fukuda T, Nagai K, Tomoda H (2012) (±)-Tylopilusins, diphenolic metabolites from the fruiting bodies of *Tylopilus eximius*. J Nat Prod 75:2228–2231. https://doi.org/10.1021/np300428r

Gao L, Han J, Si J et al (2017) Cryptoporic acid E from *Cryptoporus volvatus* inhibits influenza virus replication in vitro. Antivir Res 143:106–112. https://doi.org/10.1016/j.antiviral.2017.02.010

Gehrt A, Erkel G, Anke T, Sterner O (1998) Nitidon, a new bioactive metabolite from the basidiomycete *Junghuhnia nitida* (Pers.: Fr.) Ryv. Z Naturforsch C J Biosci 53:89–92

Geraci C, Neri P, Paterno C et al (2000) An unusual nitrogenous terphenyl derivative from fruiting bodies of the basidiomycete *Sarcodon leucopus*. J Nat Prod 63:347–351. https://doi.org/10.1021/np990293d

Gilardoni G, Clericuzio M, Tosi S et al (2007) Antifungal acylcyclopentenediones from fruiting bodies of *Hygrophorus chrysodon*. J Nat Prod 70:137–139. https://doi.org/10.1021/np060512c

Gill M (1999) Pigments of fungi (Macromycetes). Nat Prod Rep 16:301–317. https://doi.org/10.1039/a705730j

Gill M, Steglich W (1987) Pigments of fungi (Macromycetes). In: Zechmeier L (ed) Progress in the chemistry of organic natural products, vol 51. Springer, Heidelberg, pp 1–297

Hadváry P, Lengsfeld H, Wolfer H (1988) Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. Biochem J 256:357–361

Han J-J, Bao L, He L-W et al (2013a) Phaeolschidins A-E, five hispidin derivatives with antioxidant activity from the fruiting body of *Phaeolus schweinitzii* collected in the tibetan plateau. J Nat Prod 76:1448–1453. https://doi.org/10.1021/np400234u

Han JJ, Chen YH, Bao L et al (2013b) Anti-inflammatory and cytotoxic cyathane diterpenoids from the medicinal fungus *Cyathus africanus*. Fitoterapia 84:22–31. https://doi.org/10.1016/j.fitote.2012.10.001

Han J-J, Zhang L, Xu J-K et al (2015) Three new cyathane diterpenoids from the medicinal fungus *Cyathus africanus*. J Asian Nat Prod Res 17:541–549. https://doi.org/10.1080/10286020.2015.1043900

Hanpude P, Bhattacharya S, Dey A, Maiti T (2015) Critical review: Deubiquitinating enzymes in cellular signaling and disease regulation enzymatic mechanism of DUBs. IUBMB Life 67:544–555. https://doi.org/10.1002/iub.1402

Hasegawa M, Akabori Y, Akabori S (1974) New indanone compounds from *Onychium japonicum*. Phytochemistry 13:509–511. https://doi.org/10.1016/S0031-9422(00)91246-9

Hashimoto T, Tori M, Mizuno Y, Asakawa Y (1987) Cryptoporic acids A and B, novel bitter drimane sesquiterpenoid ethers of isocitric acid, from the fungus *Cryptoporus volvatus* (Polyporaceae). Tetrahedron Lett 28:6303–6304. https://doi.org/10.1016/S0040-4039(01)91358-9

Hashimoto T, Tori M, Mizuno Y et al (1989) The superoxide release inhibitors, cryptoporic acids C, D, and E; dimeric drimane sesquiterpenoid ethers of isocitric acid from the fungus *Cryptoporus volvatus*. J Chem Soc Chem Commun 1989:258–259. https://doi.org/10.1039/c39890000258

He L, Han J, Li B et al (2016) Identification of a new cyathane diterpene that induces mitochondrial and autophagy-dependent apoptosis and shows a potent in vivo anti-colorectal cancer activity. Eur J Med Chem 111:183–192. https://doi.org/10.1016/j.ejmech.2016.01.056

Hellwig V, Dasenbrock J, Gräf C et al (2002) Calopins and cyclocalopins – bitter principles from *Boletus calopus* and related mushrooms. Eur J Org Chem 2002:2895–2904. https://doi.org/10.1002/1099-0690(200209)2002:17<2895::AID-EJOC2895>3.0.CO;2-S

Hendriks IA, Vertegaal ACO (2016) A comprehensive compilation of SUMO proteomics. Nat Rev Mol Cell Biol 17:581–595. https://doi.org/10.1038/nrm.2016.81

Hill RA, Connolly JD (2015) Triterpenoids. Nat Prod Rep 32:273–327. https://doi.org/10.1039/C4NP00101J

Hirota M, Morimura K, Shibata H (2002) Anti-inflammatory compounds from the bitter mushroom, *Sarcodon scabrosus*. Biosci Biotechnol Biochem 66:179–184. https://doi.org/10.1271/bbb.66.179

Hirotani M, Furuya T, Shiro M (1991) Studies on the metabolites of higher fungi. Part 10. Cryptoporic acids H and I, drimane sesquiterpenes from *Ganoderma neo-japonicum* and *Cryptoporus volvatus*. Phytochemistry 30:1555–1559. https://doi.org/10.1016/0031-9422(91)84208-A

Ho C-L, Cheng H, Chen MC-M, Ettinger DS (2015) Antroquinonol to block Ras and Rho signaling via the inhibition of protein isoprenyltransferase activity in cancer cells. J Clin Oncol 33:e18526–e18526. https://doi.org/10.1200/jco.2015.33.15_suppl.e18526

Högenauer G (1979) Tiamulin and pleuromutilin. In: Hahn F (ed) Mechanism of action of antibacterial agents. Springer, Berlin, Heidelberg, pp 344–360

Hoppmann CD, Kurz MD, Müller G, Toti LD (2001) Inventors; Aventis pharma Deutschland GmbH, assignee. Percyquinnin, a process for its production and its use as a pharmaceutical. European patent EP1142886 (A1)

Hsu C-S, Chou H-H, Fang J-M (2015) A short synthesis of (±)-antroquinonol in an unusual scaffold of 4-hydroxy-2-cyclohexenone. Org Biomol Chem 13:5510–5519. https://doi.org/10.1039/C5OB00411J

Hu D, Li W, Zhao Z et al (2014) Highly unsaturated pyranone derivatives from the basidiomycete *Junghuhnia nitida*. Tetrahedron Lett 55:6530–6533. https://doi.org/10.1016/j.tetlet.2014.09.132

Huang Z, Dan Y, Huang Y et al (2004) Sesquiterpenes from the mycelial cultures of *Dichomitus squalens*. J Nat Prod 67:2121–2123. https://doi.org/10.1021/np0497144

Huang L, Han J, Ben-Hail D, He L, Li B, Chen Z, Wang Y, Yang Y, Liu L, Zhu Y, Shoshan-Barmatz V, Liu H, Chen Q (2015) A new fungal diterpene induces VDAC1-dependent apoptosis in bax/bak-deficient cells. J Biol Chem 290(39):23563–23578

IndexFungorum Index Fungorum. www.indexfungorum.org. Accessed 20 June 2017

Isaka M, Srisanoh U, Sappan M et al (2012) Sterostreins F–O, illudalanes and norilludalanes from cultures of the basidiomycete *Stereum ostrea* BCC 22955. Phytochemistry 79:116–120. https://doi.org/10.1016/j.phytochem.2012.04.009

Isaka M, Chinthanom P, Danwisetkanjana K, Choeyklin R (2014a) A new cryptoporic acid derivative from cultures of the basidiomycete *Poria albocincta* BCC 26244. Phytochem Lett 7:97–100. https://doi.org/10.1016/j.phytol.2013.10.009

Isaka M, Chinthanom P, Sappan M, Supothina S (2014b) Phenylglycol metabolites from cultures of the basidiomycete *Mycena pruinosoviscida* BCC 22723. Helv Chim Acta 97:909–914

Isaka M, Chinthanom P, Sappan M, Danwisetkanjana K (2016a) Antitubercular lanostane triterpenes from cultures of the basidiomycete *Ganoderma* sp. BCC 16642. J Nat Prod 79(1):161–169. https://doi.org/10.1021/acs.jnatprod.5b00826

Isaka M, Palasarn S, Sappan M et al (2016b) Hirsutane sesquiterpenes from cultures of the basidiomycete *Marasmiellus* sp. BCC 22389. Nat Prod Bioprospect 6:257–260. https://doi.org/10.1007/s13659-016-0105-7

Isaka M, Palasarn S, Supothina S et al (2016c) Seco-Tremulanes from cultures of the basidiomycete *Flavodon flavus* BCC 17421. Helv Chim Acta 99:232–236. https://doi.org/10.1002/hlca.201500249

Ishikawa NK, Yamaji K, Tahara S et al (2000) Highly oxidized cuparene-type sesquiterpenes from a mycelial culture of *Flammulina velutipes*. Phytochemistry 54:777–782. https://doi.org/10.1016/S0031-9422(00)00189-8

Ishikawa NK, Fukushi Y, Yamaji K et al (2001) Antimicrobial cuparene-type sesquiterpenes, enokipodins C and D, from a mycelial culture of *Flammulina velutipes*. J Nat Prod 64:932–934. https://doi.org/10.1021/np000593r

Ito-Kobayashi M, Aoyagi A, Tanaka I et al (2008) Sterenin A, B, C and D, novel 11β-hydroxysteroid dehydrogenase type 1 inhibitors from *Stereum* sp. SANK 21205. J Antibiot 61:128–135. https://doi.org/10.1038/ja.2008.121

Jaeger RJR, Spiteller P (2010) Mycenaaurin A, an antibacterial polyene pigment from the fruiting bodies of *Mycena aurantiomarginata*. J Nat Prod 73:1350–1354. https://doi.org/10.1021/np100155z

Jaeger RJR, Lamshöft M, Gottfried S et al (2013) HR-MALDI-MS imaging assisted screening of beta-Carboline alkaloids discovered from *Mycena metata*. J Nat Prod 76:127–134. https://doi.org/10.1021/np300455a

JGI (2016) Joint Genome Institute. http://genome.jgi.doe.gov/programs/fungi/index.jsf. Accessed 12 December 2016

Jiang M-Y, Wang F, Yang X-L et al (2008) Derivatives of vibralactone from cultures of the basidiomycete *Boreostereum vibrans*. Chem Pharm Bull 56:1286–1288. https://doi.org/10.1248/cpb.56.1286

Jiang M-Y, Zhang L, Liu R et al (2009) Speciosins A-K, oxygenated cyclohexanoids from the basidiomycete *Hexagonia speciosa*. J Nat Prod 72:1405–1409. https://doi.org/10.1021/np900182m

Jiang M, Zhang L, Dong Z et al (2010) Vibralactones D-F from cultures of the basidiomycete *Boreostereum vibrans*. Chem Pharm Bull (Tokyo) 58:113–116. https://doi.org/10.1248/cpb.58.113

Jiang M-Y, Li Y, Wang F, Liu J-K (2011) Isoprenylated cyclohexanoids from the basidiomycete *Hexagonia speciosa*. Phytochemistry (Elsevier) 72:923–928. https://doi.org/10.1016/j.phytochem.2011.03.011

Johansson M, Sterner O, Labischinski H, Anke T (2001) Coprinol, a new antibiotic cuparane from a *Coprinus* species. Z Naturforsch C J Biosci 56:31–34

Jones RN, Fritsche TR, Sader HS, Ross JE (2006) Activity of retapamulin (SB-275833), a novel pleuromutilin, against selected resistant gram-positive cocci. Antimicrob Agents Chemother 50:2583–2586. https://doi.org/10.1128/AAC.01432-05

Jülich C, Nord CL, Menkis A et al (2013) Protoilludane sesquiterpenes from the wood decomposing fungus *Granulobasidium vellereum* (Ellis & Cragin) Jülich. Phytochemistry 90:128–134. https://doi.org/10.1016/j.phytochem.2013.02.015

Kahner L, Dasenbrock J, Spiteller P et al (1998) Polyene pigments from fruit-bodies of *Boletus laetissimus* and *B. rufo-aureus* (Basidiomycetes). Phytochemistry 49:1693–1697. https://doi.org/10.1016/S0031-9422(98)00319-7

Kalb D, Lackner G, Hoffmeister D (2013) Fungal peptide synthetases: an update on functions and specificity signatures. Fungal Biol Rev 27:43–50. https://doi.org/10.1016/j.fbr.2013.05.002

Kamo T, Imura Y, Hagio T et al (2004) Anti-inflammatory cyathane diterpenoids from *Sarcodon scabrosus*. Biosci Biotechnol Biochem 68:1362–1365. https://doi.org/10.1271/bbb.68.1362

Kang H-S, Kim J-P (2016) Ostalactones A–C, β- and ε-lactones with lipase inhibitory activity from the cultured basidiomycete *Stereum ostrea*. J Nat Prod 79:3148–3151. https://doi.org/10.1021/acs.jnatprod.6b00647

Kavanagh F, Hervey A, Robbins WJ (1951) Antibiotic substances from basidiomycetes: VIII. *Pleurotus multilus* (Fr.) Sacc. and *Pleurotus passeckerianus* Pilat. Proc Natl Acad Sci USA 37:570–574

Kawagishi H, Zhuang C (2008) Compounds for dementia from *Hericium erinaceum*. Drugs Future 33:149. https://doi.org/10.1358/dof.2008.033.02.1173290

Kawai T, Mizutani S, Enoki T et al (2005) Inventors; Takara Bio Inc., assignee. Antitumor agent. WO2005115364 A1

Kettering M, Sterner O, Anke T (2004) Antibiotics in the chemical communication of fungi. Z Naturforsch C J Biosci 59:816–823

Ki D-W, Kim D-W, Hwang BS et al (2015) New antioxidant sesquiterpenes from a culture broth of *Coprinus echinosporus*. J Antibiot 68:351–353. https://doi.org/10.1038/ja.2014.158

Kim H-J, Vinale F, Ghisalberti EL et al (2006) An antifungal and plant growth promoting metabolite from a sterile dark ectotrophic fungus. Phytochemistry 67:2277–2280. https://doi.org/10.1016/j.phytochem.2006.07.022

Kim KH, Moon E, Choi SU et al (2013) Lanostane triterpenoids from the mushroom *Naematoloma fasciculare*. J Nat Prod 76:845–851. https://doi.org/10.1021/np300801x

Kim SO, Sakchaisri K, Asami Y et al (2014) Illudins C2 and C3 stimulate lipolysis in 3T3-L1 adipocytes and suppress adipogenesis in 3T3-L1 preadipocytes. J Nat Prod 77:744–750. https://doi.org/10.1021/np400520a

Kita T, Takaya Y, Oshima Y et al (1998) Scabronines B, C, D, E, F, novel diterpenoids showing stimulating activity of nerve growth factor-synthesis, from the mushroom *Sarcodon scabrosus*. Tetrahedron 54:11877–11886. https://doi.org/10.1016/S0040-4020(98)83045-7

Klaiklay S, Rukachaisirikul V, Phongpaichit S et al (2012) Flavodonfuran: a new difuranylmethane derivative from the mangrove endophytic fungus *Flavodon flavus* PSU-MA201. Nat Prod Res 27:1722. https://doi.org/10.1080/14786419.2012.750315

Kokubun T, Scott-Brown A, Kite GC, Simmonds MSJ (2016) Protoilludane, illudane, illudalane, and norilludane sesquiterpenoids from *Granulobasidium vellereum*. J Nat Prod 79:1698–1701. https://doi.org/10.1021/acs.jnatprod.6b00325

Kornsakulkarn J, Thongpanchang C, Chainoy R et al (2010) Bioactive metabolites from cultures of basidiomycete *Favolaschia tonkinensis*. J Nat Prod 73:759–762

Lee I, Jeong C, Cho S et al (1996) Illudins C2 and C3, new illudin C derivatives from *Coprinus atramentarius* ASI20013. J Antibiot 49:821–822

Lee T-H, Lee C-K, Tsou W-L et al (2007) A new cytotoxic agent from solid-state fermented mycelium of *Antrodia camphorata*. Planta Med 73:1412–1415. https://doi.org/10.1055/s-2007-990232

Lee JS, Maarisit W, Abdjul DB et al (2016) Structures and biological activities of triterpenes and sesquiterpenes obtained from *Russula lepida*. Phytochemistry 127:63–68. https://doi.org/10.1016/j.phytochem.2016.03.014

Levy LM, Cabrera GM, Wright JE, Seldes AM (2000) Indole alkaloids from a culture of the fungus *Aporpium caryae*. Phytochemistry 54:941–943. https://doi.org/10.1016/S0031-9422(00)00127-8

Levy LM, Cabrera GM, Wright JE, Seldes AM (2003) 5H-Furan-2-ones from fungal cultures of *Aporpium caryae*. Phytochemistry (Elsevier) 62:239–243. https://doi.org/10.1016/S0031-9422(02)00455-7

Li G-H, Duan M, Yu Z-F et al (2008) Stereumin A-E, sesquiterpenoids from the fungus *Stereum* sp.

CCTCC AF 207024. Phytochemistry 69:1439–1445. https://doi.org/10.1016/j.phytochem.2008.01.012

Li G, Liu F, Shen L et al (2011) Stereumins H-J,-stereumane-type sesquiterpenes from the fungus *Stereum* sp. J Nat Prod 74:296–299. https://doi.org/10.1021/np100813f

Liermann JC, Schüffler A, Wollinsky B et al (2010) Hirsutane-type sesquiterpenes with uncommon modifications from three basidiomycetes. J Org Chem 75:2955–2961. https://doi.org/10.1021/jo100202b

Liermann JC, Thines E, Opatz T, Anke H (2012) Drimane sesquiterpenoids from *Marasmius* sp. Inhibiting the conidial germination of plant-pathogenic fungi. J Nat Prod 75:1983–1986. https://doi.org/10.1021/np300337w

Lin J, Wang R, Xu G et al (2016) New cadinane sesquiterpenoids from the basidiomycetous fungus *Pholiota* sp. RSC Adv 6:112527–112533. https://doi.org/10.1039/C6RA22448B

List A, Zeiler E, Gallastegui N et al (2014) Omuralide and vibralactone: differences in the proteasome-beta-lactone-gamma-lactam binding scaffold alter target preferences. Angew Chem Int Ed 53:571–574. https://doi.org/10.1002/anie.201308567

Liu J-K (2006) Natural terphenyls: developments since 1877. Chem Rev 106:2209–2223. https://doi.org/10.1021/cr050248c

Liu D-Z (2014) A review of ergostane and cucurbitane triterpenoids of mushroom origin. Nat Prod Res 28:1099–1105. https://doi.org/10.1080/14786419.2014.900767

Liu D-Z, Wang F, Liao T-G et al (2006) Vibralactone: a lipase inhibitor with an unusual fused β-lactone produced by cultures of the basidiomycete *Boreostereum vibrans*. Org Lett 8:5749–5752. https://doi.org/10.1021/ol062307u

Liu D-Z, Wang F, Liu J-K (2007) Sesquiterpenes from cultures of the basidiomycete *Conocybe siliginea*. J Nat Prod 70:1503–1506. https://doi.org/10.1021/np070140n

Liu D-Z, Jia R-R, Wang F, Liu J-K (2008a) A new spiroaxane sesquiterpene from cultures of the basidiomycete *Pholiota adiposa*. Z Naturforsch B Chem Sci 63:111–113

Liu DZ, Wang F, Jia RR, Liu JK (2008b) A novel sesquiterpene from the basidiomycete *Boletus calopus*. Z Naturforsch B J Chem Sci 63:114–116

Liu YJ, Liu Y, Zhang KQ (2008c) Xanthothone, a new nematicidal N-compound from *Coprinus xanthothrix*. Chem Nat Compd 44:203–205. https://doi.org/10.1007/s10600-008-9014-2

Liu F-F, Li G-H, Yang Z-S et al (2010) Two new sesquiterpenes from the fungus *Stereum* sp. Helv Chim Acta 93:1737–1741. https://doi.org/10.1002/hlca.200900433

Liu L, Shi X-W, Zong S-C et al (2012) Scabronine M, a novel inhibitor of NGF-induced neurite outgrowth from PC12 cells from the fungus *Sarcodon scabrosus*. Bioorg Med Chem Lett 22:2401–2406. https://doi.org/10.1016/j.bmcl.2012.02.031

Liu L-Y, Li Z-H, Si J et al (2013) Two new sesquiterpenoids from the fungus *Ceriporia alachuana*. J Asian Nat Prod Res 15:300–304. https://doi.org/10.1080/10286020.2013.763798

Long KS, Hansen LH, Jakobsen L, Vester B (2006) Interaction of pleuromutilin derivatives with the ribosomal peptidyl transferase center. Antimicrob Agents Chemother 50(4):1458–1462

Lopez-Gallego F, Agger SA, Abate-Pella D et al (2010) Sesquiterpene synthases Cop4 and Cop6 from *Coprinus cinereus*: catalytic promiscuity and cyclization of farnesyl pyrophosphate geometric isomers. ChemBioChem 11:1093–1106. https://doi.org/10.1002/cbic.200900671

Lorenzen K, Anke T (1998) Biologically active metabolites from basidiomycetes. Curr Org Chem 2:329–364

Lu M-C,El-Shazly M, Wu T-Y et al (2013) Recent research and development of *Antrodia cinnamomea*. Pharmacol Ther 139:124–156. https://doi.org/10.1016/j.pharmthera.2013.04.001

Luana G, Fabiano S, Fabio G, Paolo G (2015) Comparing visual inspection of trees and molecular analysis of internal wood tissues for the diagnosis of wood decay fungi. Forestry 88:465–470. https://doi.org/10.1093/forestry/cpv015

Lübken T (2006) Hygrophorone, neue antifungische Cyclopentenonderivate aus Hygrophorus-Arten. PhD thesis, University of Halle, Halle, Germany

Lübken T, Schmidt J, Porzel A et al (2004) Hygrophorones A-G: fungicidal cyclopentenones from *Hygrophorus* species (Basidiomycetes). Phytochemistry 65:1061–1071. https://doi.org/10.1016/j.phytochem.2004.01.023

Lübken T, Arnold N, Wessjohann L et al (2006) Analysis of fungal cyclopentenone derivatives from *Hygrophorus* spp. by liquid chromatography/electrospray-tandem mass spectrometry. J Mass Spectrom 41:361–371. https://doi.org/10.1002/jms.996

Ma B-J, Liu J-K (2005) An unusual nitrogenous terphenyl derivative from fruiting bodies of the basidiomycete *Sarcodon scabrosus*. Z Naturforsch B Chem Sci 60:565–568

Ma B-J, Ruan Y (2008) Scabronine J, a new cyathane-type diterpenoid from the basidiomycete *Sarcodon scabrosus*. J Antibiot 61:86–88. https://doi.org/10.1038/ja.2008.115

Ma B-J, Zhu H-J, Liu J-K (2004) Isolation and characterization of new bitter diterpenoids from the basidiomycete *Sarcodon scabrosus*. Helv Chim Acta 87:2877–2881. https://doi.org/10.1002/hlca.200490259

Ma B-J, Shen J-W, Yu H-Y et al (2010) Hericenones and erinacines: stimulators of nerve growth factor (NGF) biosynthesis in *Hericium erinaceus*. Mycology 1:92–98. https://doi.org/10.1080/21501201003735556

Ma K, Bao L, Han J et al (2014a) New benzoate derivatives and hirsutane type sesquiterpenoids with antimicrobial activity and cytotoxicity from the solid-state fermented rice by the medicinal mushroom *Stereum hirsutum*. Food Chem 143:239–245. https://doi.org/10.1016/j.foodchem.2013.07.124

Ma K, Han J, Bao L et al (2014b) Two sarcoviolins with antioxidative and α-glucosidase inhibitory activity from the edible mushroom *Sarcodon leucopus* collected in Tibet. J Nat Prod 77:942–947. https://doi.org/10.1021/np401026b

Ma K, Ren J, Han J et al (2014c) Ganoboninketals A-C, antiplasmodial 3,4-seco-27-norlanostane triterpenes from *Ganoderma boninense* Pat. J Nat Prod 77:1847–1852. https://doi.org/10.1021/np5002863

Malagòn O, Porta A, Clericuzio M et al (2014) Structures and biological significance of lactarane sesquiterpenes from the European mushroom *Russula nobilis*. Phytochemistry 107:126–134. https://doi.org/10.1016/j.phytochem.2014.08.018

Marcotullio MC (2011) *Sarcodon* mushrooms: biologically active metabolites. In: Rasooli I (ed) Phytochemicals – bioactivities and impact on health. InTech, Rijeka

Matsunaga S, Furuya-Suguri H, Nishiwaki S et al (1991) Differential effects of cryptoporic acids D and E, inhibitors of superoxide anion radical release, on tumor promotion of okadaic acid in mouse skin. Carcinogenesis 12:1129–1131. https://doi.org/10.1093/carcin/12.6.1129

McMorris TC, Anchel M (1965) Fungal metabolites. The structures of the novel sesquiterpenoids illudin-S and -M. J Am Chem Soc 87:1594–1600

McMorris TC, Staake MD, Kelner MJ (2004) Synthesis and biological activity of enantiomers of antitumor irofulven. J Org Chem 69:619–623. https://doi.org/10.1021/jo035084j

Mehta G, Pallavi K (2006) Total synthesis of the putative structure of the novel triquinane based sesquiterpenoid natural product dichomitol. Tetrahedron Lett 47:8355–8360. https://doi.org/10.1016/j.tetlet.2006.09.084

Melkonyan FS, Topolyan AP, Karchava AV, Yurovskaya MA (2008) Simple synthesis of methyl 1-(1,1-dimethyl-prop-2-en-1-yl)-1h-indole-3-carboxylate. Chem Heterocycl Compd 44:1288–1290. https://doi.org/10.1007/s10593-009-0183-0

Meng J, Li Y-Y, Ou Y-X et al (2011) New sesquiterpenes from *Marasmius cladophyllus*. Mycology 2:30–36. https://doi.org/10.1080/21501203.2011.554908

Mudalungu CM, Richter C, Wittstein K et al (2016) Laxitextines A and B, cyathane xylosides from the tropical fungus *Laxitextum incrustatum*. J Nat Prod 79:894–898. https://doi.org/10.1021/acs.jnatprod.5b00950

Nakada M (2014) Enantioselective total syntheses of cyathane diterpenoids. Chem Rec (New York, NY) 14:641–662. https://doi.org/10.1002/tcr.201402019

Narisawa T, Fukaura Y, Kotanagi H, Asakawa Y (1992) Inhibitory effect of cryptoporic acid E, a product from fungus *Cryptoporus volvatus*, on colon carcinogenesis induced with N-methyl-N-nitrosourea in rats and with 1,2-dimethylhydrazine in mice. Jpn J Cancer Res 83:830–834. https://doi.org/10.1111/j.1349-7006.1992.tb01987.x

Nishimura H, Tsuda S, Shimizu H et al (2008) De novo synthesis of (Z)- and (E)-7-hexadecenylitaconic acids by a selective lignin-degrading fungus, *Ceriporiopsis subvermispora*. Phytochemistry 69:2593–2602. https://doi.org/10.1016/j.phytochem.2008.07.014

Nishimura H, Murayama K, Watanabe T et al (2009) Absolute configuration of ceriporic acids, the iron redox-silencing metabolites produced by a selective lignin-degrading fungus, *Ceriporiopsis subvermispora*. Chem Phys Lipids 159:77–80. https://doi.org/10.1016/j.chemphyslip.2009.03.006

Nishimura H, Setogawa Y, Watanabe T et al (2011) Epoxy ceriporic acid produced by selective lignin-degrading fungus *Ceriporiopsis subvermispora*. Chem Phys Lipids 164:707–712. https://doi.org/10.1016/j.chemphyslip.2011.07.005

Nishimura H, Murayama K, Watanabe T et al (2012a) Diverse rare lipid-related metabolites including ω-7 and ω-9 alkenylitaconic acids (ceriporic acids) secreted by a selective white rot fungus, *Ceriporiopsis subvermispora*. Chem Phys Lipids 165:97–104. https://doi.org/10.1016/j.chemphyslip.2011.10.007

Nishimura H, Sasaki M, Seike H et al (2012b) Alkadienyl and alkenyl itaconic acids (ceriporic acids G and H) from the selective white-rot fungus *Ceriporiopsis subvermispora*: a new class of metabolites initiating ligninolytic lipid peroxidation. Org Biomol Chem 10:6432–6442. https://doi.org/10.1039/c2ob25415h

Nord C, Menkis A, Broberg A (2014a) Cytotoxic illudalane sesquiterpenes from the wood-decay fungus *Granulobasidium vellereum* (Ellis & Cragin) Jülich. Molecules 19:14195–14203. https://doi.org/10.3390/molecules190914195

Nord CL, Menkis A, Lendel C et al (2014b) Sesquiterpenes from the saprotrophic fungus *Granulobasidium vellereum* (Ellis & Cragin) Jülich. Phytochemistry 102:197–204. https://doi.org/10.1016/j.phytochem.2014.03.012

Nord C, Menkis A, Broberg A (2015) Cytotoxic illudane sesquiterpenes from the fungus *Granulobasidium vellereum* (Ellis and Cragin) Jülich. J Nat Prod 78:2559–2564. https://doi.org/10.1021/acs.jnatprod.5b00500

Norikura T, Fujiwara K, Narita T et al (2011) Anticancer activities of thelephantin O and vialinin A isolated from *Thelephora aurantiotincta*. J Agric Food Chem 59:6974–6979. https://doi.org/10.1021/jf200461j

Norikura T, Fujiwara K, Yanai T et al (2013) P-terphenyl derivatives from the mushroom *Thele-*

phora aurantiotincta suppress the proliferation of human hepatocellular carcinoma cells via iron chelation. J Agric Food Chem 61:1258–1264. https://doi.org/10.1021/jf3041098

Obara Y, Nakahata N, Kita T et al (1999) Stimulation of neurotrophic factor secretion from 1321N1 human astrocytoma cells by novel diterpenoids, scabronines A and G. Eur J Pharmacol 370:79–84. https://doi.org/10.1016/S0014-2999(99)00077-1

Obara Y, Kobayashi H, Ohta T et al (2001) Scabronine G-methylester enhances secretion of neurotrophic factors mediated by an activation of protein kinase C-ζ. Mol Pharmacol 59:1287–1297

Ohashi Y, Kan Y, Watanabe T et al (2007) Redox silencing of the Fenton reaction system by an alkylitaconic acid, ceriporic acid B produced by a selective lignin-degrading fungus, *Ceriporiopsis subvermispora*. Org Biomol Chem 5:840. https://doi.org/10.1039/b614379b

Ohta T, Kita T, Kobayashi N et al (1998) Scabronine A, a novel diterpenoid having potent inductive activity of the nerve growth factor synthesis, isolated from the mushroom, *Sarcodon scabrosus*. Tetrahedron Lett 39:6229–6232. https://doi.org/10.1016/S0040-4039(98)01282-9

Okada K, Ye YQ, Taniguchi K et al (2013) Vialinin A is a ubiquitin-specific peptidase inhibitor. Bioorg Med Chem Lett 23:4328–4331. https://doi.org/10.1016/j.bmcl.2013.05.093

Oliveira AG, Stevani CV (2009) The enzymatic nature of fungal bioluminescence. Photochem Photobiol Sci 8:1416–1421. https://doi.org/10.1039/b908982a

Onose J, Xie C, Ye YQ et al (2008) Vialinin A, a novel potent inhibitor of TNF-α production from RBL-2H3 cells. Biol Pharm Bull 31:831–833. https://doi.org/10.1248/bpb.31.831

Onose J, Yoshioka Y, Qi Y et al (2012) Inhibitory effects of vialinin A and its analog on tumor necrosis factor- a release and production from RBL-2H3 cells. Cell Immunol 279:140–144. https://doi.org/10.1016/j.cellimm.2012.10.008

Opatz T, Kolshorn H, Anke H (2008) Sterelactones: new isolactarane type sesquiterpenoids with antifungal activity from *Stereum* sp. IBWF 01060. J Antibiot (Tokyo) 61:563–567

Otto A, Porzel A, Schmidt J et al (2015) A study on the biosynthesis of hygrophorone B12 in the mushroom *Hygrophorus abieticola* reveals an unexpected labelling pattern in the cyclopentenone moiety. Phytochemistry 118:174–180. https://doi.org/10.1016/j.phytochem.2015.08.018

Otto A, Porzel A, Schmidt J et al (2016) Structure and absolute configuration of pseudohygrophorones A12 and B12, alkyl cyclohexenone derivatives from *Hygrophorus abieticola* (Basidiomycetes). J Nat Prod 79:74–80. https://doi.org/10.1021/acs.jnatprod.5b00675

Otto A, Porzel A, Westermann B et al (2017) Structural and stereochemical elucidation of new hygrophorones from *Hygrophorus abieticola* (Basidiomycetes). Tetrahedron 73:1682–1690. https://doi.org/10.1016/j.tet.2017.02.013

Paci A, Rezai K, Deroussent A et al (2006) Pharmacokinetics, metabolism, and routes of excretion of intravenous irofulven in patients with advanced solid tumors. Drug Metab Dispos 34:1918–1926. https://doi.org/10.1124/dmd.106.010512

Palermo JA, Rodríguez Brasco MF, Spagnuolo C, Seldes AM (2000) Illudalane sesquiterpenoids from the soft coral *Alcyonium paessleri*: The first natural nitrate esters. J Org Chem 65:4482–4486. https://doi.org/10.1021/jo991740x

Pei D, Xu J, Zhuang Q et al (2010) Secondary metabolites from higher fungi: discovery, bioactivity, and bioproduction. Adv Biochem Eng Biotechnol 123:127–141

Peters S, Spiteller P (2007a) Sanguinones A and B, blue pyrroloquinoline alkaloids from the fruiting bodies of the mushroom *Mycena sanguinolenta*. J Nat Prod 70:1274–1277. https://doi.org/10.1021/np070179s

Peters S, Spiteller P (2007b) Mycenarubins A and B, red pyrroloquinoline alkaloids from the mushroom *Mycena rosea*. Eur J Org Chem 2007(10):1571–1576. https://doi.org/10.1002/ejoc.200600826

Peters S, Jaeger RJR, Spiteller P (2008) Red pyrroloquinoline alkaloids from the mushroom *Mycena haematopus*. Eur J Org Chem 2008(2):319–323. https://doi.org/10.1002/ejoc.200700739

Pettit GR, Meng Y, Pettit RK et al (2010a) Antineoplastic agents 582. Part 1: Isolation and structure of a cyclobutane-type sesquiterpene cancer cell growth inhibitor from *Coprinus cinereus* (Coprinaceae). Bioorg Med Chem 18:4879–4883. https://doi.org/10.1016/j.bmc.2010.06.023

Pettit GR, Meng Y, Pettit RK et al (2010b) Antineoplastic agents. 556. Isolation and structure of Coprinastatin 1 from *Coprinus cinereus*. J Nat Prod 73:388–392. https://doi.org/10.1021/np900371j

Pinedo C, Wang C-M, Pradier J-M et al (2008) Sesquiterpene synthase from the botrydial biosynthetic gene cluster of the phytopathogen *Botrytis cinerea*. ACS Chem Biol 3:791–801. https://doi.org/10.1021/cb800225v

Prateeptongkum S, Driller KM, Jackstell R, Beller M (2010) Iron-catalyzed carbonylation as a key step in the short and efficient syntheses of himanimide A and B. Chem Asian J 5:2173–2176. https://doi.org/10.1002/asia.201000384

Pulte A, Wagner S, Kogler H, Spiteller P (2016) Pelianthinarubins A and B, red pyrroloquinoline alkaloids from the fruiting bodies of the mushroom *Mycena pelianthina*. J Nat Prod 79:873–878. https://doi.org/10.1021/acs.jnatprod.5b00942

Purtov KV, Petushkov VN, Baranov MS et al (2015) The chemical basis of fungal bioluminescence. Angew Chem Int Ed 54:8124–8128. https://doi.org/10.1002/anie.201501779

Qin X, Shao H, Dong Z, Liu J (2008) Six new induced sesquiterpenes from the cultures of ascomycete

Daldinia concentrica. J Antibiot (Tokyo) 61:556–562

Quang DN, Hashimoto T, Hitaka Y et al (2003a) Thelephantins D-H: five p-terphenyl derivatives from the inedible mushroom *Thelephora aurantiotincta*. Phytochemistry (Elsevier) 63:919–924. https://doi.org/10.1016/S0031-9422(03)00220-6

Quang DN, Hashimoto T, Nukada M et al (2003b) Thelephantins A, B and C: three benzoyl p-terphenyl derivatives from the inedible mushroom *Thelephora aurantiotincta*. Phytochemistry 62:109–113

Quang DN, Hashimoto T, Hitaka Y et al (2004) Thelephantins I-N;p-terphenyl derivatives from the inedible mushroom *Hydnellum caeruleum*. Phytochemistry 65:1179–1184. https://doi.org/10.1016/j.phytochem.2004.02.018

Quin MB, Flynn CM, Wawrzyn GT et al (2013a) Mushroom hunting by using bioinformatics: application of a predictive framework facilitates the selective identification of sesquiterpene synthases in basidiomycota. Chembiochem 14:2480–2491. https://doi.org/10.1002/cbic.201300349

Quin MB, Wawrzyn G, Schmidt-Dannert C (2013b) Purification, crystallization and preliminary X-ray diffraction analysis of Omp6, a protoilludene synthase from *Omphalotus olearius*. Acta Crystallogr Sect F: Struct Biol Cryst Commun 69:574–577. https://doi.org/10.1107/S1744309113010749

Quin MB, Flynn CM, Schmidt-Dannert C (2014) Traversing the fungal terpenome. Nat Prod Rep 31:1449–1473. https://doi.org/10.1039/c4np00075g

Radulović N, Quang DN, Hashimoto T et al (2005) Terrestrins A-G:p-terphenyl derivatives from the inedible mushroom *Thelephora terrestris*. Phytochemistry 66(9):1052. https://doi.org/10.1016/j.phytochem.2005.03.008

Rahmawati N, Ohashi Y, Watanabe T et al (2005) Ceriporic acid B, an extracellular metabolite of *Ceriporiopsis subvermispora*, suppresses the depolymerization of cellulose by the fenton reaction. Biomacromolecules 6:2851–2856. https://doi.org/10.1021/bm050358t

Richter C, Helaly SE, Thongbai B et al (2016) Pyristriatins A and B: pyridino-cyathane antibiotics from the basidiomycete *Cyathus* cf. *striatus*. J Nat Prod 79:1684–1688. https://doi.org/10.1021/acs.jnatprod.6b00194

Rieger PH, Liermann JC, Opatz T et al (2010) Caripyrin, a new inhibitor of infection-related morphogenesis in the rice blast fungus *Magnaporthe oryzae*. J Antibiot 63:285–289. https://doi.org/10.1038/ja.2010.31

Ríos J-L, Andújar I, Recio M-C, Giner R-M (2012) Lanostanoids from fungi: a group of potential anticancer compounds. J Nat Prod 75(11):2016–2044. https://doi.org/10.1021/np300412h

Robbins WJ, Kavanagh F, Hervey A (1947) Antibiotic substances from basidiomycetes: I. *Pleurotus griseus*. Proc Natl Acad Sci USA 33:171–176. https://doi.org/10.1073/pnas.33.6.171

Sadorn K, Saepua S, Boonyuen N et al (2016) Antimicrobial activity and cytotoxicity of polyketides isolated from the mushroom *Xerula* sp. BCC56836. RSC Adv 6:94510–94523. https://doi.org/10.1039/C6RA21898A

Schmidt-Dannert C (2014) Biosynthesis of terpenoid natural products in fungi. In: Biotechnology of isoprenoids. Springer, pp 19–61

Schneider P, Bouhired S, Hoffmeister D (2008) Characterization of the atromentin biosynthesis genes and enzymes in the homobasidiomycete *Tapinella panuoides*. Fungal Genet Biol 45:1487–1496. https://doi.org/10.1016/j.fgb.2008.08.009

Schüffler A, Anke T (2014) Fungal natural products in research and development. Nat Prod Rep 31(10):1425–1448. https://doi.org/10.1039/C4NP00060A

Schüffler A, Wollinsky B, Anke T et al (2012) Isolactarane and sterpurane sesquiterpenoids from the basidiomycete *Phlebia uda*. J Nat Prod 75:1405–1408. https://doi.org/10.1021/np3000552

Schultes R, Hofmann A (1980) Pflanzen der Götter. Hallwag, Bern

Schwenk D, Nett M, Dahse H et al (2014) Injury-induced biosynthesis of methyl-branched polyene pigments in a white-rotting basidiomycete. J Nat Prod 77(12):2658–2663

Schwenk D, Brandt P, Blanchette RA et al (2016) Unexpected metabolic versatility in a combined fungal fomannoxin/vibralactone biosynthesis. J Nat Prod 79:1407–1414. https://doi.org/10.1021/acs.jnatprod.6b00147

Selles P (2005) Synthesis and biological evaluation of himanimide C and unnatural analogues. Org Lett 7:605–608. https://doi.org/10.1021/ol047664o

Shi X-W, Li X-J, Gao J-M, Zhang X-C (2011a) Fasciculols H and I, two lanostane derivatives from Chinese mushroom *Naematoloma fasciculare*. Chem Biodivers 8:1864–1870. https://doi.org/10.1002/cbdv.201000203

Shi XW, Liu L, Gao JM, Zhang AL (2011b) Cyathane diterpenes from Chinese mushroom *Sarcodon scabrosus* and their neurite outgrowth-promoting activity. Eur J Med Chem 46:3112–3117. https://doi.org/10.1016/j.ejmech.2011.04.006

Shi X-W, Zhang A-L, Pescitelli G, Gao J-M (2012) Secoscabronine M, a novel diterpenoid from the Chinese bitter mushroom *Sarcodon scabrosus*. Chirality 24:386–390. https://doi.org/10.1002/chir.22031

Shibata H, Irie A, Morita Y (1998) New antibacterial diterpenoids from the *Sarcodon scabrosus* fungus. Biosci Biotechnol Biochem 62:2450–2452. https://doi.org/10.1271/bbb.62.2450

Stadler M, Hoffmeister D (2015) Fungal natural products-the mushroom perspective. Front Microbiol 6:1–4. https://doi.org/10.3389/fmicb.2015.00127

Stadler M, Sterner O (1998) Production of bioactive secondary metabolites in the fruit bodies of macrofungi as a response to injury. Phytochemistry 49:1013–1019. https://doi.org/10.1016/S0031-9422(97)00800-5

Stipkovits L, Ripley PH, Tenk M, Glávits R, Molnár T, Fodor L (2005) The efficacy of valnemulin

(Econor) in the control of disease caused by experimental infection of calves with *Mycoplasma bovis*. Res Vet Sci 78(3):207–215

Sulake RS, Chen C (2015) Total synthesis of (+)-antroquinonol and (+)-antroquinonol D. Org Lett 17:1138–1141. https://doi.org/10.1021/acs.orglett.5b00046

Sulake RS, Lin H-H, Hsu C-Y et al (2015) Synthesis of (+)-antroquinonol: an antihyperglycemic agent. J Org Chem 80:6044–6051. https://doi.org/10.1021/acs.joc.5b00345

Sun R, Zheng X, Wang X et al (2011) Two new benzofuran derivatives from the fungus *Stereum* sp. YMF1.1684. Phytochem Lett 4:320–322. https://doi.org/10.1016/j.phytol.2011.06.003

Suresh M, Kumar N, Veeraraghavaiah G et al (2016) Total synthesis of coprinol. J Nat Prod 79 (10):2740–2743. https://doi.org/10.1021/acs.jnatprod.6b00277

Suzuki S, Murayama T, Shiono Y (2005) Illudalane sesquiterpenoids, echinolactones A and B, from a mycelial culture of *Echinodontium japonicum*. Phytochemistry 66:2329–2333. https://doi.org/10.1016/j.phytochem.2005.06.018

Tanaka N, Kitamura A, Mizushina Y et al (1998) Fomitellic acids, triterpenoid inhibitors of eukaryotic DNA polymerases from a basidiomycete, *Fomitella fraxinea*. J Nat Prod 61:193–197. https://doi.org/10.1021/np970127a

Tang H-Y, Yin X, Zhang C-C et al (2015) Structure diversity, synthesis, and biological activity of cyathane diterpenoids in higher fungi. Curr Med Chem 22:2375–2391. https://doi.org/10.2174/0929867322666150521091333

Tao Q, Ma K, Yang Y et al (2016a) Bioactive sesquiterpenes from the edible mushroom *Flammulina velutipes* and their biosynthetic pathway confirmed by genome analysis and chemical evidence. J Org Chem 81:9867–9877. https://doi.org/10.1021/acs.joc.6b01971

Tao QQ, Ma K, Bao L et al (2016b) New sesquiterpenoids from the edible mushroom *Pleurotus cystidiosus* and their inhibitory activity against α-glucosidase and PTP1B. Fitoterapia 111:29–35. https://doi.org/10.1016/j.fitote.2016.04.007

Tao QQ, Ma K, Bao L et al (2016c) Sesquiterpenoids with PTP1B inhibitory activity and cytotoxicity from the edible mushroom *Pleurotus citrinopileatus*. Planta Med 82:639–644. https://doi.org/10.1055/s-0041-111629

Tateishi K, Hoshi H, Matsunaga K (2005) Inventors; Kureha Corporation, assignee. Antiallergic agent. WO2005095412 A1

Tian MQ, Liu R, Li JF et al (2016) Three new sesquiterpenes from the fungus *Stereum* sp. YMF1.1686. Phytochem Lett 15:186–189. https://doi.org/10.1016/j.phytol.2016.01.006

Tian M-Q, Wu Q-L, Wang X et al (2017) A new compound from *Stereum insigne* CGMCC5.57. Nat Prod Res 31:932–937. https://doi.org/10.1080/14786419.2016.1255889

Tzeng YM, Geethangili M (2011) Review of pharmacological effects of *Antrodia camphorata* and its bioactive compounds. Evid Based Complement Alternat Med. https://doi.org/10.1093/ecam/nep108

Villaume MT, Sella E, Saul G et al (2015) Antroquinonol A: scalable synthesis and preclinical biology of a phase 2 drug candidate. ACS Cent Sci 2(1):27–31. https://doi.org/10.1021/acscentsci.5b00345

Wackler B, Lackner G, Chooi YH, Hoffmeister D (2012) Characterization of the *Suillus grevillei* quinone synthetase GreA supports a nonribosomal code for aromatic α-keto acids. ChemBioChem 13(1798–1804):S1798/1–S1798/5. https://doi.org/10.1002/cbic.201200187

Wang G-Q, Wei K, Feng T et al (2012a) Vibralactones G-J from cultures of the basidiomycete *Boreostereum vibrans*. J Asian Nat Prod Res 14:115–120. https://doi.org/10.1080/10286020.2011.636037

Wang Y, Bao L, Yang X et al (2012b) Bioactive sesquiterpenoids from the solid culture of the edible mushroom *Flammulina velutipes* growing on cooked rice. Food Chem 132:1346–1353. https://doi.org/10.1016/j.foodchem.2011.11.117

Wang G-Q, Wei K, Li Z-H et al (2013a) Three new vibralactone-related compounds from cultures of Basidiomycete *Boreostereum vibrans*. J Asian Nat Prod Res 15:950–955. https://doi.org/10.1080/10286020.2013.824429

Wang S, Bao L, Han J et al (2013b) Pleurospiroketals A-E, perhydrobenzannulated 5,5-spiroketal sesquiterpenes from the edible mushroom *Pleurotus cornucopiae*. J Nat Prod 76:45–50. https://doi.org/10.1021/np3006524

Wang B, Han J, Xu W et al (2014a) Production of bioactive cyathane diterpenes by a birds nest fungus *Cyathus gansuensis* growing on cooked rice. Food Chem 152:169–176. https://doi.org/10.1016/j.foodchem.2013.11.137

Wang BT, Qi QY, Ma K et al (2014b) Depside α-glucosidase inhibitors from a culture of the mushroom *Stereum hirsutum*. Planta Med 80:918–924. https://doi.org/10.1055/s-0034-1382828

Wang S-C, Lee T-H, Hsu C-H et al (2014c) Antroquinonol D, isolated from *Antrodia camphorata*, with DNA demethylation and anticancer potential. J Agric Food Chem 62:5625–5635. https://doi.org/10.1021/jf4056924

Wang SM, Han JJ, Ma K et al (2014d) New alpha-glucosidase inhibitors with p-terphenyl skeleton from the mushroom *Hydnellum concrescens*. Fitoterapia 98:149–155. https://doi.org/10.1016/j.fitote.2014.07.019

Wang F, Ma H, Hu Z et al (2016a) Secondary metabolites from *Colletotrichum capsici*, an endophytic fungus derived from *Siegesbeckia pubescens* Makino. Nat Prod Res 31(16):1849–1854. https://doi.org/10.1080/14786419.2016.1261346

Wang J-C, Li G-Z, Lv N et al (2016b) Cryptoporic acid S, a new drimane-type sesquiterpene ether of isocitric acid from the fruiting bodies of *Cryptoporus volvatus*. J Asian Nat Prod Res 6020:1–6. https://doi.org/10.1080/10286020.2016.1240170

Wangun HVK, Hertweck C (2007) Squarrosidine and Pinillidine: 3,3′-fused bis(styrylpyrones) from *Pholiota squarrosa* and *Phellinus pini*. Eur J Org Chem 2007(20):3292–3295. https://doi.org/10.1002/ejoc.200700090

Wawrzyn GT, Bloch SE, Schmidt-Dannert C (2012a) Discovery and characterization of terpenoid biosynthetic pathways of fungi, 1st edn. Elsevier, Amsterdam

Wawrzyn GT, Quin MB, Choudhary S et al (2012b) Draft genome of *Omphalotus olearius* provides a predictive framework for sesquiterpenoid natural product biosynthesis in basidiomycota. Chem Biol 19:772–783. https://doi.org/10.1016/j.chembiol.2012.05.012

Wilkins WH, Harris GCM (1943) Investigation into the production of bacteriostatic substances by fungi. Ann Appl Biol 30:226–229. https://doi.org/10.1111/j.1744-7348.1943.tb06193.x

Williams R (2013) Discontinued drugs in 2012: oncology drugs. Expert Opin Investig Drugs 22:1627–1644. https://doi.org/10.1517/13543784.2013.847088

Wittstein K, Rascher M, Rupcic Z et al (2016) Corallocins A-C, nerve growth and brain-derived neurotrophic factor inducing metabolites from the mushroom *Hericium coralloides*. J Nat Prod 79:2264–2269. https://doi.org/10.1021/acs.jnatprod.6b00371

Wu W, Zhao F, Bao L et al (2011a) Two new cryptoporic acid derivatives from the fruiting bodies of *Cryptoporus sinensis*. Helv Chim Acta 94:2020–2026. https://doi.org/10.1002/hlca.201100100

Wu W, Zhao F, Ding R et al (2011b) Four new cryptoporic acid derivatives from the fruiting bodies of *Cryptoporus sinensis*, and their inhibitory effects on nitric oxide production. Chem Biodivers 8:1529–1538. https://doi.org/10.1002/cbdv.201000184

Xia Q, Zhang H, Sun X et al (2014) A comprehensive review of the structure elucidation and biological activity of Triterpenoids from *Ganoderma* spp. Molecules 19:17478–17535. https://doi.org/10.3390/molecules191117478

Xiao H, Zhong JJ (2016) Production of useful terpenoids by higher-fungus cell factory and synthetic biology approaches. Trends Biotechnol 34:242–255. https://doi.org/10.1016/j.tibtech.2015.12.007

Xie C, Koshino H, Esumi Y et al (2005) Vialinin A, a novel 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger from an edible mushroom in China. Biosci Biotechnol Biochem 69:2326–2332. https://doi.org/10.1271/bbb.69.2326

Xie C, Koshino H, Esumi Y et al (2008) Vialinins A and B: novel bioactive compounds from *Thelephora vialis*, an edible mushroom in China. ACS Symp Ser 993:465–472. https://doi.org/10.1021/bk-2008-0993.ch041

Xie HH, Xu XY, Dan Y, Wei XY (2011) Novel sesquiterpenes from the mycelial cultures of *Dichomitus squalens*. Helv Chim Acta 94:868–874. https://doi.org/10.1002/hlca.201000328

Xu Z, Yan S, Bi K et al (2013) Isolation and identification of a new anti-inflammatory cyathane diterpenoid from the medicinal fungus *Cyathus hookeri* Berk. Fitoterapia 86:159–162. https://doi.org/10.1016/j.fitote.2013.03.002

Yabuta T, Kobe K, Hayashi T (1934) Biochemical studies of the "bakanae" fungus of rice. I. Fusarinic acid, a new product of the "Bakanae" fungus. Z Pflanzenkrankh (Pflanzenpathol) Pflanzenschutz 10:1059–1068

Yamaoka M, Fukatsu Y, Nakazaki A, Kobayashi S (2009a) Synthetic study of fomitellic acids: construction of the AB ring moiety. Tetrahedron Lett 50:3849–3852. https://doi.org/10.1016/j.tetlet.2009.04.039

Yamaoka M, Nakazaki A, Kobayashi S (2009b) Total synthesis of fomitellic acid B. Tetrahedron Lett 50:6764–6768. https://doi.org/10.1016/j.tetlet.2009.09.088

Yang SS, Wang GJ, Wang SY et al (2009) New constituents with iNOS inhibitory activity from mycelium of *Antrodia camphorata*. Planta Med 75:512–516. https://doi.org/10.1055/s-0029-1185305

Yang SX, Yu ZC, Lu QQ et al (2012) Toxic lanostane triterpenes from the basidiomycete *Ganoderma amboinense*. Phytochem Lett 5:576–580. https://doi.org/10.1016/j.phytol.2012.05.017

Yang N, Ma Q, Huang S, Dai H (2014a) Two androstane derivatives from the cultures of fungus *Marasmiellus ramealis* (Bull.) Singer. Bull Kor Chem Soc 35:3224–3226

Yang X-Y, Li Z-H, Dong Z-J et al (2014b) Three new sesquiterpenoids from cultures of the basidiomycete *Conocybe siliginea*. J Asian Nat Prod Res 17:1–6. https://doi.org/10.1080/10286020.2014.939072

Yang N, Huang S, Dai H et al (2015) Chemical constituents from cultures of the fungus *Marasmiellus ramealis* (Bull.) Singer. J Braz Chem Soc 26:9–13. https://doi.org/10.5935/0103-5053.20140206

Yang L, Wang C, Zhou J, Kim S-W (2016) Combinatorial engineering of hybrid mevalonate pathways in *Escherichia coli* for protoilludene production. Microb Cell Factories 15:14. https://doi.org/10.1186/s12934-016-0409-7

Yaoita Y, Hiraob M, Kikuchi M, Machida K (2012) Three new lactarane sesquiterpenoids from the mushroom *Russula sanguinea*. Nat Prod Commun 7:1133–1135

Ye YQ, Koshino H, Onose J et al (2009) Structural revision of thelephantin G by total synthesis and the inhibitory activity against TNF-r production. J Org Chem 74(12):4642–4645. https://doi.org/10.1021/jo900638b

Ying YM, Shan WG, Zhang LW, Zhan ZJ (2013) Ceriponols A-K, tremulane sesquitepenes from

Ceriporia lacerate HS-ZJUT-C13A, a fungal endophyte of *Huperzia serrata*. Phytochemistry 95:360–367. https://doi.org/10.1016/j.phytochem.2013.07.025

Yoshikawa K, Kaneko A, Matsumoto Y et al (2006) Russujaponols A-F illudoid sesquiterpenes from the fruiting body of *Russula japonica*. J Nat Prod 69:1267–1270. https://doi.org/10.1021/np068006a

Yoshikawa K, Matsumoto Y, Hama H et al (2009) Russujaponols G-L, illudoid sesquiterpenes, and their neurite outgrowth promoting activity from the fruit body of *Russula japonica*. Chem Pharm Bull 57:311–314

Yoshikawa K, Koso K, Shimomura M et al (2013) Yellow pigments, fomitellanols A and B, and drimane sesquiterpenoids, cryptoporic acids P and Q, from *Fomitella fraxinea* and their inhibitory activity against COX and 5-LO. Molecules 18:4181–4191. https://doi.org/10.3390/molecules18044181

Yoshioka Y, Namiki D, Makiuchi M et al (2016) Vialinin A and thelephantin G, potent inhibitors of tumor necrosis factor-α production, inhibit sentrin/SUMO-specific protease 1 enzymatic activity. Bioorg Med Chem Lett 26:4237–4240. https://doi.org/10.1016/j.bmcl.2016.07.051

Yun BS, Lee IK, Kim JP, Yoo ID (2000) Curtisians A-D, new free radical scavengers from the mushroom *Paxillus curtisii*. J Antibiot 53:114–122

Zähner H, Anke H, Anke T (1983) Evolution of secondary pathways. In: Bennett J, Ciegler A (eds) Secondary metabolites and differentiation in fungi. Dekker, New York, pp 153–171

Zeiler E, Braun N, Böttcher T et al (2011) Vibralactone as a tool to study the activity and structure of the ClpP1P2 complex from *Listeria monocytogenes*. Angew Chem Int Ed 50:11001–11004. https://doi.org/10.1002/anie.201104391

Zhao PJ, Yang YL, Du L et al (2013) Elucidating the biosynthetic pathway for vibralactone: a pancreatic lipase inhibitor with a fused bicyclic β-lactone. Angew Chem Int Ed 52:2298–2302. https://doi.org/10.1002/anie.201208182

Zheng Y, Shen Y (2009) Clavicorolides A and B, sesquiterpenoids from the fermentation products of edible fungus *Clavicorona pyxidata*. Org Lett 11:109–112. https://doi.org/10.1021/ol8024549

Zheng X, Li G-H, Xie M-J et al (2013) Stereumins K–P, sesquiterpenes from the fungus *Stereum* sp. CCTCC AF 2012007. Phytochemistry 86:144–150. https://doi.org/10.1016/j.phytochem.2012.10.014

Zheng Y, Wang J, Pang H (2014) Inventor. Sesquiterpene compounds with antitumor activity and preparation method of sesquiterpene compounds. Chinese patent CN104059040 A. 2014 Sep 24

Zheng Y, Pang H, Wang J et al (2015) New apoptosis-inducing sesquiterpenoids from the mycelial culture of Chinese edible fungus *Pleurotus cystidiosus*. J Agric Food Chem 63:545–551. https://doi.org/10.1021/jf504931n

Zhong J-J, Xiao J-H (2009) Secondary metabolites from higher fungi: discovery, bioactivity, and bioproduction. In: Biotechnology in China I. pp 79–150

Zhou Z-Y, Liu J-K (2010) Pigments of fungi (macromycetes). Nat Prod Rep 27:1531–1570. https://doi.org/10.1039/c004593d

Zhou Q, Snider BB (2008a) Synthesis of (±)-vibralactone. Org Lett 10:1401–1404. https://doi.org/10.1021/ol800118c

Zhou Q, Snider BB (2008b) Synthesis of (±)- and (−)-vibralactone and vibralactone C. J Org Chem 73:8049–8056. https://doi.org/10.1021/jo8015743

Zhou Z-Y, Tang J-G, Wang F et al (2008) Sesquiterpenes and aliphatic diketones from cultures of the basidiomycete *Conocybe siliginea*. J Nat Prod 71:1423–1426. https://doi.org/10.1021/np8002657

Zhou L-Y, Yu X-H, Lu B, Hua Y (2016) Bioassay-guided isolation of cytotoxic isocryptoporic acids from *Cryptoporus volvatus*. Molecules 21:1692. https://doi.org/10.3390/molecules21121692

Identification of Fungicide Targets in Pathogenic Fungi

Andrew J. Foster[1]

CONTENTS

I. Introduction

Many fungal species are beneficial to mankind. Notable examples are the application of *Saccharomyces cerevisiae* in bread and beer making or the production of penicillin by *Penicillium* species. There are however several species that pose a problem to mankind as pathogens of plants and animals. To combat fungal diseases, there are many fungicides on the market which target particular aspects of fungal metabolism and kill the pathogen or severely limit its proliferation. For clarification, for the purposes of the present discussion, fungicides, as the name suggests, act against the fungi but also refer to chemicals used against oomycete pathogens. Additionally the present discussion will refer to targets in the sense of the molecular targets of a fungicide within a pathogen, although some discussion is included of what the industry refers to as 'targets' with the meaning of the species to which a fungicide is directed.

As with antibiotics used to combat bacterial infections, due mainly to the risk of resistance development but also with the threat of withdrawal from the use of some older chemicals, there is a pressing need to find novel fungicides with new targets. With an emphasis on the molecular targets of fungicides (modes of action), this chapter will consider what chemicals are currently available and how they were discovered and will review the impact that the rapid developments in life sciences such as 'omics' technologies and next-generation sequencing have made and are likely to make in the future on the discovery process. The discussion that follows will predominantly refer to fungicide development in the agricultural sector although there is obviously in principle much in common with the discovery of medicinal antimycotics, and this area of research is also considered.

[1] University of Exeter, Biosciences, Exeter, UK; e-mail: A.J.Foster@exeter.ac.uk

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

II. Fungicide Development

Development of a fungicide is an extremely long and expensive process, and there are tight regulations which must be met before any fungicide is marketed (Oliver and Hewitt 2014). Likewise, antifungals for the treatment of human diseases must undergo stringent clinical trials before drugs are approved for sale. For fungicides developed for use in agriculture, the number of conditions which need to be met before the fungicide is considered safe increases year on year. Despite the huge development costs involved in bringing an antifungal to market, depending on the mode of action, it may only be a few years following the release before resistance develops in the 'target' organism. Within the agricultural sector, since the move away from inorganic fungicides from the 1940s onwards, fungicide development has gone through cycles of different approaches to fungicide development starting with essentially empirical approaches looking for chemicals active in field situations. Such approaches therefore start with an active compound, and an understanding of the mode of action was not considered important and was sometimes only discovered much later. Because whole organism tests are time-consuming, it is not practical to screen large numbers of compounds. Fungi are generally ideally suited for high-throughput in vivo screens although some economically relevant species are obligate pathogens and therefore require an association with the plant for the screen to be conducted. Therefore, in the 1990s industry's focus shifted to large volume combinatorial chemical screening approaches in combination with more reductionist assays such as reporter gene test systems. These high-throughput approaches typically require of the order of 140,000 different compounds for every hit (Drewes et al. 2012). Eventually frustrated with the productivity of high-throughput screening approaches (notably the problem of false positives) in the last decade, industry has begun looking to improve efficiency and to explore the potential of new technologies. The developments in life sciences over the last decade have allowed industry to use biorational design and in silico screening approaches which start with target active sites and then design the chemistry to fit (Oliver and Hewitt 2014) an approach that contrasts with the 'chemistry first' approaches of the past. It may be some years until it is clear if starting with the molecular target in this manner is an efficient approach. Additionally this approach is likely biased towards established targets and well-characterised enzymes. Despite technological advances which hold great promise for speeding the process of target identification and fungicide development, the arsenal of weapons presently available to fight fungal diseases remains limited. Furthermore, just a handful of crops are considered viable in terms of the chances of recovering a profit on the investment made (Russel 2005). The increasing cost of research and development may be offset to some degree by miniaturisation and directed chemical synthesis (Drewes et al. 2012); however, the cost aspect of fungicide development is a major brake on innovation. A fungicide takes in the region of 10 years and an average of $286 million to bring to market (McDougall 2016), and measures to limit the risk of resistance development will restrict the usage of any new chemistry and the chances of turning a profit. There is therefore less incentive for industry to take risks, and consequently improved compounds targeting existing modes of action or 'patent busting' of competitors' chemistries (Oliver and Hewitt 2014) represent cost-effective shortcuts to a product compared to the development of an agent with a novel mode of action. With food security being a major public concern for the future, it is questionable whether we should rely on industry alone to deliver the crop protective agents necessary to guarantee sufficient yields. Similarly the need for novel medicinal antifungals is an urgent pressing one to ensure effective future therapies for the increasing incidence of human fungal diseases.

III. Currently Deployed Fungicides

The currently deployed fungicides in agriculture cover many aspects of fungal metabolism (Table 1). Despite this the majority of agents presently sold act on only a limited range of

Table 1 Main targets of currently deployed fungicides

Pathway or process Group name	Mode of action (molecular target)	Examples/common name
Nucleic acid metabolism		
Phenylamides	Polymerisation of rRNA (RNA polymerase I)	Talaxyl (Ridomil©)
Hydroxy-(2-amino) pyrimidines	Purine interconversion (adenosine deaminase)	Pirimate, dimethyrimol
Heteroaromatics	DNA/RNA synthesis (precise target unclear)	Octhilinone
Carboxylic acids	DNA synthesis (topoisomerase II)	Oxolinic acid
Cytoskeleton/motor proteins		
Methyl benzimidazole carbamates	Microtubule formation (beta-tubulin)	Benomyl (Benlate©)
N-phenyl carbamates	Microtubule formation (beta-tubulin)	*N*-phenyl carbamate
Benzamides and thiazole carboxamide	Microtubule formation (beta-tubulin)	Zoxamide
Phenylureas	Cell division (precise target unclear)	Pencycuron
Benzamides	Actin filament function (spectrin-like proteins)	Fluopicolide
Cyanoacrylates	Disruption of actin assembly (myosin-5)	Benzamacril
Respiration		
Pyrimidinamines/pyrazole-MET1	Complex I NADH oxidoreductase	Diflumetorim
Succinate dehydrogenase inhibitors (SDHIs)	Complex II succinate dehydrogenase	Boscalid
Quinone outside inhibitors (QoIs)	Complex III ubiquinol oxidase	Azoxystrobin
Quinone inside inhibitors (QIIs)	Complex III ubiquinone reductase	Cyazofamid
Oxidative phosphorylation uncouplers	Uncouplers of oxidative phosphorylation	Dinocap
Organotin compounds	Inhibition of oxidative phosphorylation	Fentin acetate
Thiophene carboxamides	ATP production (proposed)	Silthiofam
Quinone outside inhibitors (QoIs)	QoSI stigmatellin binding subsite complex III ubiquinone reductase	Ametoctradin
Amino acid biosynthesis/translation		
Anilino/pyrimidines	Inhibition of cystathionine γ-synthase in methionine biosynthesis	Cyprodinil
Enopyranuronic acid (antibiotic)	Inhibition of termination step of translation	Blasticidin S
Hexopyranosyl	Inhibition of translation initiation	Kasugamycin
Glucopyranosyl	Ribosome binding (protein synthesis inhibition)	Ag streptomycin
Tetracycline	Ribosome binding (protein synthesis inhibition)	Oxytetracycline
Signal transduction		
Azanaphthalenes	Precise target unclear	Quinoxyfen
Phenylpyrroles	Hog1p MAP kinase in HOG pathway	Fludioxonil
Dicarboximides	Histidine kinase in HOG pathway	Vinclozolin
Membrane synthesis or function		
Phosphorothiolates, dithiolanes	Methyltransferase phospholipid biosynthesis	Edifenphos
Aromatic hydrocarbons		
Heteroaromatics	Lipid peroxidation	Dicloran
Carbamates	Multisite (membrane permeability proposed)	Prothiocarb
Bacillus species	Cell membrane disruption	*Bacillus subtilis*
Tea tree extract	Cell membrane disruption	Oil of *Melaleuca alternifolia*
DMI fungicides (SBI class I)	C14-sterol-demethylase in ergosterol biosynthesis	Triazole
Morpholines (SBI class II)	C8-sterol-isomerase and C14-sterol-reductase in ergosterol biosynthesis	Aldimorph
Morpholines (SBI class III)	3-keto-sterol-reductase in ergosterol biosynthesis	Fenhexamid
Thiocarbamates, allylamines	Squalene epoxidase in ergosterol biosynthesis	Terbinafine
Cell wall biosynthesis/melanin biosynthesis		
Polyoxins	Chitin synthase (gene family in all fungi)	Polyoxin D zinc salt
Carboxylic acid amides (CAAs)	Cellulose synthase (oomycetes only)	Mandipropamid (Revus©)

(continued)

Table 1 (continued)

Pathway or process Group name	Mode of action (molecular target)	Examples/common name
MBI – (reductase)	Reductases in melanin biosynthesis	Tricyclazole (beam©)
MBI – (dehydratase)	Melanin biosynthesis dehydratase	Fenoxanil
MBI – (PKS)	Polyketide synthase in melanin biosynthesis	Tolprocarb

Based on the Fungicide Resistance Action Committee's classification FRAC Code List 2016

fungal targets, and more than 50% of worldwide fungicide sales cover just three modes of action: the C14 demethylase in ergosterol biosynthesis inhibitors (DMIs; FRAC code G1), the respiration complex III quinone outside inhibitors (QoIs; FRAC code C3) and the succinate dehydrogenase in respiration complex II inhibitors (SDHIs; FRAC code C2), all of which are classified medium to high risk of resistance development (Leadbetter 2015). In fact more than 70% of worldwide fungicide sales are of chemicals considered at medium to high risk of resistance development, although it should be noted that these are often now applied in combination with lower-risk fungicides (Leadbetter 2015). In the treatment of human fungal diseases, just six different modes of action, three of which target ergosterol or its biosynthesis, are used by current drugs: inhibition of lanosterol 14 α-demethylase by azoles, sterol binding by polyenes such as nystatin or amphotericin, inhibition of the squalene epoxidase involved in ergosterol formation by allylamine/thiocarbamates, inhibition of β-1,3-glucan synthase by echinocandins, inhibition of nucleic acid synthesis by the antimetabolite 5-fluorocytosine and griseofulvin-mediated inhibition of mitosis by tubulin binding (Spampinato and Leonardi 2013). There is clearly therefore an urgent need to maintain and arguably to increase efforts to identify new antifungals ideally with novel modes of action. A look at what is currently sold is revealing: few fungicides presently used actually do target unique aspects of fungal metabolism. An important lesson from what has been successful in the past is that specificity of a fungicide is complex and the fact that the target is present in the pathogen and the host does not necessarily preclude the use of that target for effective intervention. It should also be clear though from the chemicals currently sold that there are aspects of fungal metabolism that are underexploited as fungicide targets, some of which might be predicted to be good sources of fungal-specific targets. These are considered in a later section.

IV. Blueprint for the Ideal Fungicide

For agricultural purposes, at least the attributes of the ideal fungicide would be novel mode of action, specificity for the molecular target, low ecotoxicity, low threat of resistance (durability), effectivity at a low dose, safety for the user and low cost. Specificity for one particular fungal (or oomycete) species certainly from an industry point of view is not necessarily advantageous; indeed broad-spectrum agents have shown the best returns in investment to date. In terms of the probability of compounds being effective, there are several good predictors based on the chemical attributes of current agents (Avram et al. 2014; Hao et al. 2011). However, effective fungicides which fall well outside of these 'ideal' parameters are known (Avram et al. 2014). In practice, few chemicals employed to date fit the blueprint of the ideal fungicide exactly. From the point of view of the molecular target in the pathogen, this should ideally be accessible, druggable (have a suitable binding pocket; for further information on this attribute, see Owens 2007) and distinct enough from any related protein or other target molecule in the host (Oliver and Hewitt 2014). Using the penicillin paradigm, we might imagine that specificity is best be determined by the lack of the drug target in the host. Good examples of

this 'magic bullet' approach among crop protective agents are fungicidal chemistries which target ergosterol or melanin biosynthesis. As mentioned above, many current fungicides target critical aspects of metabolism which are also present in the host and in benign species present in the environment, and there are other determinants of specificity than target conservation (Hewitt 2000). This is the case for the strobilurin class of fungicides, for example, where differential uptake between host and pathogen occurs and any effects on most, but not all, plant species are beneficial rather than detrimental (Vincelli 2002). Likewise contact fungicides of the dithiocarbamate class such as mancozeb although potentially toxic to both plant and fungal cells are not absorbed by the plant. Armed with ever more knowledge of the dynamic proteomes of host and pathogen, we are increasingly better placed to judge how specific a potential target will be and to make a rational selection of potential targets and potentially uncover a new 'magic bullet'. Another lesson from the modern era of fungicides if we consider resistance development is that specificity in terms of molecular target is a double-edged sword. Resistance development has been rapid in fungicides that act at one target site (Deising et al. 2008). Notably, it is the multisite fungicides that have endured the longest, a good example being the dithiocarbamate fungicides which, although generally requiring more applications than modern systemics, have now been used for more than 50 years with only limited emergence of fungicide resistance. Therefore, in order to assure durability, perhaps agents that act on more than one target should be the focus of future searches. Formulations which mix two fungicides with differing modes of action such as the pyraclostrobin (respiration complex III inhibitor) and boscalid (complex II inhibitor) mixture marketed by BASF are increasingly popular. Because a few large companies produce the bulk of the currently deployed fungicides and the biggest selling agents cover just a few modes of action, the threat of resistance development to these makes the identification of agents with novel modes of action essential, and a consideration of where these new targets might potentially be found is warranted.

If we look in general to the field of pharmaceutical development, we may draw some parallels with fungicide discovery. An estimate of the number of potential targets present in the products of the genes in the human genome is of the order of 10% of all proteins (Drews and Ryser 1996). If there are 20,300 genes, then this would predict around 2000 potential targets, and in 2011 it was estimated that drugs exist for around 430 different human proteins (Rask-Andersen et al. 2011) suggesting there are many new targets waiting to be discovered. The rate of release of drugs targeting novel proteins in human disease treatment is about 4 every year (Rask-Andersen et al. 2011). The release of the human genome and subsequently the genomes of many key fungal pathogens and increased investment in research and development have not increased this rate of innovation to date. In the agricultural sector, the rate of release of fungicides with a novel mode of action is of the order of 1 per year (Leadbetter 2015). Genome size is generally much greater in mammalians compared with fungal species; there are generally some 10,000–12,000 genes in filamentous fungi and typically half as many in unicellular fungi such as the human pathogen *Candida albicans*. This and the fact that there are only around 40 different modes of actions, some of which target different sites in the same molecular target, used by agricultural fungicides and only 6 different modes of action classes are used by medicinal antifungal drug make clear the potential for innovation. It is encouraging however to know that opportunities exist for novel means of control of fungal diseases. Some potentially underexploited aspects of fungal metabolism are discussed in the following sections.

V. Potentially Underexploited Aspects of Fungal Metabolism

A. The Fungal Plasma Membrane as a Target

Fungicides which target various steps in ergosterol synthesis are good examples of the exploitation aspects of metabolism present in the pathogen but not in the host. Ergosterol takes

the place of cholesterol in the cell membrane of fungal species (and some protists) and has been the target of several of most successful fungicides of the modern era. Sterol biosynthesis inhibitor (SBI) fungicides of the sterol demethylation inhibitor (DMI) type (triazoles) currently have a 29% share of the agricultural fungicide market (Leadbetter 2015), and azoles are also widely exploited in medicine for the treatment of fungal infections. It is of interest to think more generally of the fungal membrane as a potential source of new fungicide targets. Fungal glycosphingolipids are important as structural components of the cell membrane and in some cases are sufficiently divergent from mammalian glycosphingolipids that their biosynthesis could be exploited as targets for control of human fungal infections (Guimarães et al. 2014). Glucosylceramides are essential for normal *Candida albicans* infection (Noble et al. 2010), and perturbation to the biosynthesis of glucosylceramides also severely impairs the ability of *Cryptococcus neoformans* to infect in a mouse disease model (Singh et al. 2012). Promising lead compounds for specific inhibition of fungal glucosylceramide synthesis were recently reported (Mor et al. 2015). If divergence from plant host galactosyl- and glucosylceramide biosynthesis is sufficient, the fungal enzymes might also prove useful targets in the control of fungi causing agricultural diseases (Fradin et al. 2015). The antifungal PAF protein from *Penicillium chrysogenum* seems to interfere with membrane function by inducing hyperpolarisation and shows promise as a means to control human pathogenic fungal species such as *Aspergillus fumigatus* by a potentially novel membrane-associated mode of action (Marx et al. 2008).

B. The Fungal Cell Wall as a Target

Fungal cell walls are predominantly composed of β-1,3-glucan and chitin which together with glycoproteins and other minor carbohydrate constituents form a cross-linked network with aspects unique to this kingdom (Bowman and Free 2006). The potential of the cell wall as a target for control is clear. The cell walls of oomycetes meanwhile are more similar to those of plants and contain cellulose rather than glucans. The recently released mandelamide fungicide mandipropamid inhibits cell wall synthesis in *Phytophthora infestans* by targeting the PiCesA3 protein (Blum et al. 2010) and is a promising new means to control a number of diseases caused by oomycetes (Walter 2011). As an inhibitor cellulose synthesis, this chemistry's applications are limited to combating oomycetes. The echinocandins (Bowman and Free 2006; Perlin 2011) are one of the few currently deployed chemicals which exploit attributes of the fungal cell wall, and these drugs are often referred to as the 'penicillin of the fungal world' (Anaissie et al. 2009).

The biosynthesis of chitin which is absent from plant and mammalian host cell walls has been used as a fungicide target. Chitin synthesis in the cell wall is required for normal growth and virulence in several human and plant pathogens (Munro et al. 2001; Weber et al. 2006; Lenardon et al. 2010; Kong et al. 2012; Cui et al. 2013), and chitin synthase enzymes are established as a target of the polyoxin group of fungicides. The mechanism by which chitin is transported from its site of synthesis to its final destination in the cell wall is still under investigation and could also be a source of novel drug targets. Unless they target one key chitin synthase, inhibitors of these enzymes are potentially less at risk from resistance development as these enzymes form multigene families in fungi. Chitosan (deacetylated chitin) is necessary for virulence in *Cryptococcus neoformans*, and targeting the chitin deacetylases involved in the biosynthesis of chitosan therefore has some promise for developing novel means to control fungal diseases (Baker et al. 2011). Interestingly, chitosan itself has antifungal properties and induces hyperpolarisation of the plasma membrane in *Candida albicans* (Peña et al. 2013).

Poacic acid (a diferulate derived from grasses) was recently shown to be an effective inhibitor of the growth of several important plant pathogenic fungi and oomycete pathogens and targets β-1,3-glucan synthesis (Piotrowski et al. 2015). Poacic acid may therefore represent a promising lead structure for devel-

opment of commercial antifungals (Piotrowski et al. 2015). The enzyme β-1,3,-D-glucan synthase is the target of the echinocandin antifungal medicinal drugs such as caspofungin (Sawistowska-Schroder et al. 1984) but is prone to resistance development as a single-site inhibitor (Perlin 2015). This mode of action is not currently exploited in an agricultural context. α-1,3-Glucan biosynthesis is also of interest as a drug target and has been shown to be required for successful infection in several plant pathogenic species and in *Cryptococcus neoformans* (Fujikawa et al. 2012; Reese et al. 2007). As the α-1,3-glucan synthase-encoding gene is a single gene in most fungi, any fungicide targeting its product might be at risk of resistance development; however, the lack of this enzyme in hosts makes this a particularly attractive target.

A recent study suggests that minor glycans in the fungal cell wall may also play a critical role in establishing infection (Santhanam et al. 2017). A rhamnose synthase of the vascular wilt pathogen *Verticillium dahliae* was recently shown to be essential for pathogenicity (Santhanam et al. 2017) and represents an attractive potential fungicide target. Regulation of cell wall integrity (CWI) is controlled by a conserved MAP kinase signalling pathway in fungi and is likewise a promising area for targeting antifungal drugs (Valiante et al. 2015). Cell wall components are also established elicitors of immune responses in plants and humans. Some fungi have been shown to take measures to mask these potential elicitors from their hosts (Gravelat et al. 2013; Oliveira-Garcia and Deising 2013), and a better understanding of countermeasures employed by the pathogen will no doubt open up new means to control fungal diseases by countering stealth mechanisms.

Melanin is an important component of the cell wall of many fungi (Eisenman and Casadevall 2012) and is crucial to the function of the infection structures (appressoria) of several plant pathogenic fungi. Melanin is also an important virulence factor in human pathogenic fungi (Langfelder et al. 2003). Two separate pathways for melanisation have been identified: one uses 1,8-dihydroxynaphthalene (DHN) and is a polyketide synthase (PKS)-dependent process, while the other pathway uses L-3,4-dihydroxyphenylalanine (L-dopa) and is a phenoloxidase-dependent process. Three key enzymes in the PKS-dependent synthesis of melanin have been exploited as fungicide targets (Thieron et al. 1999; Hamada et al. 2014). Recent studies suggest that specialised secretory vesicles similar to mammalian melanosomes are secreted into the cell wall where they initiate melanin deposition (Franzen et al. 2008; Eisenman et al. 2009; Walker et al. 2010). Chitin seems to be the macromolecule to which melanin becomes anchored (Nosanchuk et al. 2015). As our understanding of regulation of melanin formation increases, it may be possible to target factors controlling melanin deposition or regulatory proteins which control melanisation.

Agents which target fungal cell wall synthesis are particularly attractive for many reasons discussed above. Other components of the cell wall such as mannoproteins might prove useful antifungal targets if they can be proved relevant to disease. Testing whether subsets of such related proteins can be targeted should be a goal for future studies.

C. Secretion and Secreted Factors as Drug Targets

Secretion plays a special role in fungal nutrition which relies heavily on extracellular breakdown of carbohydrates and proteins. Secretion is of course also essential for fungal growth, and components of the conserved exocyst complex are therefore necessary for viability (Heider and Muson 2012). Although, being evolutionarily conserved, the core components of the secretory machinery might not be ideal fungicide targets, however, analysis of the key proteins trafficked through the secretion system might reveal novel targets for drug intervention. Recent research has additionally highlighted the importance of secretion in suppression of plant host defences and the ability to proliferate within hosts (Giraldo et al. 2012; Zhang et al. 2014; Chavez-Dozal et al. 2015). Key proteins (effectors) which act to suppress plant defence responses have already been identified (Lyu et al. 2016; Zhu et al. 2013; Du et al. 2015; Saitoh et al. 2012; Chanclud et al.

2016; Whigham et al. 2015). In some cases, enzymatic activity is associated with the effector (Dong et al. 2011), but in other cases suitable assays to quantify suppressive activity would need to be developed to assist in the development of specific inhibitors if they were considered druggable. Such proteins could represent novel and accessible targets for fungicides. Additionally secreted proteases have been shown to be relevant for the virulence of human pathogens (Hube et al. 1997), and the accessibility of such enzymes and the potential to search for multisite inhibitors (one drug binding several different enzymes) make them attractive targets for antifungals. Furthermore, enzymes responsible for the biosynthesis or the function of fungal-specific structures such as the Spitzenkörper which is associated with the secretion of the machinery required for polarised growth (Steinberg 2007) may be targeted. As our understanding of the critical roles of secretion in the pathogenic lifestyles of key pathogens deepens, it is likely that novel disease control points will become apparent.

D. Amino Acid Biosynthesis as a Source of Potential Fungicide Targets

Exploiting amino acid biosynthetic enzymes as fungicide targets is of interest. In control of human pathogenic fungi, the potential is very clear as mammals lack the biosynthetic machinery for several amino acids (Jastrzębowska and Gabriel 2015). If an enzyme is lacking in the host, whether an inhibitor of the fungal enzyme responsible for the synthesis of the amino acid will be effective will obviously depend to some degree on the bioavailability of that amino acid in the host. In the case of plant pathogenic fungi, there is less obvious potential to exploit the amino acid biosynthetic machinery as most biosynthetic pathways for forming amino acids are common to both pathogen and host. Indeed amino acid biosynthesis is the mode of action of two common types of herbicide (glyphosate (BASTA©) a phosphoenolpyruvate analogue which targets 5-enolpyruvylshikimate-3-phosphate synthase in the shikimate pathway and sulfonylurea-type herbicides which target acetohydroxyacid synthase in isoleucine/valine synthesis, respectively). These pathways would therefore not be a promising source of targets for the control of agricultural fungal pathogens although they might, especially shikimate pathway enzymes, be exploited in the control of human fungal pathogens. Toxic analogues of intermediates of amino acid biosynthetic pathways are considered particularly promising as antifungals as they are often poor substrates for resistance-associated multidrug resistance (MDR) proteins of fungi (Jastrzębowska and Gabriel 2015).

Methionine biosynthesis (cystathionine γ-synthase) has been proposed to be the target of the anilino-pyrimidine class of fungicide (FRAC group D1; Fritz et al. 1997). The target enzyme is also present in plant hosts, and there is still some debate as to whether it is the primary target of these fungicides (Fu et al. 2013). Methionine biosynthesis is a requirement for normal virulence of the rice blast fungus (Saint-Macary et al. 2015; Balhadere et al. 1999) and the wheat head blight pathogen *Fusarium graminearum* (Fu et al. 2013) and is also required for virulence of *Cryptococcus neoformans* (Yang et al. 2002). The natural product ebelactone A has been shown to potently inhibit microbial homoserine transacetylase in the methionine biosynthetic pathway and could prove a useful lead structure for the development of antimycotics (De Pascale et al. 2011). Histidine biosynthesis is required for normal virulence of *Aspergillus fumigatus* (in a wax moth disease model) and for normal infection of rice by the rice blast fungus (Dietl et al. 2016; Sweigard et al. 1998). Although histidine biosynthesis in plants follows the same path as in fungi, there is some evidence for significant divergence at the histidinol-phosphate phosphatase-catalysed step (Petersen et al. 2010) which might allow the development of pathogen-specific inhibitors.

Some enzymatic steps in the α-aminoadipate pathway (for lysine synthesis) are unique to microorganisms and have been demonstrated to be relevant in *Candida albicans* (Shepherd 1985), *Aspergillus fumigatus* (Schöbel et al. 2010) and *Magnaporthe oryzae* (Chen et al. 2014). These would seem obvious as potential targets for antifungal therapies or crop protective fungicides.

If potential to exploit the amino acid biosynthetic machinery as a fungicide target in an agricultural context is limited, then perhaps greater potential lies with targeting its regulation. Components of these regulatory pathways such as the general cross-pathway control system which activates many amino acid biosynthetic activities via the Gcn4p transcription factor might be significantly more diverged than the amino acid biosynthetic pathways themselves and which would be promising drug targets if accessibility was not an issue.

E. Signalling Components as Fungicide Targets

Regulatory proteins (signal transduction components) have been proposed as the target of just three fungicide groupings: the azanapthalenes, the dicarboximides and the phenylpyrroles (Wheeler et al. 2003; Tanaka and Izumitsu 2010; Ochiai et al. 2002). In the case of the azanaphthalenes such as quinoxyfen (effective in control of powdery mildew), one study suggests that disruption of signal transduction processes may be indirect, and the compound has been suggested to target serine esterase activity (Lee et al. 2008). Phenylpyrroles such as fludioxonil target the MAP kinase within the high-osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway and cause cell death due to run away glycerol accumulation. Dicarboximides are proposed to target the histidine kinase enzyme in the HOG pathway (Tanaka and Izumitsu 2010). Other components of the HOG pathway such as the Sln1 histidine kinase show promise as intervention points for fungicides (Jacob et al. 2016). Given that signal transduction pathways have been intensively studied over the last few decades in fundamental research, and some of the these, notably the Pmk1-MAPK pathway, have been shown to be essential for pathogenicity across a broad range of fungi (Turrà et al. 2014; Román et al. 2007), we can anticipate future fungicides will be developed with mode of actions based on perturbations of these signal transduction mechanisms. A search for improved inhibitors of target of TOR (target of rapamycin), calcineurin and Hsp90 signalling factors also holds promise for antifungal therapy (Bastidas et al. 2008). Components of Ras signal transduction pathways might also be developed as targets for antifungal medicines in the control of *Aspergillus fumigatus* provided that such compounds show sufficient specificity (reviewed by Norton and Fortwendel 2014). pH signalling has likewise been suggested as a potential pathway where points of control for human pathogenic fungi might be found (Cornet and Gaillardin 2014). As proof of relevance already exists for many signalling components across a broad range of pathogenic fungi, there are good prospects for the development of therapeutic agents targeting signalling pathways.

Given the availability of G protein-coupled receptor (GPCR) agonists and antagonists in the pharmaceutical realm (Kostensis 2006), it is surprising that currently no agrochemical or medicinal antifungal is known to target GPCRs as a mode of action despite the fact that these are extended gene families in fungi just as they are in mammalian cells. Relevance for infection has been demonstrated in one model plant pathogen (DeZwaan et al. 1999), and a synergistic requirement for the Gpr1 with the trehalose-6-phosphate phosphatase Tps2 in *Candida albicans* infection has been reported (Maidan et al. 2008). Most fungal GPCRs have not been characterised however, and future fungicide targets are probable within these families. Similarly, protein kinases have been very extensively characterised in humans and are the target of several drugs especially in cancer treatment. In contrast the exploitation of protein kinases as fungicide targets in plant pathogenic species is limited to just two chemistries (phenylpyrroles and dicarboximides). Where sufficient conservation occurs, there would seem to be potential in exploiting mammalian protein kinase inhibitory agents to validate targets for control of fungal diseases of plants.

F. Other Metabolic Pathways and Processes with Potential Fungicide Targets

Iron uptake is another pathogen activity where there are currently no agents targeting key nodes in the pathway despite the fact that it is critical to the successful host colonisation by several fungal pathogens. Many organisms pro-

duce iron-chelating siderophores for iron acquisition, and these have been shown to be necessary for virulence in *Aspergillus fumigatus* and *Histoplasma capsulatum* (Hissen et al. 2005; Schrettl et al. 2004; Hwang et al. 2008) and for full virulence of the head blight fungus *Fusarium graminearum* (Greenshields et al. 2007). Although some plants such as grasses also secrete siderophores from their root systems, there would seem to be some promise in the development of compounds targeting enzymes involved in siderophore biosynthesis in fungi (Leal et al. 2013). Because microorganisms readily take up siderophores, there is also some interest in using them as drug delivery systems (Górska et al. 2014).

Chemistries interfering with autophagy have been considered as useful therapies in human neurodegenerative diseases and infections with bacteria and viruses (reviewed by Rubinsztein et al. 2015). As autophagy is an absolute prerequisite for pathogenic development in several human and plant pathogenic fungi (Liu et al. 2012) and no fungicides inhibiting the process are currently in use, this would appear to be an excellent aspect of metabolism which could be exploited in the future provided that the highly conserved nature of components of this process is not problematic.

Carbohydrate metabolism would also seem a very good hunting ground for novel fungicide target discovery. Although not specific to the fungi, trehalose metabolism is a key aspect of the carbohydrate metabolism of many fungi and has important roles additional to the synthesis of the trehalose disaccharide as a storage carbohydrate. Trehalase enzymes which hydrolyse the trehalose disaccharide are proposed to be the molecular target of validamycin which may induce plant defence responses (rather than being directly antifungal) by trehalose accumulation as a result of inhibition of trehalase (Goddijn et al. 1997). The enzyme trehalose-6-phosphate synthase Tps1 plays a central role in the carbohydrate and nitrogen metabolism of the rice blast fungus (Wilson et al. 2007) and is essential for pathogenicity (Foster et al. 2003). Likewise in the human pathogenic yeast, *Cryptococcus gattii* Tps1 is required for normal virulence (Ngamskulrungroj et al. 2009). Targeting trehalose synthesis would seem a promising target for disease control in fungal pathogens; indeed in silico-based approaches have led to interesting lead compounds targeting rice blast fungus Tps1 (Xue et al. 2014). Many nutritionally relevant genes form part of gene families, and deciphering their contribution to virulence has to date proved problematic using molecular genetic approaches. Gene silencing seemed to hold some promise for testing if such gene families might be useful drug targets and has been used to prove the importance of endo-β-1,4-xylanases of *Magnaporthe* for colonisation of its rice host (Nguyen et al. 2011). A more robust approach to tackling the function of gene families may prove the use of type II clustered regularly interspaced short palindromic repeats/CRISPR-associatedprotein-9 nuclease (CRISPR/Cas9) technology which has already been used to introduce simultaneous multilocus changes in the genome of *Saccharomyces cerevisiae* (Mans et al. 2015).

There are many fungal processes and metabolic activities (pathways) that differentiate them from their hosts and consequently make them attractive as sources of novel targets. For example, germination of fungal spores is a potential hunting ground. Of course germination requires proteins that participate in other developmental processes, and specificity of potential targets would need to be determined. Nevertheless it is perhaps surprising that in the molecular genetic analyses of key fungal species such as *Magnaporthe oryzae*, relatively few defined mutants that affect germination have been isolated to date. Perhaps this is a reflection of how critical this process is for the pathogen or an indication of redundancy built in as a fail-safe measure. Alternatively it could be that many of the factors required for germination are essential genes. In contrast to germination, appressorium formation by *Magnaporthe oryzae* has been very well characterised at the molecular genetic level and has already been exploited as a stage for fungicide intervention by inhibitors of melanin biosynthesis which render the infection structure inactive. Because spore germination is a critical step in the dis-

ease cycle or in many different fungal species including non-appressorium-forming species such as *Aspergillus fumigatus*and other human pathogens, a search for germination mutants as well as germination inhibitors and their targets would seem to warrant more effort. In a similar manner, sporulation plays such a critical role in the dissemination of many fungal diseases that it would seem an obvious hunting ground for targets which could be exploited for curative treatments in agriculture. Morphogenetic switches such as that which controls dimorphic switching and white-opaque switching in *Candida albicans*might also be targeted (Huang 2012). Adhesion is the very first step in the disease cycle of many pathogenic fungi yet is still relatively poorly characterised at the molecular genetic level (Tucker and Talbot 2001). Development of inhibitors of key factors involved in processes such as germination, sporulation and adhesion will require greater efforts to dissect these processes, and these are good examples of aspects of biology where lack of understanding has precluded any practical applications.

G. Terra Incognita

If we again look to human drug development, it becomes clear that current drugs target a fairly narrow range of the human proteome with GPCRs being the largest single group (Rask-Andersen et al. 2011). As one might anticipate, there is a very strong positive correlation between how well a particular protein is characterised and the availability of drugs targeting that protein (Edwards et al. 2011). Clearly there is a need to address the function of the uncharacterised and less well-characterised proteins. This is particularly true for fungi where in many species the majority of proteins would be classed this way. Genetic and chemical proteomic approaches might be used coordinately to address this goal, and, if 'proof of relevance' is obtained, this can stimulate the search for effective inhibitors of novel targets. As discussed earlier due to financial constraints, industry might be reluctant to address potential targets which would require longer development times, and 'unknowns' are likely to fall into this category. Some public funding might be necessary to stimulate research into less charted territories where novelty is guaranteed.

VI. The Impact of Genome Sequences and 'Omics' Technologies on Target Identification

How far have 'omics' technologies brought us forwards in target identification (and more generally in the development of chemicals for combating diseases)? One answer to this question might be that it is still early days. Another view is that, certainly in the field of pharmaceutical development (which has several years' advantage over the more recently acquired fungal genomes), the promise of a wave of innovation based on the new information has largely not been fulfilled (Munos and Chin 2011; Bunnage 2011). One view why this is the case is that genetic approaches have been prioritised over pharmacological approaches (Moellering and Cravatt 2012). Powerful chemical proteomic approaches are a recent development (Bantscheff and Drewes 2012), and it has been suggested that the availability of these approaches should place pharmacodynamics and pharmacokinetics much earlier in the drug development pipeline (Moellering and Cravatt 2012). Certainly the ability to really exploit the genomic resources we now have by addressing the relevance of gene products on a large scale is a recent development and one on the back of which a wave of innovation in terms of new drug targets may yet come.

VII. Clever Fungicide Formulations

The threat of resistance development and a recognition of the dangers of uncontrolled pesticide application have ended the practice of a single chemical's repeated use in agriculture and led to widespread adoption of integrated pest management (IPM) originally developed as a concept in controlling insect pest strategies (Ehler 2006). IPM aims to reduce pest populations to acceptable levels and encourages the

responsible use of pesticides in combination with other controls. Applications of fungicides should therefore be made as part of a broader control strategy and only when there is a real threat and using no more product that is absolutely necessary. IPM limits the risk of fungicide resistance development and reduces the environmental impact of fungicides. From the point of view of the chemical company, however, the potential market for any new fungicide now faces constraints which given the huge investment required might be a disincentive in the development of fungicides especially for a niche crop. Chemical companies may therefore need to be inventive in marketing durable fungicides in the future so that although they might not sell as much product, the product might sell for longer. Understanding how resistance develops may be critical here. We know that in some cases resistance development has arisen by mutation which alters the direct target of the fungicide as is the case with strobilurin resistance. In other cases, resistance mechanisms are more complex, and resistance arises more slowly, and cross-resistance to related compounds is apparent. Resistance which may develop incrementally might be associated with increased transcription of transporter genes such as ATP-binding cassette (ABC) transporters or MDRs. One possibility is to exploit these resistance mechanisms as supplementary drug targets. If it is known that a particular transporter can act on a particular drug, a clever fungicide could be applied together with an inhibitor of the drug transporter protein. In a similar manner, fungi that acquire resistance by detoxification of the antifungal via enzymes could be applied with an inhibitor of that enzyme with an aim to extending the active life of the antifungal agent and/or reducing the dose applied. Therefore, although not directly fungicide targets themselves, proteins that act to inactivate or exclude fungicides could be very usefully exploited as supplementary targets in combination with other antimycotic agents. Technologies such as haploinsufficiency screens (Giaever et al. 1999) and multicopy suppression profiling screens (Butcher and Schreiber 2006) with susceptible organisms, which are established techniques in mode of action identification, can be used to predict resistance mechanisms during the fungicide development process. Additionally, studies of the additive and synergistic interactions between fungicides are worth further consideration in the development of more durable products (Li and Rinaldi 1999).

VIII. Recognising Complexity: How Modelling Networks Might Impact Target Identification

Complexity is often overlooked when we consider potential targets. Although there are some tens of thousands of proteins, post-transcriptional modifications mean that there may be hundreds of thousands of different proteins in a cell. Added to this, there are several possible protein-protein and other interactions possible, and these will vary with cell type and from an infection perspective depending on the stage of infection. How can this complexity be addressed? Looking to the world of pharmaceuticals, there are many lessons learned there which may guide thinking. Perhaps one of the biggest lessons has been the recognition that the action of drugs is often much more complicated than we imagined before current technologies became available. Using modern chemoproteomic approaches, it has been shown that there are examples of medicinal drugs which are under trial or already on sale which have subsequently been demonstrated to act on several often unrelated targets in the human proteome (Moellering and Cravatt 2012). The ability to precisely map the promiscuity of small molecules to whole proteomes will prove beneficial in assessing 'off-target' effects in future screens for antifungals. With the implementation of 'systems biology' and the generation of the vast datasets that accompany, its use has come the emerging field of networks and its application in drug research (for a comprehensive review of these technologies and their potential in drug design, see Csermely et al. 2013). In the future, we can anticipate that modelling of networks will lead to a more rational testing of potential drug

targets and a better understanding of broader effects and resistance risks. Although it is early days, recognising the complexities of the interaction of a pathogen with its host and the wider changes that accompany disease and chemical intervention within transcriptional and proteomic networks can only reap rewards. This 'holistic' view contrasts starkly with reductionist approaches of the past. Fully exploiting this technology necessitates firstly a much better understanding of the networks that exist in pathogenic fungi and their hosts in response to infection. It may be many years, yet before there is sufficient information in fungal infection model systems to usefully exploit this knowledge. Although it might be possible to make better predictions of a drug's effects, obviously ultimately the acid test is whether the agent is actually effective and safe in use.

IX. More Elegant Genetic Tools for Fungal Research

With the availability of so many genome sequences, we are now very well placed to assess conservation of proteins across a broad range of fungal species and their hosts. We can potentially identify key factors that are specific to our 'target' species and absent from benign species and the host and then search for small molecules to target these. However, the genetic potential of an organism does not tell us much about what part of this potential is actually used under any particular condition. For example, we can see that several filamentous fungi have the ability to produce mycotoxins; however, we know in this case that few of these are actually produced and only under specific conditions (Calvo et al. 2002). Transcriptomics holds more promise than comparative genomics alone because we specifically focus on the active components of the genetic repertoire. For example, in a comparison between *Saccharomyces cerevisiae* and *Candida glabrata*response to benomyl, it was shown that the regulation of the response has diverged much more greatly than the response itself (Lelandais et al. 2008), information that would not come from comparative genomics alone. Ultimately, functional genomics is needed to establish target relevance. It seems certain that genome-wide gene deletion programmes (Noble and Johnson 2005) can deliver potential new targets. The fact that deletion of a gene is lethal or renders, the mutant non-pathogenic does not of course guarantee that the product of the gene will be a good drug target, and further validation is required by finding a specific inhibitor.

Although essential genes are for obvious reasons good fungicide targets, with many model systems, there are limited resources for molecular genetic analysis of such genes. Exceptions are species which can be stably maintained in a diploid state. Inducible promoters and conditionally lethal strains such as strains harbouring analogue-sensitive isoforms of essential enzymes (Penn et al. 2015) can be developed to assist in analysis of factors required for viability. Technologies which more closely mirror the effects of drug intervention are also desirable as gene disruption, or deletion does not mimic the effect of most drugs which quantitatively suppress protein function and are often promiscuous to some degree in their target recognition. Gene silencing has promising attributes in terms of mimicking partial suppression of gene function in a similar manner to fungicides. Nevertheless in analysis of fungi, at least the creation of constructs for stable and specific gene silencing is still time-consuming. The CRISPR/Cas9 gene editing system is rapidly being developed for model pathogenic species (Vyas et al. 2015; Fuller et al. 2015; Arazoe et al. 2015) and holds great promise for assessing whether fungal gene families or subsets of related genes could be exploited as fungicide targets (DiCarlo et al. 2013; Bao et al. 2015). Cell wall-degrading enzymes have been difficult to analyse because they tend to be large gene families; nevertheless, common catalytic mechanisms and accessibility make them attractive drug targets. Activity-based protein profiling now offers the possibility to identify related enzymes recognised by the same small molecule and can pinpoint relevant members of gene families and explore an antifungal agent's promiscuity

(Fonović and Bogyo 2008). These new technologies should aid in future verification of whether any subsets of the products of extended gene families could be exploited as good multisite fungicide targets.

XII. Conclusions and Future Prospects

With all the technologies available to us today, we have never been better placed to seek new fungicide targets. So what is holding us back? As stated earlier, fungicide development is a costly and time-consuming process. There would seem to be a need to bring academic research and industry-led drug development programmes together to seek new targets. Faced with finite funds, industry is unlikely to take on risky and costly projects that search for a few novel targets among many thousands of proteins and is likely instead to focus on developing new chemistries which act on existing targets. Likewise academics are not generally attracted by high-risk projects with no guarantee of publication. Consequently, although the products of academic and industrial research groups are very different, neither group is keen to take on high-risk/high-cost projects. Additionally both fields of research generally take a short-term view and prefer to invest time or money in projects which will take a shorter time to bear fruit. A concerted effort within both academia and industry to identify new fungicide targets would ensure that plant protectants can be developed in time to provide food security for the coming generations. Achieving this will need investment in long-term programmes that bring the expertise in industry and academia closer together to pool their skills in the identification of novel fungicide targets.

A current trend in the pharmaceutical realm is the 'repurposing' of current drugs (Strittmatter 2014). This approach has an obvious appeal in that established medicinal drugs have a history of safe use. In the case of fungal diseases of humans, the antidepressant sertraline has been shown to be effective in the treatment of cryptococcosis, and although not as potent as amphotericin, it has been trialled clinically (Rhein et al. 2016). In the application of such chemistries in an agricultural context, further tests would need to be conducted to ensure that plant host metabolism is not detrimentally perturbed. It has been suggested that because some classes of chemical have found a use both as crop protection fungicides and antifungal drugs and because the former group has a much greater diversity of modes of action, agrochemicals might serve as an inspiration for the development of new drugs to treat human fungal infections (Myung 2015; Jampillek 2016).

Genome sequencing has been extremely useful in determining evolutionary relationships and has been and will be useful in exploring resistance development; however, it has so far shed limited light on what makes pathogens pathogenic or similar questions in other fields of biology. In the future, we can expect that the focus will turn to gene products and high-throughput functional genomics. Genome-wide deletion studies could be achieved for key fungal pathogens as has been achieved in model yeast systems (Noble and Johnson 2005; Winzeler et al. 1999). A genome-wide mutant bank including a series of mutations in each gene, conditionally lethal alleles and an overexpression collection for key pathogenic species would provide a good foundation for target searches. Other approaches based on more traditional mutagenesis programmes such as TILLING on a genome-wide scale have become realistic goals as sequencing technologies develop and become more economical (Comai et al. 2004). To ensure durability, however, we must also prioritise the development of fungicides that act on multiple targets such as gene families. Projects that will generate a deep understanding of key processes such as secretion, the suppression of host defences and the fungal pathogen's nutrition in its host can be expected to yield potential new targets in the coming years. Meeting the growing demands for food supply is a challenge and one in which fungicides will continue to play a key role. Although the public perception of pesticides is generally not a positive one, never have these agents been safer than at the time of

writing, and even if genetically modified crops gain a wider acceptance in Europe, we can anticipate that fungicides will remain a key weapon in safeguarding food security for many years to come. Likewise, medical mycology will need to embrace new methodologies and maintain efforts to keep clinicians one step ahead of pathogens. With the technologies available to the researcher now, it is a very exciting time to work in the mycology and the fungicide development field, and it will be interesting to see what innovations new technologies will deliver.

References

Anaissie EJ, McGinnis MR, Pfaller MA (2009) Clinical mycology, 2nd edn. Churchill Livingstone

Arazoe T, Miyoshi K, Yamato T, Ogawa T, Ohsato S, Arie T, Kuwata S (2015) Tailor-made CRISPR/Cas system for highly efficient targeted gene replacement in the rice blast fungus. Biotechnol Bioeng 112:2543–2549. https://doi.org/10.1002/bit.25662

Avram S, Funar-Timofei S, Borota A, Chennamaneni SR, Manchala AK, Muresan S (2014) Quantitative estimation of pesticide-likeness for agrochemical discovery. J Cheminform 6:42 eCollection

Baker LG, Specht CA, Lodge JK (2011) Cell wall chitosan is necessary for virulence in the opportunistic pathogen *Cryptococcus neoformans*. Eukaryot Cell 10:1264–1268

Balhadere PV, Foster AJ, Talbot NJ (1999) Identification of pathogenicity mutants of the rice blast fungus *Magnaporthe grisea* using insertional mutagenesis. Mol Plant Microbe Interact 12:129–142

Bantscheff M, Drewes G (2012) Chemoproteomic approaches to drug target identification and drug profiling. Bioorg Med Chem 20:1973–1978

Bao Z, Xiao H, Liang J, Zhang L, Xiong X, Sun N, Si T, Zhao H (2015) Homology-integrated CRISPR-Cas(HI-CRISPR) system for one-step multigene disruption in *Saccharomyces cerevisiae*. ACS Synth Biol 4:585–594

Bastidas RJ, Reedy JL, Morales-Johansson H, Heitman J, Cardenas ME (2008) Signaling cascades as drug targets in model and pathogenic fungi. Curr Opin Investig Drugs 9:856–864

Blum M, Boehler M, Randall E, Young V, Csukai M, Kraus S, Moulin F, Scalliet G, Avrova AO, Whisson SC, Fonne-Pfister R (2010) Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. Mol Plant Pathol 11:227–243

Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. BioEssays 28:799–808

Bunnage ME (2011) Getting pharmaceutical R&D back on target. Nat Chem Biol 7:335–339

Butcher RA, Schreiber SL (2006) A microarray-based protocol for monitoring the growth of yeast overexpression strains. Nat Protoc 1:569–576

Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459

Chanclud E, Kisiala A, Emery NR, Chalvon V, Ducasse A, Romiti-Michel C, Gravot A, Kroj T, Morel JB (2016) Cytokinin production by the rice blast fungus is a pivotal requirement for full virulence. PLoS Pathog 12:e1005457. https://doi.org/10.1371/journal.ppat.1005457

Chavez-Dozal AA, Bernardo SM, Rane HS, Herrera G, Kulkarny V, Wagener J, Cunningham I, Brand AC, Gow NA, Lee SA (2015) The *Candida albicans* exocyst subunit Sec6 contributes to cell wall integrity and is a determinant of hyphal branching. Eukaryot Cell 14:684–689

Chen Y, Zuo R, Zhu Q, Sun Y, Li M, Dong Y, Ru Y, Zhang H, Zheng X, Zhang Z (2014) MoLys2 is necessary for growth, conidiogenesis, lysine biosynthesis, and pathogenicity in *Magnaporthe oryzae*. Fungal Genet Biol 67:51–57

Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR, Henikoff S (2004) Efficient discovery of DNA polymorphisms in natural populations by ecotilling. Plant J 37:778–786

Cornet M, Gaillardin C (2014) pH signaling in human fungal pathogens: a new target for antifungal strategies. Eukaryot Cell 13:342–352

Csermely P, Korcsmáros T, Kiss HJ, London G, Nussinov R (2013) Structure and dynamics of molecular networks: a novel paradigm of drug discovery: a comprehensive review. Pharmacol Ther 138:333–408. https://doi.org/10.1016/j.pharmthera.2013.01.016

Cui Z, Wang Y, Lei N, Wang K, Zhu T (2013) *Botrytis cinerea* chitin synthase BcChsVI is required for normal growth and pathogenicity. Curr Genet 59:119–128

De Pascale G, Nazi I, Harrison PH, Wright GD (2011) β-lactone natural products and derivatives inactivate homoserine transacetylase, a target for antimicrobial agents. J Antibiot (Tokyo) 64:483–487

Deising HB, Reimann S, Pascholati SF (2008) Mechanisms and significance of fungicide resistance. Braz J Microbiol 39:286–295

DeZwaan TM, Carroll AM, Valent B, Sweigard JA (1999) Magnaporthe grisea pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues. Plant Cell 11:2013–2030

DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM (2013) Genome engineering in *Saccharomyces*

cerevisiae using CRISPR-Cas systems. Nucleic Acids Res 41:4336–4343

Dietl AM, Amich J, Leal S, Beckmann N, Binder U, Beilhack A, Pearlman E, Haas H (2016) Histidine biosynthesis plays a crucial role in metal homeostasis and virulence of *Aspergillus fumigatus*. Virulence 7:465–476

Dong S, Yin W, Kong G, Yang X, Qutob D, Chen Q, Kale SD, Sui Y, Zhang Z, Dou D, Zheng X, Gijzen M, Tyler BM, Wang Y (2011) Phytophthora sojae avirulence effector Avr3b is a secreted NADH and ADP-ribose pyrophosphorylase that modulates plant immunity. PLoS Pathog 7:e1002353. https://doi.org/10.1371/journal.ppat.1002353

Drewes M, Tietjen K, Sparks TC (2012) High-throughput screening in agrochemical research. In: Jeschke P, Kramer W, Schirmer U, Witschel M (eds) Modern methods in crop protection research, 1st edn. Wiley-VCH, New York, pp 1–20

Drews J, Ryser ST (1996) An innovation deficit in the pharmaceutical industry. Drug Inf J 30:97–108

Du Y, Mpina MH, Birch PR, Bouwmeester K, Govers F (2015) Phytophthora Infestans RXLR effector AVR1 interacts with exocyst component Sec5 to manipulate plant immunity. Plant Physiol 169:1975–1990

Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, FH Y (2011) Too many roads not taken. Nature 470:163–165

Ehler LE (2006) Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Manag Sci 62:787–789

Eisenman HC, Casadevall A (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 93:931–940. https://doi.org/10.1007/s00253-011-3777-2

Eisenman HC, Frases S, Nicola AM, Rodrigues ML, Casadevall A (2009) Vesicle-associated melanisation in *Cryptococcus neoformans*. Microbiology 155:3860–3867

Fonović M, Bogyo M (2008) Activity-based probes as a tool for functional proteomic analysis of proteases. Expert Rev Proteomics 5:721–730. https://doi.org/10.1586/14789450.5.5.721

Foster AJ, Jenkinson JM, Talbot NJ (2003) Trehalose synthesis and metabolism are required at different stages of plant infection by *Magnaporthe grisea*. EMBO J 22:225–235

Fradin C, Bernardes ES, Jouault T (2015) Candida albicans phospholipomannan: a sweet spot for controlling host response/inflammation. Semin Immunopathol 37:123–130

Franzen AJ, Cunha MM, Miranda K, Hentschel J, Plattner H, da Silva MB, Salgado CG, de Souza W, Rozental S (2008) Ultrastructural characterization of melanosomes of the human pathogenic fungus *Fonsecaea pedrosoi*. J Struct Biol 162:75–84

Fritz R, Lanen C, Colas V, Leroux P (1997) Inhibition of methionine biosynthesis in *Botrytis cinerea* by the anilinopyrimidine fungicide pyrimethanil. Pestic Sci 49:40–46

Fu J, Wu J, Jiang J, Wang Z, Ma Z (2013) Cystathionine gamma-synthase is essential for methionine biosynthesis in *Fusarium graminearum*. Fungal Biol 117:13–21. https://doi.org/10.1016/j.funbio.2012.11.001

Fujikawa T, Sakaguchi A, Nishizawa Y, Kouzai Y, Minami E, Yano S, Koga H, Meshi T, Nishimura M (2012) Surface α-1,3-glucan facilitates fungal stealth infection by interfering with innate immunity in plants. PLoS Pathog 8:e1002882. https://doi.org/10.1371/journal.ppat.1002882

Fuller KK, Chen S, Loros JJ, Dunlap JC (2015) Development of the CRISPR/Cas9 system for targeted gene disruption in *Aspergillus fumigatus*. Eukaryot Cell 14:1073–1080

Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, Astromoff A, Davis RW (1999) Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat Genet 21:278–283

Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B (2012) Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. Nat Commun 4:1996. https://doi.org/10.1038/ncomms2996

Goddijn OJ, Verwoerd TC, Voogd E, Krutwagen RW, de Graaf PT, van Dun K, Poels J, Ponstein AS, Damm B, Pen J (1997) Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol 113:181–190

Górska A, Sloderbach A, Marszałł MP (2014) Siderophore-drug complexes: potential medicinal applications of the 'Trojan horse' strategy. Trends Pharmacol Sci 35:442–449

Gravelat FN, Beauvais A, Liu H, Lee MJ, Snarr BD, Chen D, Xu W, Kravtsov I, Hoareau CM, Vanier G, Urb M, Campoli P, Al Abdallah Q, Lehoux M, Chabot JC, Ouimet MC, Baptista SD, Fritz JH, Nierman WC, Latgé JP, Mitchell AP, Filler SG, Fontaine T, Sheppard DC (2013) *Aspergillus* galactosaminogalactan mediates adherence to host constituents and conceals hyphal β-glucan from the immune system. PLoS Pathog 9:e1003575. https://doi.org/10.1371/journal.ppat.1003575

Greenshields DL, Liu G, Feng J, Selvaraj G, Wei Y (2007) The siderophore biosynthetic gene *SID1*, but not the ferroxidase gene *FET3*, is required for full *Fusarium graminearum* virulence. Mol Plant Pathol 8:411–421. https://doi.org/10.1111/j.1364-3703.2007.00401.x

Guimarães LL, Toledo MS, Ferreira FAS, Straus AH, Takahashi HK (2014) Structural diversity and biological significance of glycosphingolipids in pathogenic and opportunistic fungi. Front Cell Infect Microbiol 4:138. https://doi.org/10.3389/fcimb.2014.00138

Hamada T, Asanagi M, Satozawa T, Araki N, Banba S, Higashimura N, Akase T, Hirase K (2014) Action mechanism of the novel rice blast fungicide tol-

procarb distinct from that of conventional melanin biosynthesis inhibitors. J Pest Sci 39:152–158
Hao G, Dong Q, Yang G (2011) A comparative study on the constitutive properties of marketed pesticides. Mol Inform 30:614–622. https://doi.org/10.1002/minf.201100020
Heider MR, Munson M (2012) Exorcising the exocyst complex. Traffic 13:898–907
Hewitt G (2000) New modes of action of fungicides. Pest Outlook 11:28–32. https://doi.org/10.1039/B006311H
Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM (2005) The *Aspergillus fumigatus* siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun 73:5493–5503
Huang G (2012) Regulation of phenotypic transitions in the fungal pathogen *Candida albicans*. Virulence 3:251–261
Hube B, Sanglard D, Odds FC, Hess D, Monod M, Schäfer W, Brown AJ, Gow NA (1997) Disruption of each of the secreted aspartyl proteinase genes *SAP1*, *SAP2*, and *SAP3* of *Candida albicans* attenuates virulence. Infect Immun 65:3529–3538
Hwang LH, Seth E, Gilmore SA, Sil A (2008) SRE1 regulates iron-dependent and -independent pathways in the fungal pathogen *Histoplasma capsulatum*. Eukaryot Cell 11:16–25
Jacob S, Schüffler A, Thines E (2016) Hog1p activation by marasmic acid through inhibition of the histidine kinase Sln1p. Pest Manag Sci 72:1268–1274
Jampillek J (2016) Potential of agricultural fungicides for antifungal drug discovery. Expert Opin Drug Discov 11:1–9. https://doi.org/10.1517/17460441.2016.1110142
Jastrzębowska K, Gabriel I (2015) Inhibitors of amino acids biosynthesis as antifungal agents. Amino Acids 47:227–249
Kong L-A, Yang J, Li G-T, Qi L-L, Zhang Y-J, Wang C-F et al (2012) Different chitin synthase genes are required for various developmental and plant infection processes in the rice blast fungus *Magnaporthe oryzae*. PLoS Pathog 8:e1002526. https://doi.org/10.1371/journal.ppat.1002526
Kostensis E (2006) G proteins in drug screening: from analysis of receptor-G protein specificity to manipulation of GPCR-mediated signalling pathways. Curr Pharm Des 12:1703–1715
Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158
Leadbetter A (2015) Recent developments and challenges in chemical disease control. Plant Protect Sci 51:163–169
Leal SM Jr, Roy S, Vareechon C, Sd C, Clark H, Lopez-Berges MS, Di Pietro A, Schrettl M, Beckmann N, Redl B, Haas H, Pearlman E (2013) Targeting iron acquisition blocks infection with the fungal pathogens *Aspergillus fumigatus* and *Fusarium oxysporum*. PLoS Pathog 9:e1003436. https://doi.org/10.1371/journal.ppat.1003436
Lee S, Gustafson G, Skamnioti P, Baloch R, Gurr S (2008) Host perception and signal transduction studies in wild-type *Blumeria graminis* f. Sp. *hordei* and a quinoxyfen-resistant mutant implicate quinoxyfen in the inhibition of serine esterase activity. Pest Manag Sci 64:544–555. https://doi.org/10.1002/ps.1538
Lelandais G, Tanty V, Geneix C, Etchebest C, Jacq C, Devaux F (2008) Genome adaptation to chemical stress: clues from comparative transcriptomics in *Saccharomyces cerevisiae* and *Candida glabrata*. Genome Biol 9:R164. https://doi.org/10.1186/gb-2008-9-11-r164
Lenardon MD, Munro CA, Gow NAR (2010) Chitin synthesis and fungal pathogenesis. Curr Opin Micobiol 13:416–423. https://doi.org/10.1016/j.mib.2010.05.002
Li RK, Rinaldi MG (1999) In vitro antifungal activity of nikkomycin Z in combination with fluconazole or itraconazole. Antimicrob Agents Chemother 43:1401–1405
Liu XH, Gao HM, Xu F, Lu JP, Devenish RJ, Lin FC (2012) Autophagy vitalizes the pathogenicity of pathogenic fungi. Autophagy 8:1415–1425. https://doi.org/10.4161/auto.21274
Lyu X, Shen C, Fu Y, Xie J, Jiang D, Li G, Cheng J (2016) A small secreted virulence related protein is essential for the necrotrophic interactions of *Sclerotinia sclerotiorum* with its host plants. PLoS Pathog 12: e1005435. https://doi.org/10.1371/journal.ppat.1005435
Maidan MM, De Rop L, Relloso M, Diez-Orejas R, Thevelein JM, Van Dijck P (2008) Combined inactivation of the *Candida albicans GPR1* and *TPS2* genes results in avirulence in a mouse model for systemic infection. Infect Immun 76:1686–1694
Mans R, van Rossum HM, Wijsman M, Backx A, Kuijpers NG, van den Broek M, Daran-Lapujade P, Pronk JT, van Maris AJ, Daran JM (2015) CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*. FEMS Yeast Res 15:pii: fov004. https://doi.org/10.1093/femsyr/fov004
Marx F, Binder U, Leiter E, Pócsi I (2008) The *Penicillium chrysogenum* antifungal protein PAF, a promising tool for the development of new antifungal therapies and fungal cell biology studies. Cell Mol Life Sci 65:445–454
McDougall P (2016) The cost of new agrochemical product discovery, development and registration in 1995 2000 2005 and 2010 to 2014. http://www.croplifeamerica.org/wp-content/uploads/2016/04/Phillips-McDougall-Final-Report_4.6.16.pdf. Cited 17 May 2016

Moellering RE, Cravatt BF (2012) How chemo proteomics can enable drug discovery and development. Chem Biol 19:11–22

Mor V, Rella A, Farnoud AM, Singh A, Munshi M, Bryan A, Naseem S, Konopka JB, Ojima I, Bullesbach E, Ashbaugh A, Linke MJ, Cushion M, Collins M, Ananthula HK, Sallans L, Desai PB, Wiederhold NP, Fothergill AW, Kirkpatrick WR, Patterson T, Wong LH, Sinha S, Giaever G, Nislow C, Flaherty P, Pan X, Cesar GV, de Melo Tavares P, Frases S, Miranda K, Rodrigues ML, Luberto C, Nimrichter L, Del Poeta M (2015) Identification of a new class of antifungals targeting the synthesis of fungal sphingolipids. MBio 6:e00647. https://doi.org/10.1128/mBio.00647-15

Munos BH, Chin WW (2011) How to revive breakthrough innovation in the pharmaceutical industry. Sci Transl Med 3:89cm16. https://doi.org/10.1126/scitranslmed.3002273

Munro CA, Winter K, Buchan A, Henry K, Becker JM, Brown AJ, Bulawa CE, Gow NA (2001) Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. Mol Microbiol 39:1414–1426

Myung K (2015) Can agricultural fungicides accelerate the discovery of human antifungal drugs? Drug Discov Today 20:7–10. https://doi.org/10.1016/j.drudis.2014.08.010

Ngamskulrungroj P, Himmelreich U, Breger JA, Wilson C, Chayakulkeeree M, Krockenberger MB, Malik R, Daniel HM, Toffaletti D, Djordjevic JT, Mylonakis E, Meyer W, Perfect JR (2009) The trehalose synthesis pathway is an integral part of the virulence composite for *Cryptococcus gattii*. Infect Immun 77:4584–4596

Nguyen QB, Itoh K, Van Vu B, Tosa Y, Nakayashiki H (2011) Simultaneous silencing of endo-β-1,4 xylanase genes reveals their roles in the virulence of *Magnaporthe oryzae*. Mol Microbiol 81:1008–1019. https://doi.org/10.1111/j.1365-2958.2011.07746.x

Noble SM, Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. Eukaryot Cell 4:298–309

Noble SM, French S, Kohn LA, Chen V, Johnson AD (2010) Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet 42:590–598

Norton TS, Fortwendel JR (2014) Control of Ras-mediated signaling in *Aspergillus fumigatus*. Mycopathologia 178:325–330. https://doi.org/10.1007/s11046-014-9765-1

Nosanchuk JD, Stark RE, Casadevall A (2015) Fungal melanin: what do we know about structure? Front Microbiol 6:1463. https://doi.org/10.3389/fmicb.2015.01463

Ochiai N, Fujimura M, Oshima M, Motoyama T, Ichiishi A, Yamada-Okabe H, Yamaguchi I (2002) Effects of iprodione and fludioxonil on glycerol synthesis and hyphal development in *Candida albicans*. Biosci Biotechnol Biochem 66:2209–2215

Oliveira-Garcia E, Deising HB (2013) Infection structure-specific expression of β-1,3-glucan synthase is essential for pathogenicity of *Colletotrichum graminicola* and evasion of β-glucan-triggered immunity in maize. Plant Cell 25:2356–2378

Oliver RP, Hewitt HG (2014) Fungicides in crop protection, 2nd edn. CABI, Oxfordshire, UK

Owens J (2007) Determining druggability. Nat Rev Drug Discov 6:187–187

Peña A, Sánchez NS, Calahorra M (2013) Effects of chitosan on *Candida albicans*: conditions for its antifungal activity. Biomed Res Int 2013:527549. https://doi.org/10.1155/2013/527549

Penn TJ, Wood ME, Soanes DM, Csukai M, Corran AJ, Talbot NJ (2015) Protein kinase C is essential for viability of the rice blast fungus *Magnaporthe oryzae*. Mol Microbiol 98:403–419. https://doi.org/10.1111/mmi.13132

Perlin DS (2011) Current perspectives on echinocandin class drugs. Future Microbiol 6:441–457. https://doi.org/10.2217/fmb.11.19

Perlin DS (2015) Mechanisms of echinocandin antifungal drug resistance. Ann N Y Acad Sci 1354:1–11. https://doi.org/10.1111/nyas.12831

Petersen LN, Marineo S, Mandalà S, Davids F, Sewell BT, Ingle RA (2010) The missing link in plant histidine biosynthesis: *Arabidopsis* myoinositol monophosphatase-like2 encodes a functional histidinol-phosphate phosphatase. Plant Physiol 152:1186–1196. https://doi.org/10.1104/pp.109.150805

Piotrowski JS, Okada H, Lu F, Li SC, Hinchman L, Ranjan A, Smith DL, Higbee AJ, Ulbrich A, Coon JJ, Deshpande R, Bukhman YV, McIlwain S, Ong IM, Myers CL, Boone C, Landick R, Ralph J, Kabbage M, Ohya Y (2015) Plant-derived antifungal agent poacic acid targets beta-1,3-glucan. Proc Natl Acad Sci U S A 112:1490–1497. https://doi.org/10.1073/pnas.1410400112

Rask-Andersen M, Almén MS, Schiöth HB (2011) Trends in the exploitation of novel drug targets. Nat Rev Drug Discov 10:579–590. https://doi.org/10.1038/nrd3478

Reese AJ, Yoneda A, Breger JA, Beauvais A, Liu H, Griffith CL, Bose I, Kim M-J, Skau C, Yang S, Sefko JA, Osumi M, Latge J-P, Mylonakis E, Doering TL (2007) Loss of cell wall alpha(1–3) glucan affects *Cryptococcus neoformans* from ultrastructure to virulence. Mol Microbiol 63:1385–1398. https://doi.org/10.1111/j.1365-2958.2006.05551.x

Rhein J, Morawski BM, Hullsiek KH, Nabeta HW, Kiggundu R, Tugume L, Musubire A, Akampurira A, Smith KD, Alhadab A, Williams DA, Abassi M, Bahr NC, Velamakanni SS, Fisher J, Nielsen K, Meya DB, Boulware DR (2016) Efficacy of adjunctive sertraline for the treatment of HIV-associated

cryptococcal meningitis: an open-label dose-ranging study. Lancet Infect Dis. pii:S1473-3099(16)00074–8. doi: https://doi.org/10.1016/S1473-3099(16)00074-8

Román E, Arana DM, Nombela C, Alonso-Monge R, Pla J (2007) MAP kinase pathways as regulators of fungal virulence. Trends Microbiol 15:181–190. https://doi.org/10.1016/j.tim.2007.02.001

Rubinsztein DC, Bento CF, Deretic V (2015) Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. J Exp Med 212:979–990. https://doi.org/10.1084/jem.20150956

Russel PE (2005) A century of fungicide evolution. J Agric Sci 143:11–25

Saint-Macary ME, Barbisan C, Gagey MJ, Frelin O, Beffa R, Lebrun MH, Droux M (2015) Methionine biosynthesis is essential for infection in the rice blast fungus *Magnaporthe oryzae*. PLoS One 10: e0111108. https://doi.org/10.1371/journal.pone.0111108

Saitoh H, Fujisawa S, Mitsuoka C, Ito A, Hirabuchi A, Ikeda K, Irieda H, Yoshino K, Yoshida K, Matsumura H, Tosa Y, Win J, Kamoun S, Takano Y, Terauchi R (2012) Large-scale gene disruption in *Magnaporthe oryzae* identifies MC69, a secreted protein required for infection by monocot and dicot fungal pathogens. PLoS Pathog 8:e1002711. https://doi.org/10.1371/journal.ppat.1002711

Santhanam P, Boshoven JC, Salas O, Bowler K, Islam T, Keykha Saber M, van den Berg GC, Bar-Peled M, Thomma BP (2017) Rhamnose synthase activity is required for pathogenicity of the vascular wilt fungus *Verticillium dahliae*. Mol Plant Pathol 18:347–362. https://doi.org/10.1111/mpp.12401

Sawistowska-Schroder ET, Kerridge D, Perry H (1984) Echinocandin inhibition of 13-beta-D-glucan synthase from *Candida albicans*. FEBS Lett 173:134–138. https://doi.org/10.1016/0014-5793(84)81032-7

Schöbel F, Jacobsen ID, Brock M (2010) Evaluation of lysine biosynthesis as an antifungal drug target: biochemical characterization of *Aspergillus fumigatus* homocitrate synthase and virulence studies. Eukaryot Cell 9:878–893. https://doi.org/10.1128/EC.00020-10

Schrettl M, Bignell E, Kragl C, Joechl C, Rogers T, Arst HN Jr, Haynes K, Haas H (2004) Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. J Exp Med 200:1213–1219

Shepherd MG (1985) Pathogenicity of morphological and auxotrophic mutants of *Candida albicans* in experimental infections. Infect Immun 50:541–544

Singh A, Wang H, Silva LC, Na C, Prieto M, Futerman AH, Luberto C, Del Poeta M (2012) Methylation of glycosylated sphingolipid modulates membrane lipid topography and pathogenicity of *Cryptococcus neoformans*. Cell Microbiol 14:500–516. https://doi.org/10.1111/j.1462-5822.2011.01735.x

Spampinato C, Leonardi D (2013) Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Res Int 2013:204237. https://doi.org/10.1155/2013/204237

Steinberg G (2007) Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. Eukaryot Cell 6:351–360

Strittmatter SM (2014) Old drugs learn new tricks. Nat Med 20:590–591. https://doi.org/10.1038/nm.3595

Sweigard JA, Carroll AM, Farrall L, Chumley FG, Valent B (1998) *Magnaporthe grisea* pathogenicity genes obtained through insertional mutagenesis. Mol Plant Microbe Interact 11:404–412

Tanaka C, Izumitsu K (2010) Two-component signaling system in filamentous fungi and the mode of action of dicarboximide and phenylpyrrole fungicides. In: Carisse O (ed) Fungicides. InTech. https://doi.org/10.5772/13774

Thieron M, Pontzen R, Kurahashi Y (1999) Carpropamid: a rice fungicide with two different modes of action. In: Lyr H, Russell PE, Dehne H-W, Sisler HD (eds) Modern fungicides and antifungal compounds II. In: 12th International Reinhardsbrunn symposium, Friedrichroda, Thuringia, Germany, 24–29 May 1998, pp 101–109

Tucker SL, Talbot NJ (2001) Surface attachment and pre-penetration stage development by plant pathogenic fungi. Annu Rev Phytopathol 39: 385–417

Turrà D, Segorbe D, Di Pietro A (2014) Protein kinases in plant-pathogenic fungi: conserved regulators of infection. Annu Rev Phytopathol 52:267–288

Valiante V, Macheleidt J, Föge M, Brakhage AA (2015) The *Aspergillus fumigatus* cell wall integrity signaling pathway: drug target, compensatory pathways, and virulence. Front Microbiol 6:325. https://doi.org/10.3389/fmicb.2015.00325

Vincelli P (2002) QoI (Strobilurin) fungicides: benefits and risks. Plant Health Instructor. doi: https://doi.org/10.1094/PHI-I-2002-0809-02. Updated 2012

Vyas VK, Barrasa MI, Fink GR (2015) A *Candida albicans* CRISPR system permits genetic engineering of essential genes and gene families. Sci Adv 1: e1500248

Walker CA, Gómez BL, Mora-Montes HM, Mackenzie KS, Munro CA, Brown AJ, Gow NA, Kibbler CC, Odds FC (2010) Melanin externalization in *Candida albicans* depends on cell wall chitin structures. Eukaryot Cell 9:1329–1342

Walter H (2011) New fungicides and modes of action. In: Dehne HW, Deising HB, Gisi U, Kuck KH, Russell PE, Lyr H (eds) Modern fungicides and antifungal compounds VI. Deutsche phytomedizinischen Gesellschaft, Brunswick, Braunschweig, pp 47–54

Weber I, Assmann D, Thines E, Steinberg G (2006) Polar localizing class V myosin chitin synthases are essential during early plant infection in the

plant pathogenic fungus *Ustilago maydis*. Plant Cell 18:225–242

Wheeler IE, Hollomon DW, Gustafson G, Mitchell JC, Longhurst C, Zhang Z, Gurr SJ (2003) Quinoxyfen perturbs signal transduction in barley powdery mildew (*Blumeria graminis* f.Sp. *hordei*). Mol Plant Pathol 4:177–186

Whigham E, Qi S, Mistry D, Surana P, Xu R, Fuerst G, Pliego C, Bindschedler LV, Spanu PD, Dickerson JA, Innes RW, Nettleton D, Bogdanove AJ, Wise RP (2015) Broadly conserved fungal effector BEC1019 suppresses host cell death and enhances pathogen virulence in powdery mildew of barley (*Hordeum vulgare* L.). Mol Plant-Microbe Interact 28:968–983

Wilson RA, Jenkinson JM, Gibson RP, Littlechild JA, Wang ZY, Talbot NJ (2007) *Magnaporthe* Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence. EMBO J 26:3673–3685

Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connelly C, Davis K, Dietrich F, Dow SW, El Bakkoury M, Foury F, Friend SH, Gentalen E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Liebundguth N, Lockhart DJ, Lucau-Danila A, Lussier M, M'Rabet N, Menard P, Mittmann M, Pai C, Rebischung C, Revuelta JL, Riles L, Roberts CJ, Ross-MacDonald P, Scherens B, Snyder M, Sookhai-Mahadeo S, Storms RK, Véronneau S, Voet M, Volckaert G, Ward TR, Wysocki R, Yen GS, Yu K, Zimmermann K, Philippsen P, Johnston M, Davis RW (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901–906

Xue Y, Shui G, Wenk MR (2014) TPS1 drug design for rice blast disease in *Magnaporthe oryzae*. Spring 3:18. https://doi.org/10.1186/2193-1801-3-18

Yang Z, Pascon RC, Alspaugh A, Cox GM, McCusker JH (2002) Molecular and genetic analysis of the *Cryptococcus neoformans MET3* gene and a *met3* mutant. Microbiol 148:2617–2625

Zhang Z, Qin G, Li B, Tian S (2014) Knocking out Bcsas1 in *Botrytis cinerea* impacts growth, development, and secretion of extracellular proteins, which decreases virulence. Mol Plant Microbe Interact 27:590–600

Zhu W, Wei W, Fu Y, Cheng J, Xie J, Li G, Yi X, Kang Z, Dickman MB, Jiang D (2013) A secretory protein of necrotrophic fungus *Sclerotinia sclerotiorum* that suppresses host resistance. PLoS One 8: e53901. https://doi.org/10.1371/journal.pone.0053901

Helmint Electron Transport Inhibitors Produced by Fungi

Mihoko Mori[1], Kenichi Nonaka[1], Rokuro Masuma[1], Satoshi Ōmura[1], Kazuro Shiomi[1]

CONTENTS

[1] The Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan; e-mail: morim@lisci.kitasato-u.ac.jp; ken@lisci.kitasato-u.ac.jp; masuma-f@wj8.so-net.ne.jp; omuras@inst.kitasato-u.ac.jp; shiomi@lisci.kitasato-u.ac.jp

I. Introduction

The electron transport chain is present in mitochondria (eukaryotes) or plasma membrane (prokaryotes) and is linked with oxidative phosphorylation to produce ATP. The chain consists of complex I (NADH-ubiquinone reductase), complex II (succinate-ubiquinone reductase), complex III (ubiquinol–cytochrome-*c* reductase, cytochrome bc_1 complex), complex IV (cytochrome-*c* oxidase), and complex V (ATP synthase, F_OF_1-ATPase) which culminates in ATP via oxidative phosphorylation (Saraste 1999). Electrons generated from NADH and $FADH_2$ pass through complexes I–IV to produce a proton gradient, which is harnessed by complex V (Fig. 1).

Inhibitors of electron transport and oxidative phosphorylation enzymes are used to study the mechanism of energy conversion. Some of them have been developed into antifungal, insecticidal, antiparasite, and other anti-infectious agents. For example, carboxin (**1**, complex II inhibitor) and azoxystrobin (**2**, complex III inhibitor) are used against plant-pathogenic fungi, fenpyroximate (**3**, complex I inhibitor) and chlorfenapyr (**4**, uncoupler) are used to combat insects or acari, and bithionol (**5**, complex II inhibitor) and atovaquone (**6**, complex III inhibitor) are used against various parasites (Fig. 2). Though they are synthetic compounds, several are derived from natural compounds. Compound **2** is an analog of strobilurin A (Fig. 3, **7**) produced by a basidiomycete (Sauter et al. 1999), and the origin of **4** is dioxapyrrolomycin (Fig. 3, **8**), produced by *Streptomyces* spp. (Addor et al. 1992).

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

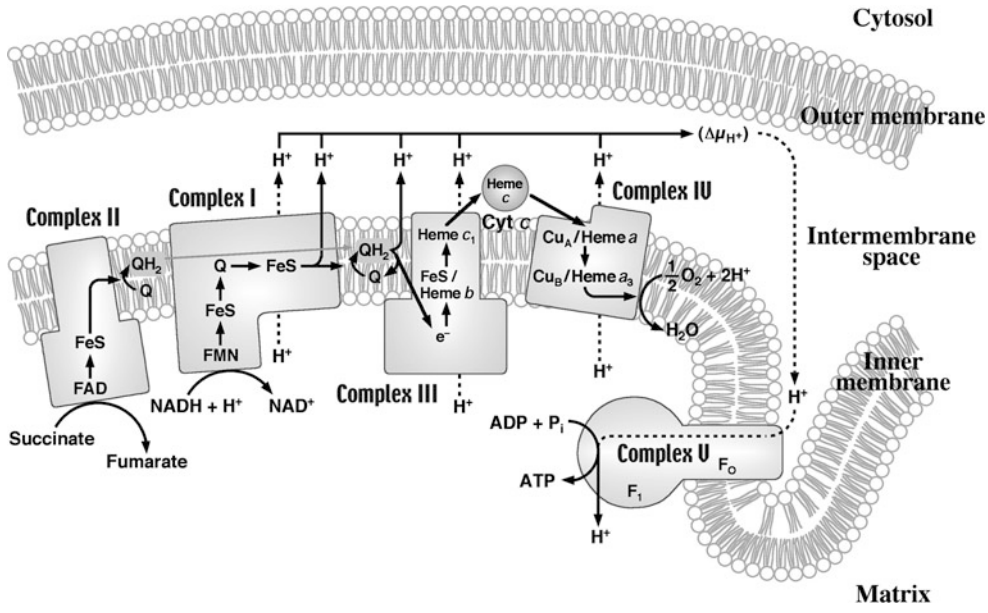


Fig. 1 Electron transport and oxidative phosphorylation in mitochondria

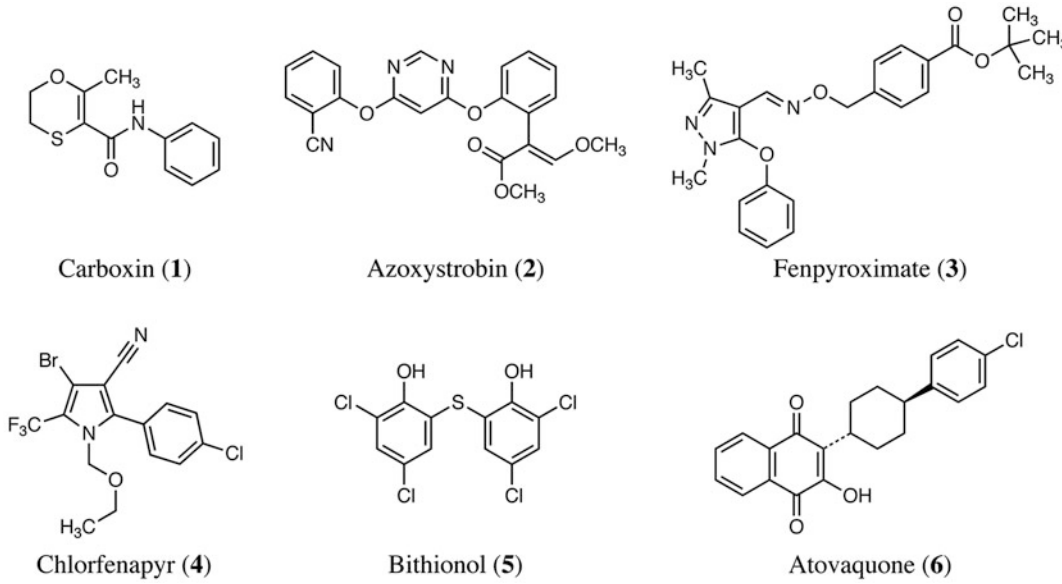
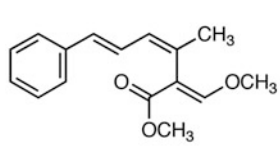
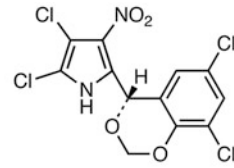


Fig. 2 Structures of practically used electron transport inhibitors

Many electron transport inhibitors and oxidative phosphorylation enzyme inhibitors have been isolated from natural origins (Lardy 1980; Degli Esposti 1998; Ueki et al. 2000). A famous complex I inhibitor, rotenone (Fig. 4, **9**), is a plant metabolite and used effectively as an insecticide. Piericidin A (Fig. 4, **10**) and antimycin A_{3a} (Fig. 4, **11**) are produced by *Streptomyces* spp.

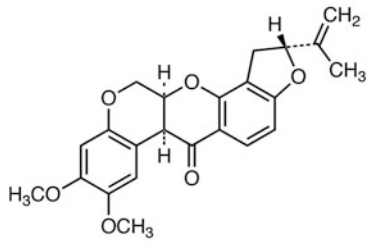


Strobilurin A (**7**)

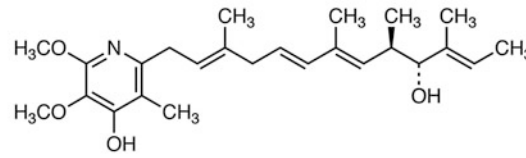


Dioxapyrrolomycin (**8**)

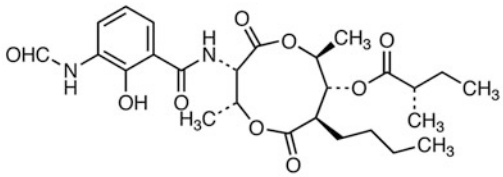
Fig. 3 Structures of strobilurin A and dioxapyrrolomycin



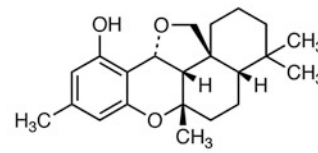
Rotenone (**9**)



Piericidin A (**10**)



Antimycin A_{3a} (**11**)



Siccanin (**12**)

Fig. 4 Popular natural electron transport inhibitors

and widely used as complex I and complex III inhibitors respectively. Siccanin (Fig. 4, **12**), also produced by a fungus, inhibits complex II and is used against surface mycosis.

During the course of screening for anthelmintic antibiotics, some new compounds isolated from the culture broths of fungi were found to be NADH-fumarate reductase (NFRD) inhibitors (Shiomi and Ōmura 2004; Kita et al. 2007; Ōmura and Shiomi 2007). Among them, some compounds show specific inhibition against complex I of anaerobic helminths. One compound, atpenin A5, inhibited complex II potently. Here, we review electron transport and oxidative phosphorylation inhibitors of fungal origin, including the NFRD inhibitors. These inhibitors are summarized in Table 1.

II. Inhibitors of Complex I

Complex I (NADH-ubiquinone reductase) is a relatively massive enzyme. In mammals, complex I consists of 45 different subunits with a combined molecular mass approaching 1 MDa, together with noncovalently bound FMN and eight iron–sulfur clusters (Carroll et al. 2006). Electrons from NADH are accepted by ubiquinone through complex I, ubiquinone being reduced to ubiquinonol. In this process, protons are transferred from the mitochondrial matrix to the intermembrane space, thus producing the electrochemical proton gradient. This is the first step of the electron transport system.

All known high-affinity inhibitors of complex I act at the terminal electron transfer step (quinone-binding site). They are classified into

Table 1 Electron transport and oxidative phosphorylation inhibitors produced by fungi

Compound	Producer	Biological activity
Complex I inhibitors		
Cochlioquinones A (**16**) and B (**17**)	*Cochliobolus miyabeanus*	Anthelmintic, phytotoxic, etc.
Isocochlioquinone A (**18**)	*Bipolaris bicolor*	Phytotoxic
Stemphone A (**19**)	*Stemphylium sarcinaeforme*	Phytotoxic, antibacterial
Pterulone (**20**) and pterulinic acid (**21**)	*Pterula* sp.	Antifungal
Nafuredin (**22**)	*Aspergillus niger*	Anthelmintic
Paecilaminol (**33**)	*Paecilomyces* sp.	Anthelmintic, insecticidal
Verticipyrone (**37**)	*Verticillium* sp.	Anthelmintic, insecticidal
Ukulactones A (**52**), B (**53**) and C (**54**)	*Talaromyces* sp.	Anthelmintic
Prugosene A1 (**55**)	*Talaromyces rugulosus*	
Complex II inhibitors		
Harzianopyridone (**59**)	*Trichoderma harzianum*	Antifungal
Atpenins A4 (**60**) and A5 (**61**)	*Penicillium* sp.	Antifungal
Siccanin (**12**)	*Helminthosporium siccans*	Antifungal
Anhydrofulvic acid (**72**)	*Penicillium* spp.	Antifungal
Complex III inhibitors		
Strobilurin A (**7**)	*Strobilurus tenacellus*	Antifungal
Ilicicolin H (**65**)	*Cylindrocladium ilicicola*	Antifungal, cytotoxic
Funiculosin (**66**)	*Penicillium funiculosum*	Antifungal, antiviral
Sambutoxin (**67**)	*Fusarium sambucinum*	Platelet aggregation inhibitor
AS2077715 (**74**)	*Capnodium* sp.	Antifungal
Oudemansin A (**76**)	*Oudemansiella mucida*	Antifungal
Acrebol (**78**)	*Acremonium exuviarum*	Cytotoxic
Ascochlorin (**79**)	*Ascochyta viciae*	Antiviral, antifungal
Complex IV inhibitor		
Metarhizin A (**82**)	*Metarhizium flavoviride*	Cytotoxic
Complex V inhibitors		
Aurovertin B (**84**)	*Calcarisporium arbuscula*	Apoptosis inducer
Citreoviridin (**85**)	*Penicillium citreoviride*	Neurotoxic
Asteltoxin (**86**)	*Aspergillus stellatus*	Toxic
Efrapeptin D (**87**)	*Tolypocladium niveum*	Antimalarial, antifungal, insecticidal
Tentoxin (**88**)	*Alternaria tenuis*	Chloroplast F_1-ATPase inhibitor, phytotoxic
Uncouplers		
ACR-toxin I (**89**)	*Alternaria citri*	Phytotoxic
Leucinostatin A (**90**)	*Penicillium lilacinum*	Antifungal, antimalarial

two or three groups (Friedrich et al. 1994; Degli Esposti and Ghelli 1999; Okun et al. 1999). Type A (class I) inhibitors, including piericidin A (**10**) and fenpyroximate (**3**), are quinone antagonists and inhibit in a partially competitive manner with regard to ubiquinone. Type B (class II) inhibitors, including rotenone (**9**), aureothin (Fig. 5, **13**), and phenoxan (Fig. 5, **14**), are semiquinone antagonists and inhibit in a noncompetitive fashion (Washizu et al. 1954; Kunze et al. 1992). Aureothin is produced by *Streptomyces* sp. and **14** is produced by myxobacteria. Type C inhibitors are quinol antagonists, such as capsaicin (Fig. 5, **15**), a pungent principle of chili peppers (Shimomura et al. 1989; Yagi 1990). However, this classification does not mean the existence of two or three distinct inhibitors and quinone-binding sites. Okun et al. (1999) has proposed the existence of only one large inhibitor binding pocket in the hydrophobic part of complex I.

Many natural complex I inhibitors have been reported, especially from plants and myxobacteria, whereas the number of reported fungal complex I inhibitors is very small.

Cochlioquinones (Fig. 6) are isolated from *Cochliobolus miyabeanus* and some other fungi

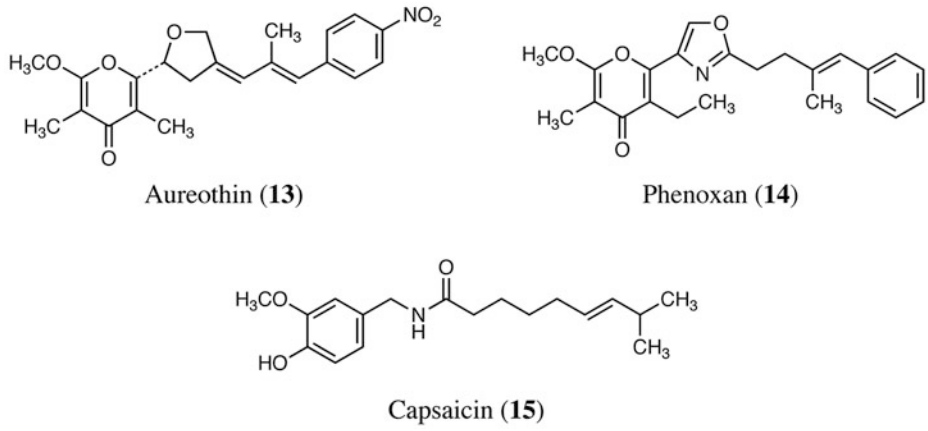


Fig. 5 Structures of aureothin, phenoxan, and capsaicin

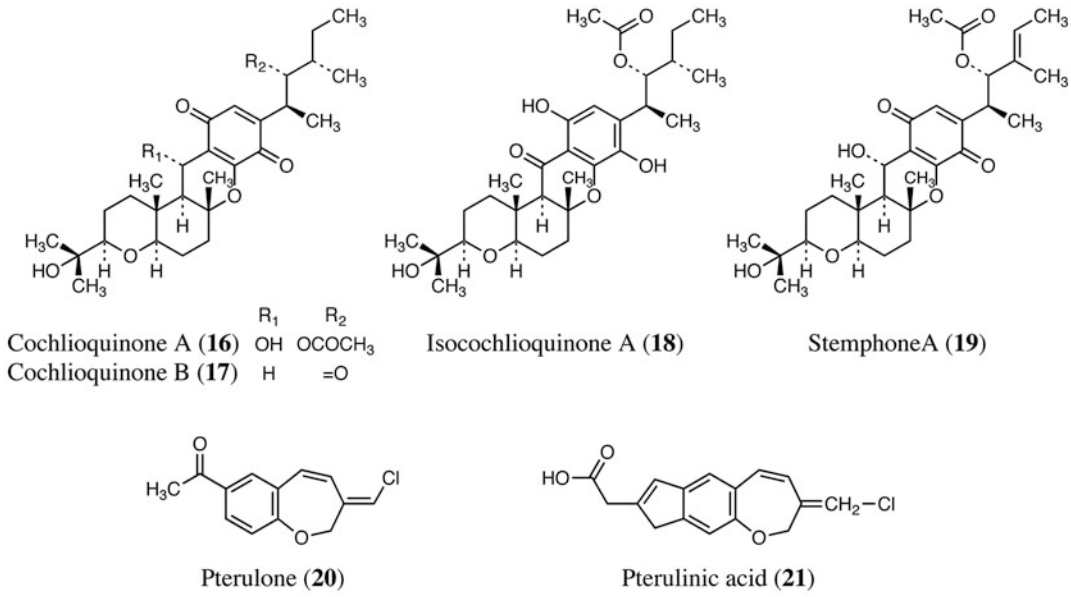


Fig. 6 Structures of complex I inhibitors produced by fungi

(Carruthers et al. 1971). They have a structure of benzoquinone joined to a sesquiterpene, and they show various biological activities, such as nematocidal (against *Caenorhabditis elegans*), phytotoxic and anti-angiogenic properties and competitive inhibition of specific [^{3}H]ivermectin binding (Schaeffer et al. 1990). Inhibitory activities (IC_{50} values) of cochlioquinones A (**16**) and B (**17**), isocochlioquinone A (**18**), and stemphone A (**19**) against bovine heart NADH oxidase (complexes I + III + IV) were 115, 83, 56, and 160 nmol/mg of protein, respectively (Lim et al. 1996). Compound **17** inhibited complex I at an IC_{50} value of 370 nmol/mg of protein. It did not inhibit the other complexes, which indicated **17** was a specific complex I inhibitor. The inhibition against NADH is uncompetitive but inhibition against quinone changes from noncompetitive to competitive when the exogenous quinone concentration increases. A similar complicated inhibition against complex I was reported for capsaicin

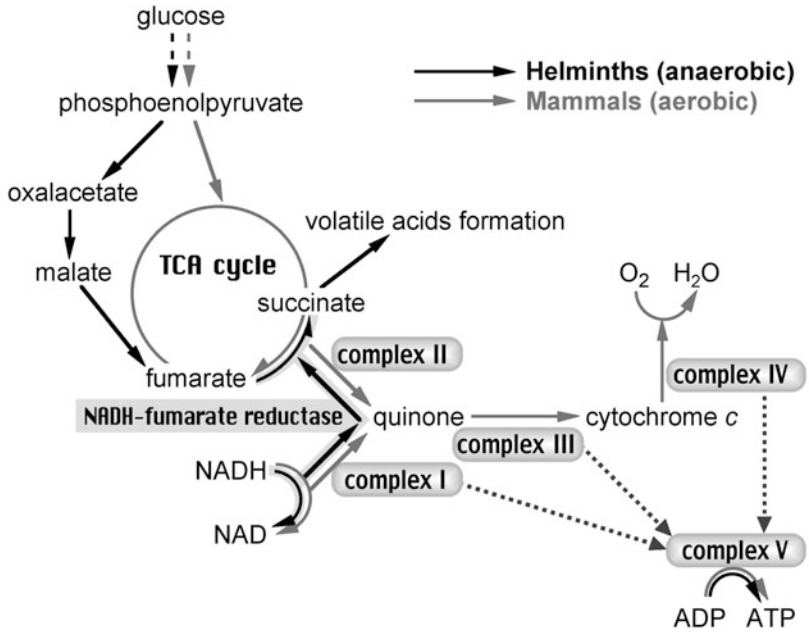


Fig. 7 Difference of energy metabolism between aerobic mammals and anaerobic helminths

(**15**), a type C inhibitor of complex I (Yagi 1990).

Pterulone (Fig. 6, **20**) and pterulinic acid (Fig. 6, **21**) are produced by the submerged culture of the basidiomycete *Pterula* sp. (Engler et al. 1997). They have a 1-benzoxepin ring with chloromethylidene. Though **20** is a single *E* isomer, **21** is a 5:1 mixture of *E*:*Z* that cannot be separated from each other. The compounds inhibit the growth of fungi. The IC_{50} values of **20** and **21** against bovine heart NADH oxidase were 36 and 450 μM, respectively, while they did not inhibit succinate oxidase (complexes II + III + IV). Therefore, they may be complex I inhibitors.

Since complex I reduces ubiquinone and complex III oxidizes ubiquinol, both complexes have quinone-binding sites. Therefore, some inhibitors targeting quinone-binding sites of complex I inhibit complex III and vice versa (Degli Esposti et al. 1993). Strobilurin A (**7**) inhibits complex III potently (see Section "Inhibitors of Complex III") and reportedly inhibits bovine heart complex I weakly (15.5% inhibition at 3.3 μM; Degli Esposti et al. 1993).

III. Inhibitors of Helminth Complex I

A. NADH-Fumarate Reductase

Energy metabolism in many adult helminths differs from that in larvae and host (Kita et al. 2001; Komuniecki and Tielens 2003). They produce ATP in low oxygen concentration using a special respiratory system (Fig. 7). Phosphoenolpyruvate produced via an anaerobic glycolytic pathway is converted to oxalacetate by phosphoenolpyruvate carboxykinase, and oxalacetate is metabolized to malate and then to fumarate. Electrons from NADH are accepted by rhodoquinone through complex I (NADH-rhodoquinone reductase) and then transferred to fumarate through complex II (rhodoquinol-fumarate reductase). Therefore, the anaerobic complex II catalyzes the reverse reaction of aerobic complex II (succinate-ubiquinone reductase). The end products of this glucose catabolism are volatile fatty acids, such as 2-methylpentanoate. This anaerobic electron transport system can provide ATP in the absence of oxygen. Rhodoquinone is used for this anaerobic respiration. Having a

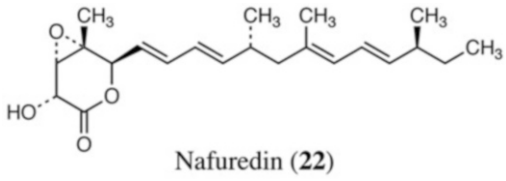


Fig. 8 Structure of nafuredin

lower redox potential than ubiquinone enables rhodoquinone to be used for the reverse reaction of aerobic complex II. NFRD, composed of the above complex I and complex II, plays an important role in the anaerobic respiratory system. Therefore, NFRD inhibitors have been sought from microbial origin to produce anthelmintics, and some helminth-specific complex I inhibitors have been discovered.

B. Nafuredin

1. Producing Strain and Fermentation

Nafuredin (Fig. 8, **22**) was isolated as an NFRD inhibitor (Ōmura et al. 2001; Ui et al. 2001). The producing fungal strain, FT-0554, was isolated from a marine sponge collected in the Palau Islands, Republic of Palau. The strain FT-0554 was identified as *Aspergillus niger* from morphological characteristics. *A. niger* is well known as a terrestrial fungus, so the effect of seawater concentration in a culture medium on fungal growth and nafuredin production was studied (Masuma et al. 2001).

The mycelial growth and the production of **22** were evaluated in different natural seawater concentrations (0–100%) after incubation at 25 °C for 7 days on potato-dextrose agar or broth. The mycelial growth of strain FT-0544 increased with natural seawater concentration, and the addition of natural seawater (25–100%) enhanced the production of nafuredin. The strain FT-0554 also grew abundantly on medium containing 4% of sodium chloride. Generally, the growth of terrestrial fungi tends to be suppressed in the presence of natural seawater. Therefore, *A. niger* FT-0554 is suggested to be adapted to the marine environment.

After the isolation of **22**, more than five fungal strains have been found as producers of **22** during the screening of NFRD inhibitors. It is interesting that all strains were terrestrial fungi, and moreover, all were *Trichoderma* spp.

2. Structure

The structure of **22** was elucidated by NMR and mass spectra analysis (Ui et al. 2001). The total synthesis of **22** revealed its absolute configuration (Takano et al. 2001a, b). It has a β,γ-epoxy-δ-lactone ring with an alkenyl side chain. The biosynthesis study by Ui et al. (2001) suggested that **22** is composed of nine acetates and four methionines (branched four methyl carbons).

Many natural δ-lactones have been reported. δ-Decalactone (Fig. 9, **23**) is found in several kinds of foods, and it is also produced by fungi (Nago et al. 1993). It is used for flavoring and fragrance. With the exception of the simple lactones, natural alkenyl-δ-lactones are usually very cytotoxic. Leptomycin B (Fig. 9, **24**) and kazusamycin A (Fig. 9, **25**), produced by *Streptomyces* spp., are antitumor and antifungal compounds (Hamamoto et al. 1983; Umezawa et al. 1984). They inhibit nuclear export signal-dependent nuclear export of proteins (Wolff et al. 1997). Fostriecin (Fig. 9, **26**) and pironetin (Fig. 9, **27**) are also antitumor compounds produced by *Streptomyces* spp. (Tunac et al. 1983; Kobayashi et al. 1994). The former inhibits protein phosphatase 2A (Roberge et al. 1994), and the latter causes microtubule disassembly (Kondoh et al. 1999). Aurovertins, citreoviridin, and asteltoxin are fungal metabolites possessing alkenyl-δ-lactones. They are mycotoxins and inhibit F_1-ATPase of complex V (see Section "Inhibitors of Complex IV"). ACR-toxin I is a fungal phytotoxin produced by *Alternaria* spp. It is an uncoupler (as shown in Section "Inhibitors of Complex V").

Natural compounds having a β,γ-epoxy-δ-lactone moiety have only been found in clero-

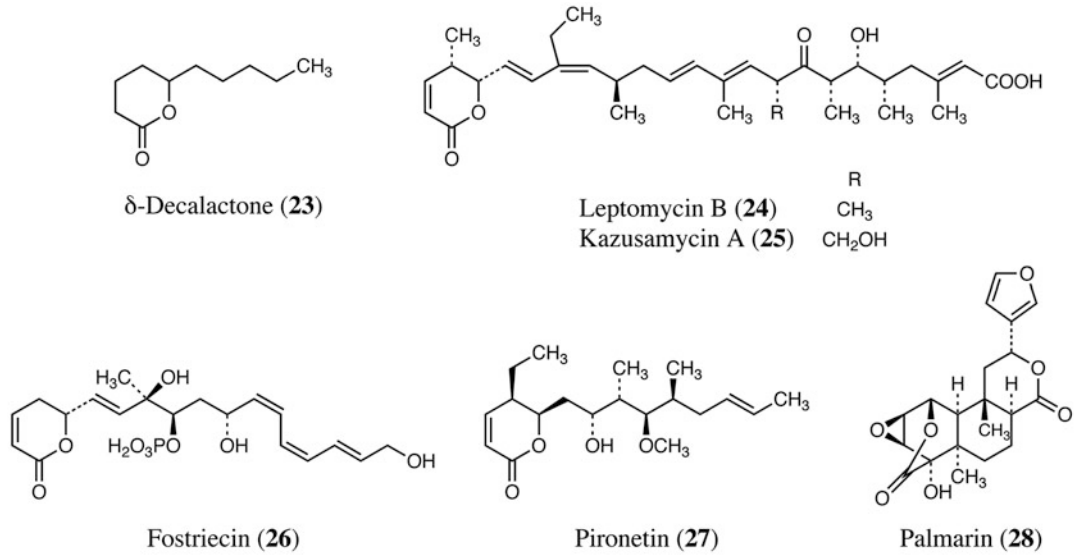


Fig. 9 Structures of δ-lactones produced by microorganisms

Table 2 Effects of nafuredin on electron transport enzymes of nematodes and rat

Enzyme	Complex	IC_{50} (nM)			
		Ascaris suum (adult)	*Ascaris suum* (L2)	*Haemonchus contortus* (adult)	Rat liver
NADH-fumarate reductase	I + II	12	NT	NT	1000
NADH-ubiquinone reductase	I	8	8.9	0.86	10,000
NADH-rhodoquinone reductase	I	24	9.0	195	>100,000
Rhodoquinol-fumarate reductase	II	80,000	NT	NT	NT
Succinate-ubiquinone reductase	II	>100,000	NT	NT	>100,000

NT not tested

dane furanoditerpene group, such as palmarin (Fig. 9, **28**), isolated as a bitter component of Calumba root (Wessely et al. 1936), but their δ-lactones are parts of fused rings (Yonemitsu et al. 1989). Therefore, **22** is the first natural compound having nonfused β,γ-epoxy-δ-lactone.

3. Enzyme Inhibition and Biological Activity

The screening for NFRD inhibitors was carried out using *Ascaris suum* (roundworm) mitochondria. Compound **22** inhibited NFRD at an IC_{50} value of 12 nM (Table 2; Ōmura et al. 2001). Since NFRD consists of NADH-rhodoquinone reductase (complex I) and rhodoquinol-fumarate reductase (complex II), inhibitory activity against each enzyme of *A. suum* was evaluated. As shown in Table 2, **21** potently inhibited complex I (IC_{50} = 24 nM) and the inhibition against complex II was very weak (IC_{50} = 80 μM). It is interesting that **22** inhibited not only anaerobic adult complex I of *A. suum* (NADH-rhodoquinone reductase) but also larval complex I (NADH-ubiquinone reductase), which has an aerobic energy metabolism like that of mammals. The IC_{50} value against larval complex I was 8.9 nM. Therefore, **22** is a complex I inhibitor. However, **22** only inhibited rat

liver complex I at very high concentration (IC_{50} = 10 μM). Therefore, **22** seems to be a selective inhibitor of helminth complex I. From a kinetic study against NADH-rhodoquinone reductase of adult *A. suum*, **22** revealed competitive inhibition with rhodoquinone and non-competitive inhibition with NADH, which indicated that the site of inhibition of **22** is the quinone-binding domain in complex I.

Haemonchus contortus (barberpole worm) is reported to have an NFRD system (Van Hellemond et al. 1995). Therefore, in vivo anthelmintic activity of **22** was evaluated using *H. contortus*-infected sheep (Ōmura et al. 2001). As shown in Table 3, **22** (2 mg/kg p.o.) significantly reduced the number of fecal eggs of infected sheep. This anthelmintic activity may be due to the inhibition of complex I, because **22** also inhibits the enzyme of *H. contortus* (Table 2). Moreover, **22** was effective in mice infected with dwarf tapeworm, *Hymenolepis nana*. There were no signs of any side effects and no loss of body weight during tests in either sheep (2 mg/kg p.o.) or mice (50 mg/kg p.o. and i.p.).

Table 3 Effects of treatment with nafuredin on fecal egg counts in sheep infected with *Haemonchus contortus*

	Number of eggs per gram of feces	
	Treated	Control
Day −1	5689 ± 1120	5200 ± 754
Day 4	1489 ± 655	5100 ± 424
Day 11	289 ± 214	4034 ± 801

Nafuredin (2 mg/kg) was given orally at day 0. Values are means of three experiments (treated animals) or two experiments (controls) ± SD

4. Nafuredin-γ and Its Analogs

During total synthesis of **22**, it was found to convert into a novel γ-lactone derivative, named nafuredin-γ (Fig. 10, **29**), under mild basic conditions (Nagamitsu et al. 2003; Shiomi et al. 2005). The epoxide of **22** opens and the δ-lactone recyclizes to form γ-lactone with keto–enol tautomerism. Compound **29** inhibited NADH-rhodoquinone reductase of adult *A. suum* with an IC_{50} value of 2.3 nM and did not inhibit bovine liver NADH-ubiquinone reductase at 10 μM. It showed anthelmintic activity against *H. contortus* using treatment with two oral doses each of 2 mg/kg. Thus, **29** has similar enzyme inhibitory and anthelmintic activities as those of **22**. Though **22** was converted into **29** in basic condition, only a part of **22** was converted in neutral buffer. Therefore, it is not likely that **21** was converted into **29** and showed inhibitory activity. Both **22** and **29** may inhibit complex I directly.

Since the lactone moiety synthesis of **29** is simpler than that of **22**, **29** is useful for studying structure–activity relationships. The total synthesis of **29** has been achieved (Nagamitsu et al.

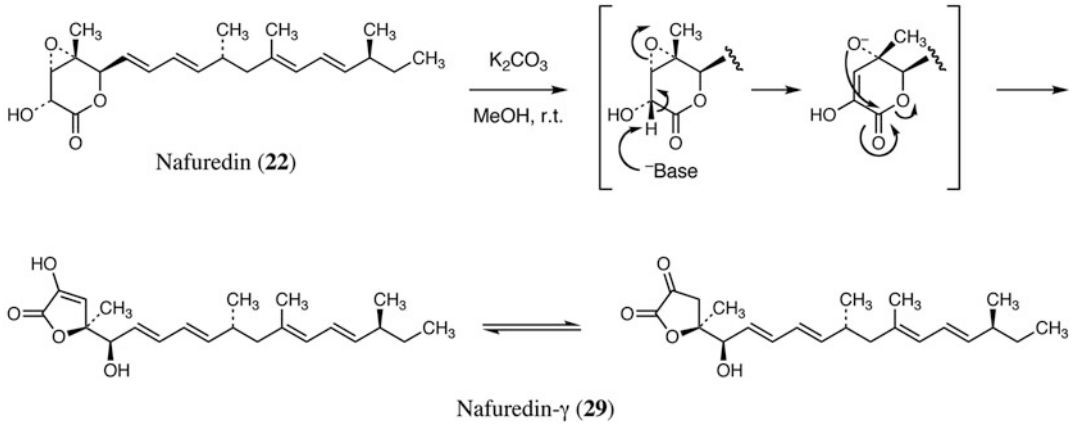


Fig. 10 Proposed mechanism of the conversion of nafuredin to nafuredin-γ

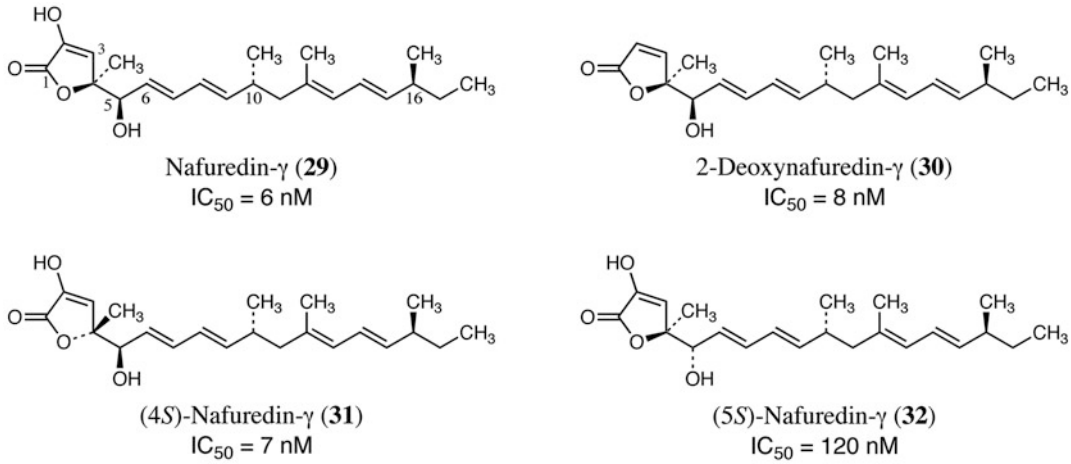


Fig. 11 NADH-fumarate reductase inhibitory activities of nafuredin-γ analogs

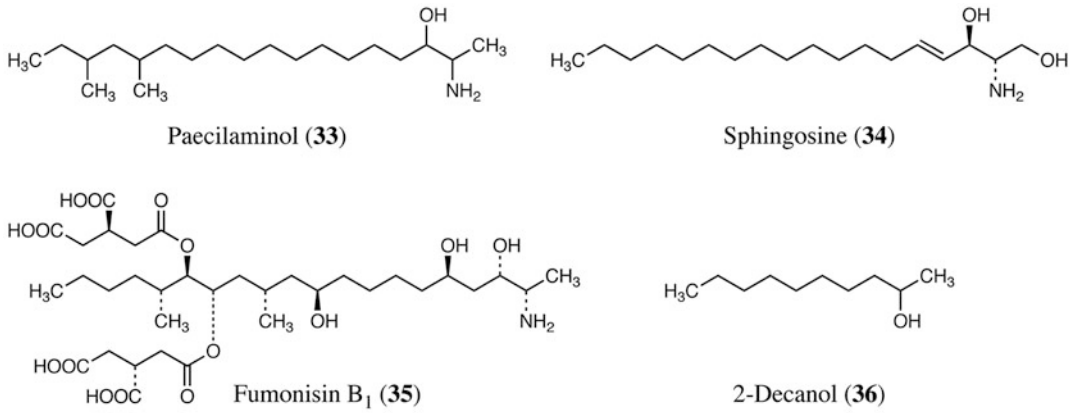


Fig. 12 Structures of paecilaminol and its related compounds

2003), and Nagamitsu et al. (2008) prepared some analogs (Fig. 11). The inhibitory activities of the 2-deoxy derivative (**30**) and C4 epimer (**31**) against NFRD of adult *A. suum* were similar to that of **29**. Therefore, the enol (or 2-ketone) functionality and the C4 stereochemistry of **29** are not responsible for the inhibitory activity. However, the NFRD inhibitory activity of the C5 epimer (**32**) was weakened 20-fold, which suggests that the stereochemistry of the C5 hydroxy group is important for the inhibitory activity of **29**.

C. Paecilaminol

Paecilaminol (Fig. 12, **33**) was produced by a fungus, *Paecilomyces* sp. FKI-0550, isolated from a soil sample collected on Miyakojima Island, Okinawa Prefecture, Japan (Ui et al. 2006a). The structure of **33** was elucidated by NMR and mass spectra as 2-amino-14,16-dimethyl-3-octadecanol. Compound **32** inhibited the growth of free-living nematode *C. elegans* and brine shrimp *Artemia salina* at the MIC values of 20 μg/ml and 5 μg/ml, respectively.

The adult *Ascaris suum* NFRD inhibitory activity of **33** was moderate (IC_{50} = 5.1 μM), but the inhibition was about four times more potent than that of bovine heart NADH oxidase (complexes I + III + IV, IC_{50} = 19.8 μM). The IC_{50} values of **33** against NADH-rhodoquinone reductase (complex I) and rhodoquinol-fumarate reductase (complex II) of *A. suum* were 23 μM and 35 μM, respectively. The values against NADH-ubiquinone reductase (complex I) and ubiquinol–cytochrome-*c* reductase (complex III) of bovine heart were 16 μM and 20 μM, respectively. However, **33** does not inhibit bovine succinate-ubiquinone reductase (complex II) at 100 μM. Therefore, **33** shows similar inhibitory activities against complexes I, II, and III of *A. suum* and bovine heart, except bovine complex II. It is not common for electron transport inhibitors to show such low selectivity. The only group that shows such wide inhibitions are 2-alkyl-4,6-dinitrophenols, with their inhibitory activities against complex II also being weaker than that for complexes I and III (Tan et al. 1993). The low selectivity of **33** may be due to its linear structure, because both amino and hydroxy groups can freely rotate and be attached to enzymes.

Compound **33** is an amino alcohol. The NFRD inhibitory activity of a similar amino alcohol sphingosine (Fig. 12, **34**), a long-chain base of sphingolipids, was weaker (IC_{50} = 28 μM) than **33**. However, Fumonisin B_1 (Fig. 12, **35**), an amino alcohol produced by the fungus *Fusarium moniliforme* (Gelderblom et al. 1988), did not inhibit NFRD at 100 μM. Desai et al. (2002) reported that fumonisins inhibited ceramide synthase (sphingosine *N*-acyltransferase). A simple alcohol 2-decanol (Fig. 12, **36**) also showed no inhibition against NFRD at 100 μM.

D. Verticipyrone

Verticipyrone (Fig. 13, **37**) was produced by a fungus, *Verticillium* sp. FKI-1083 (recently re-identified as *Metapochonia rubescens*: Nonaka et al. 2013; Kepler et al. 2014), isolated from a soil sample collected on Yakushima Island, Kagoshima Prefecture, Japan (Ui et al. 2006b). The structure was shown to be (*E*)-2-methoxy-3,5-dimethyl-6-(3-methyl-2-undecenyl)-4*H*–pyran-4-one by NMR and mass spectra studies. MIC values of **37** against *Caenorhabditis elegans* and *A. salina* were 20 μg/ml and 2.0 μg/ml, respectively.

Compound **37** inhibited NFRD from *A. suum* with an IC_{50} value of 4.1 nM and the inhibition was specific to NADH-rhodoquinone reductase (complex I) as shown in Table 4. However, its inhibitory activity against NADH-ubiquinone reductase (complex I) from bovine heart was similar to that of *A. suum* complex I.

The total synthesis of **37** has been accomplished and some analogs of **37** were prepared (Shimamura et al. 2007), as shown in Fig. 13. Olefin isomers **38** and **39** and alkyl side chain analog **40** showed *Ascaris* NFRD inhibitory activities similar to **37**, which suggests that the olefin in the side chain is not important. The two alcohols **41** and **42** showed much potent NFRD inhibitory activities than **37** suggesting the newly introduced hydroxy group on the side chain may contribute to the inhibition. However, **43** (didemethyl **41**) showed no NFRD inhibition. Therefore, 3,5-dimethyl groups on the γ-pyrone moiety may be essential for NFRD inhibition. The γ-pyrone **44** showed no NFRD inhibition, which suggests that the long side chain is also important for the inhibition.

Among the analogs, **41** showed >20-fold more potent inhibition against *Ascaris* complex I compared with that of **37**, whereas inhibition of **41** against bovine heart complex I was four times less potent than that of **37** (Table 4). Therefore **41** is a selective inhibitor of *Ascaris* complex I.

Alveolar echinococcosis caused by larval *Echinococcus multilocularis* is a life-threatening parasitic zoonosis. *E. multilocularis* was found to use an anaerobic NADH-fumarate reductase system for its energy metabolic pathways (Matsumoto et al. 2008). Therefore, the efficacy of **42** (the most potent NFRD inhibitor among the analogs of **37**) against larval *E. multilocularis* was evaluated. The viability of the *E. multilocularis* protoscolex was progressively reduced during in vitro treatment of the parasites with **42**, and more than 90% of the parasites were

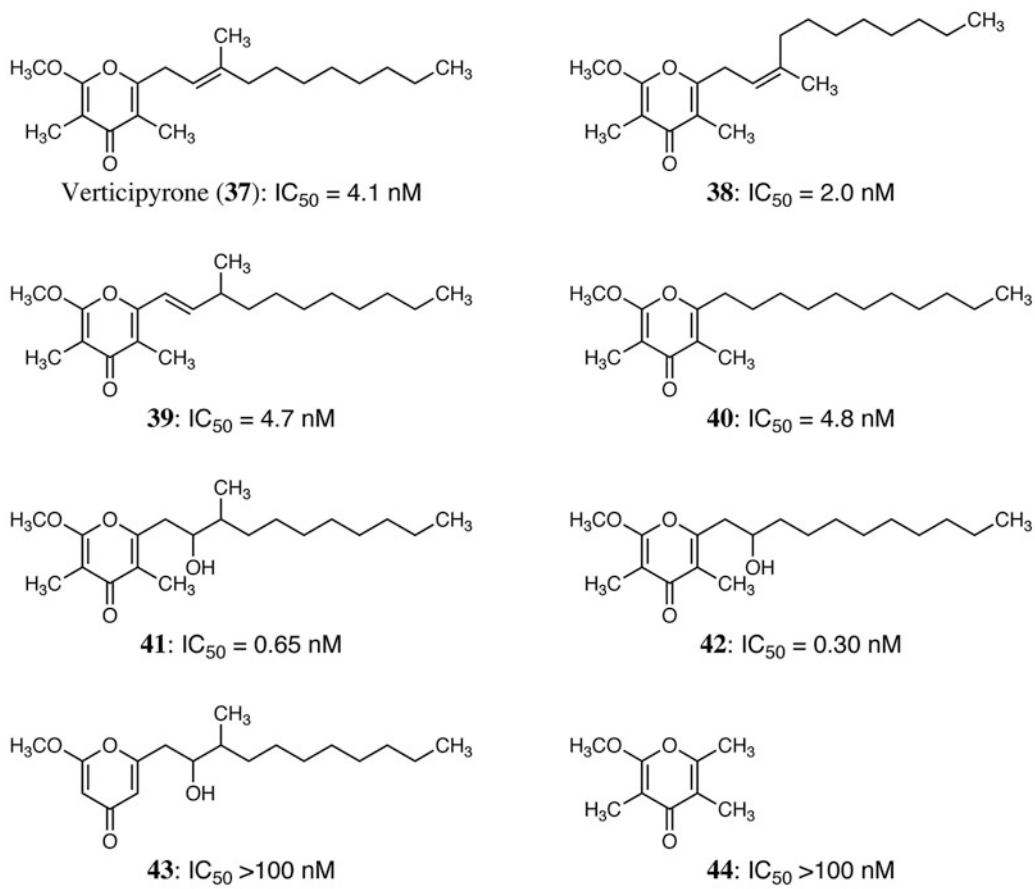


Fig. 13 NADH-fumarate reductase inhibitory activities of verticipyrone and its analogs

Table 4 Effects of verticipyrone and its analog **41** on electron transport enzymes

Enzyme		Complex	IC_{50} (nM)	
			Verticipyrone	**41**
Ascaris suum	NADH-fumarate reductase	I + II	4.1	0.65
	NADH-rhodoquinone reductase	I	49	2.0
	Rhodoquinol-fumarate reductase	II	>100,000	>100,000
Bovine heart	NADH oxidase	I + III + IV	1.3	20
	NADH-ubiquinone reductase	I	46	200
	Succinate-ubiquinone reductase	II	>100,000	>100,000
	Ubiquinol–cytochrome-*c* reductase	III	26,000	80,000

eliminated on days 5 and 18 when **42** was used at 50 μM and 5 μM, respectively (Matsumoto et al., unpublished data).

Compound **37** has the structure of 2-methoxy-3,5-dimethyl-γ-pyrone with a side chain at C6. Aureothin (**13**) produced by *Streptomyces thioluteus* has the same skeleton and inhibits complex I as shown in Section "Inhibitors of Complex I". Neoaureothin (spectinabilin; Fig. 14, **45**), an analog of **13** produced

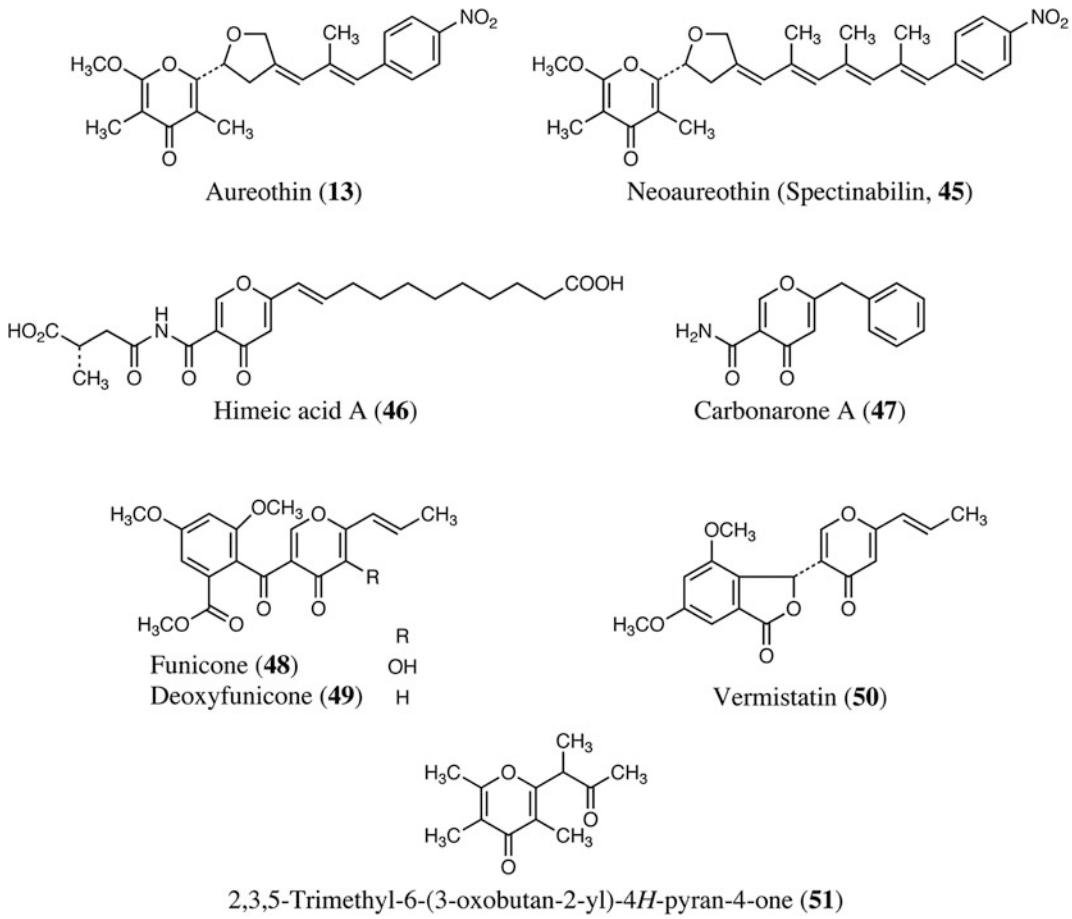


Fig. 14 Structures of verticipyrone related γ-pyrones

by *Streptomyces* spp. (Cassinelli et al. 1966), was found to inhibit *Ascaris* NFRD with an IC_{50} value of 15 nM (Ui et al. 2006b).

Some γ-pyrones with a side chain at C6 have been also isolated from the culture broths of fungi. Himeic acid A (Fig. 14, **46**), produced by *Aspergillus* sp. (Tsukamoto et al. 2005), inhibited ubiquitin-activating enzyme (E1). Carbonarone A (Fig. 14, **47**) produced by *Aspergillus carbonarius* (Zhang et al. 2007) showed moderate cytotoxicity. The producing strains of **46** and **47** were both marine-derived fungi. Funicone (**48**), deoxyfunicone (**49**), and vermistatin (**50**) have (*E*)-propenyl side chains at C6 of the γ-pyrone (Fig. 14). They were produced by *Penicillium funiculosum*, *P. vermiculatum*, and some other fungi (Merlini et al. 1970; Fuska et al. 1979; Sassa et al. 1991). Compounds **48** and **49** showed antifungal activities and **50** showed cytotoxicity. Compounds **49** and **50** also potentiated the antifungal activity of miconazole against *Candida albicans* (Arai et al. 2002). 2,3,5-Trimethyl-6-(3-oxobutan-2-yl)-4*H*–pyran-4-one (Fig. 14, **51**), produced by *Aspergillus sydowi* isolated from the deep sea, was not cytotoxic (Li et al. 2007).

E. Ukulactones

1. Structures and Biological Activities

Ukulactones A and B (Fig. 15, **52** and **53**) were produced by a fungus, *Penicillium* sp. FKI-3389, isolated from a soil sample collected on Hawaii Island, Hawaii, United States (Ōmura et al. 2007). They have the same planar structure, with 2-oxabicyclo[2.2.1]heptane-3,5-dione and 5,6-dihydro-2*H*-pyran linked by

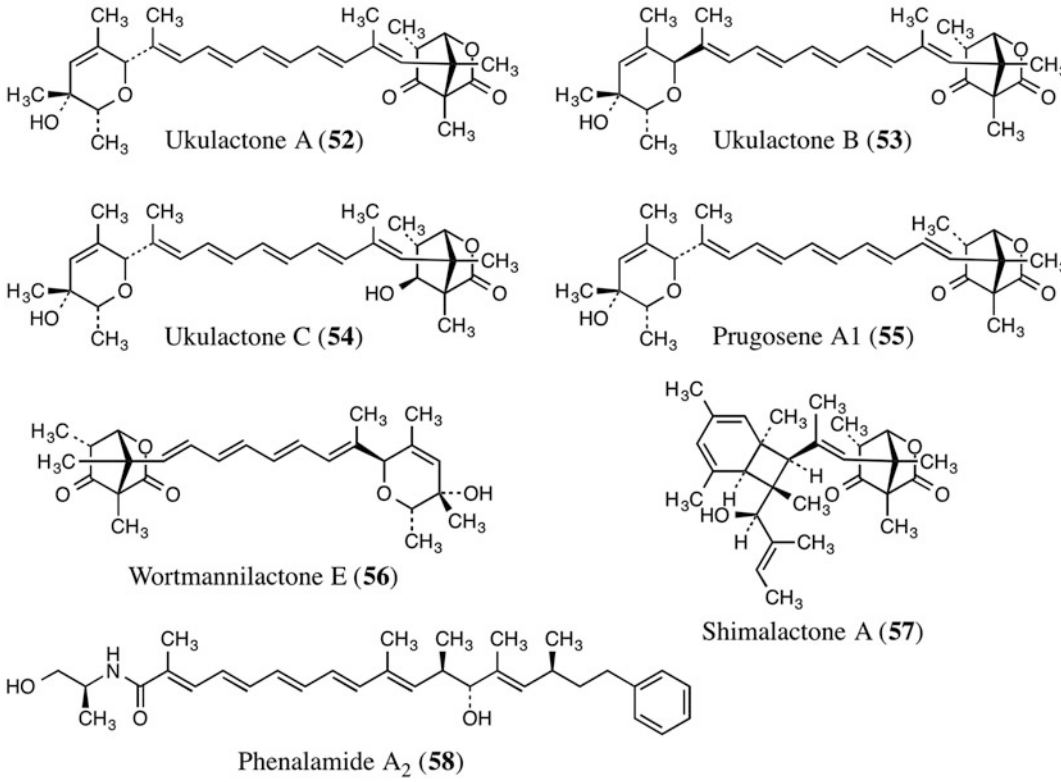


Fig. 15 Structures of ukulactones and their related compounds

Table 5 Effects of ukulactones on electron transport enzymes

Enzyme		Complex	IC_{50} (nM)	
			Ukulactone A	Ukulactone B
Ascaris suum	NADH-fumarate reductase	I + II	2.4	470
	NADH-rhodoquinone reductase	I	55	NT
	Rhodoquinol-fumarate reductase	II	>100,000	NT
Bovine heart	NADH oxidase	I + III + IV	9000	16,000
	Succinate–cytochrome-*c* reductase	II + III	68,000	30,000
	NADH-ubiquinone reductase	I	28,000	NT
	Succinate-ubiquinone reductase	II	>100,000	NT
	Ubiquinol–cytochrome-*c* reductase	III	32,000	NT

NT not tested

pentaene. The configurations at C2 of the pyran (junction of the pyran and the pentaene) are opposite between **52** and **53**. The absolute configurations of **52** and **53** have not yet been elucidated. Compound **52** inhibited the growth of parasite nematode *Nippostrongylus brasiliensis* at 1 μg/ml in vitro.

Compound **52** inhibited *Ascaris* NFRD (Table 5) and is an NADH-rhodoquinone reductase (complex I)-specific inhibitor (IC_{50} = 55 nM). Its inhibition against bovine heart NADH-ubiquinone reductase (complex I) was about 500 times weaker (IC_{50} = 28 μM) than that of *Ascaris* complex I. The inhibitions

against bovine complexes II and III were also weak. Therefore, **52** is a selective inhibitor of *Ascaris* complex I, comparable to nafuredin (**22**). The difference between **52** and **53** is the configuration of only one carbon. However, the inhibition of **53** against *Ascaris* NFRD was about 200 times weaker than that of **52** (Table 5). Thus, the configuration at C2 of the pyran may be very important for the inhibitory activity.

Recently, a new ukulactone analog, ukulactone C (**54**), was obtained from the cultured broth of *Talaromyces* sp. FKI-6713. The 3-ketone moiety on a bicyclo ring of **54** was reduced (Kaifuchi et al. 2015). The configuration at C2 of the pyran is the same as that of **52**; therefore, **54** retained the potent inhibitory activity against *Ascaris* NFRD ($IC_{50} = 62$ nM).

Demethyl **52** compound, prugosene A1 (Fig. 15, **55**), was isolated from the surface-cultured mycelium of a marine sponge-derived *Penicillium rugulosum* by Lang et al. (2007). The configuration at C2 of the pyran of **55** was the same as that of **52**. Compound **55** showed *Ascaris* NFRD inhibitory activity as potent as **52** (Mori et al. 2011).

Dong et al. (2009) isolated wortmannilactone E (Fig. 15, **56**) along with its analogs from the culture broth of *Talaromyces wortmannii*. The pentaene moiety of **55** is altered to tetraene in **56**, and the configuration at C7 of the oxabicyclo ring moiety was opposite to those of prugosenes and ukulactones.

2-Oxabicyclo[2.2.1]heptane-3,5-dione is also found in the structure of shimalactone A (Fig. 15, **57**) produced by the marine fungus *Emericella variecolor* (Wei et al. 2005). Compound **57** induced neuritogenesis against neuroblastoma cells. Compounds **52** and **53** have an (all-*E*)-2,10-dimethyldodeca-2,4,6,8,10-pentaene moiety, which is also found in phenalamide A_2 (**58**) produced by the myxobacterium *Myxococcus stipitatus* (Trowitzsch-Kienast et al. 1992). It suppressed HIV-1 replication in cell cultures and inhibited NADH-ubiquinone reductase (complex I) potently (Friedrich et al. 1994). It is interesting that mammalian complex I was inhibited potently by **58**, but not by **52** and **53**.

2. Taxonomy of Ukulactone-Related Polyene Compound-Producing *Talaromyces*

Ukulactones A and B (**52** and **53**) were isolated as *Ascaris* complex I-specific inhibitors from a broth of a fungal strain of *Penicillium* sp., and they had a unique partial structure of an oxabicyclo[2.2.1]heptane ring. Only a few compounds containing an oxabicyclo[2.2.1]heptane ring have been reported: shimalactone A (**57**), ukulactones A and B, prugosenes (Lang et al. 2007), wortmannilactones (Dong et al. 2009), and coccidiostatin A (Jayasuriya et al. 2007). Except for **57**, these compounds have a polyene chain in their structures. The producers of ukulactones A and B, prugosenes and coccidiostatin A were identified as *Penicillium* species by taxonomic studies. Since *Penicillium* subgenus *Biverticillium* was transferred to the genus *Talaromyces* (Houbraken and Samson 2011; Samson et al. 2011), the ukulactone-producing fungus was revised to *Talaromyces allahabadensis*, and the producer of prugosenes and coccidiostatin A, *Penicillium rugulosum* (Jayasuriya et al. 2007; Lang et al. 2007), was also revised to *Talaromyces rugulosus*. Further oxabicyclo[2.2.1]heptane ring-containing tetraene compounds, wortmannilactones E-H were isolated from the culture broth of *Talaromyces wortmannii* (Dong et al. 2009). Thus, all producers of oxabicyclo[2.2.1]heptane ring-containing polyene compounds are *Talaromyces* species.

Talaromyces species are suggested to be capable of producing unique and specific secondary metabolites, especially many kinds of polyketides and meroterpenoids (Samson et al. 2011; Frisvad 2015). Therefore, the secondary metabolites of *Talaromyces* species have been considered to be of use in taxonomical analysis (Frisvad et al. 1990; Samson et al. 2011; Yilmaz et al. 2014, 2016).

Talaromyces species were classified into three clades; clade 1, clade 2A, and clade 2B by phylogenetic analysis (Samson et al. 2011). About 80% of *Talaromyces* strains belonged to clade 1. However, all of *Talaromyces* species which produced oxabicyclo[2.2.1]heptane ring-containing polyene compounds were classified into one of minor clades, clade 2A. Recently, Yilmaz et al. (2014) proposed a new

sectional classification for the 88 accepted *Talaromyces* species. Strains belonging to clade 1 were classified into four sections: section *Talaromyces*, section *Helici*, section *Purpurei*, and section *Trachyspermi*. The clade 2A species were placed into a single section, section *Islandici*, and the clade 2B species were divided into two sections, section *Bacillispori* and section *Subinflati*. Therefore, all producers of oxabicyclo[2.2.1]heptane ring-containing polyene compounds mentioned above are now grouped in section *Islandici*.

To clarify whether the species in section *Islandici* of the genus *Talaromyces* specifically produce oxabicyclo[2.2.1]heptane ring-containing polyene compounds, 24 *Talaromyces* strains were collected in Japan and classified into five sections (*Islandici*, *Bacillispori*, *Subinflati*, *Trachyspermi*, and *Talaromyces*) by both ITS analyses and phenotypic studies (Fig. 16). These species were cultured on solid medium, and their secondary metabolites were analyzed by HPLC. Eight strains belonging to section *Talaromyces* and four strains belonging to section *Trachyspermi* (both sections belonged to former clade 1) did not produce oxabicyclo[2.2.1]heptane ring-containing polyene compounds (Table 6). The sole strain in section *Subinflati* (former clade 2B) also did not produce these compounds. In contrast, many species in section *Islandici* (former clade 2A) did produce the ukulactone-related polyene compounds, as did all strains in section *Bacillispori* (former clade 2B). Ukulactone C (**54**) was found in the broth of a strain in section *Bacillispori*. Compound **54** contains a reduced ketone moiety on the oxabicyclo[2.2.1]heptane ring, a structural feature limited to **54** in question. Therefore, some strains in section *Bacillispori* may have the species-specific reductase.

It is difficult to find very rare oxabicyclo [2.2.1]heptane ring-containing polyene compounds by random screening of fungal secondary metabolites. However, exploiting phylogenetic taxonomy facilitates discovery of new oxabicyclo[2.2.1]heptane ring-containing polyene compounds.

IV. Inhibitors of Complex II

A. Atpenins and Harzianopyridone

1. Structures

Harzianopyridone (Fig. 17, **59**), produced by the fungus *Trichoderma* sp. was isolated during screening for NFRD inhibitors (Miyadera et al. 2003). It was originally isolated from *T. harzianum* and showed antifungal, antibacterial, and herbicidal activities, but its mode of action had not been studied (Dickinson et al.1989; Cutler and Jacyno 1991). Structurally similar antifungal antibiotics, atpenins A4, A5, and B (Fig. 17, **60–62**), have been isolated from the culture broth of the fungus *Penicillium* sp. (Ōmura et al. 1988; Kumagai et al. 1990) before the report of **59**. Since **62** was suggested to inhibit the ATP-generating system (Oshino et al. 1990), the effects of **59** together with atpenins on the inhibitory activities of electron transport enzymes were examined and were revealed to be potent and selective complex II (succinate-ubiquinone reductase) inhibitors, as shown below.

Though the structure of **59** is depicted as 2-pyridone and the structures of **60–62** are depicted as 2-pyridinol in Fig. 17, structures in the figure only reflect those of the original papers. 2-Pyridone and 2-pyridinol are tautomers, and they may be equivalent. The other compounds having the same chromophore are WF-16775 A_1 and A_2 (Fig. 17, **63**, **64**), isolated from the fungus *Chaetasbolisia erysiophoides* (Otsuka et al. 1992), and they display potent angiogenetic activity.

Fungi also produce some 2-pyridone compounds. Ilicicolin H (Fig. 18, **65**) was isolated by Hayakawa et al. (1971). Funiculosin (Fig. 18, **66**) and sambutoxin (Fig. 18, **67**) are *N*-methyl-2-pyridones (Ando et al.1969; Kim et al. 1995). Compounds **65–67** inhibit complex III (as shown in Section "Inhibitors of Complex III"). Flavipucine (Fig. 18, **68**), produced by *Aspergillus flavipes* (Findlay and Radics 1972), shows antibacterial and fungicidal activities, and apiosporamide (Fig. 18, **69**), produced by *Apiospora montagnei* (Alfatafta et al. 1994), possesses

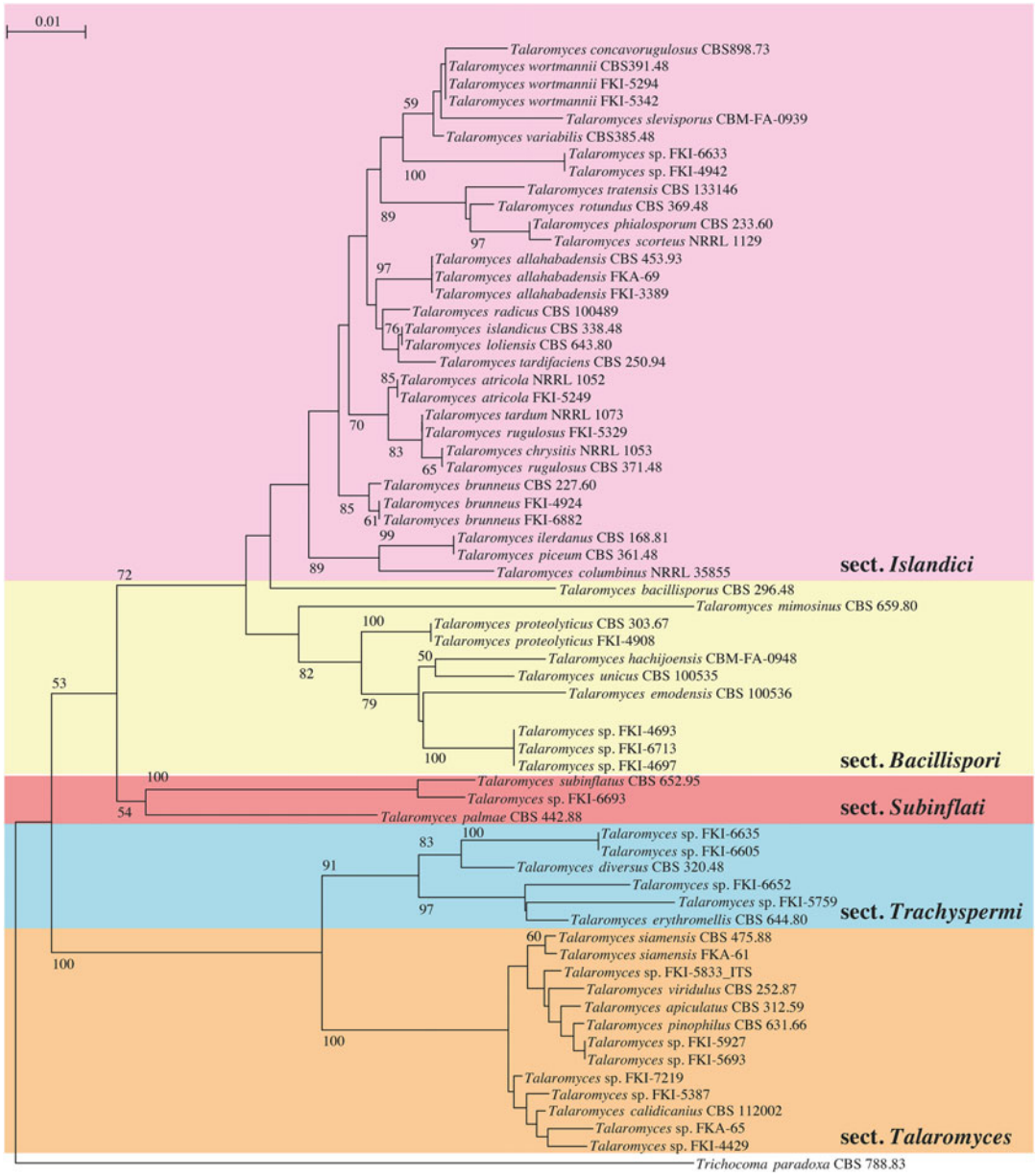


Fig. 16 Isolated *Talaromyces* strains in phylogenetic tree

antifungal activity. Militarinone D (Fig. 18, **70**), isolated from the culture broth of *Paecilomyces militaris* (Schmidt et al. 2003), exhibits cytotoxicity.

As for Actinomycetes, most 2-pyridone-type products belong to the kirromycin (Fig. 18, **71**) group. Kirromycin (mocimycin), produced by *Streptomyces collinus* (Wolf and Zähner 1972), is an antibacterial antibiotic and inhibits protein synthesis interfering with transpeptidation by acting on elongation factor Tu (Parmeggiani and Nissen 2006).

Table 6 Ukulactone-related polyene compounds productivity of *Talaromyces* species

Section	Species	Polyene compounds
Islandici	*T. wortmannii* FKI-5342	Wortmannilactone E
	Talaromyces sp. FKI-6633	Not detected
	Talaromyces sp. FKI-4942	Not detected
	T. allahabadensis FKA-69	Ukulactones
	T. atricola FKI-5249	Coccidiostatin A
	T. brunneus FKI-4924	Not detected
	T. brunneus FKI-6882	Not detected
Bacillispori	*T. proteolyticus* FKI-4908	Coccidiostatin A
	Talaromyces sp. FKI-4693	Ukulactone C
	Talaromyces sp. FKI-6713	Ukulactone C
	Talaromyces sp. FKI-4697	Ukulactone C
Subinflati	*Talaromyces* sp. FKI-6693	Not detected
Trachyspermi	*Talaromyces* sp. FKI-6635	Not detected
	Talaromyces sp. FKI-6605	Not detected
	Talaromyces sp. FKI-6652	Not detected
	Talaromyces sp. FKI-5759	Not detected
Talaromyces	*T. siamensis* FKA-61	Not detected
	Talaromyces sp. FKI-5833	Not detected
	Talaromyces sp. FKI-5927	Not detected
	Talaromyces sp. FKI-5693	Not detected
	Talaromyces sp. FKI-7219	Not detected
	Talaromyces sp. FKI-5387	Not detected
	Talaromyces sp. FKA-65	Not detected
	Talaromyces sp. FKI-4429	Not detected

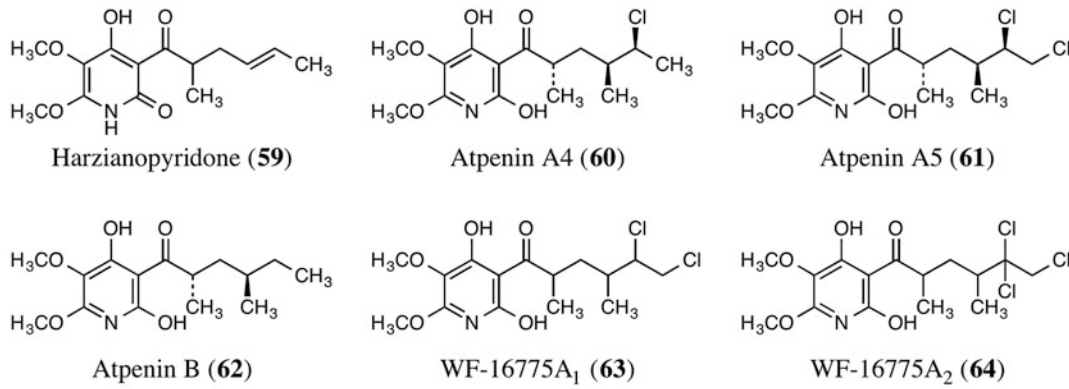
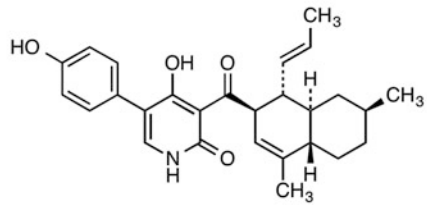


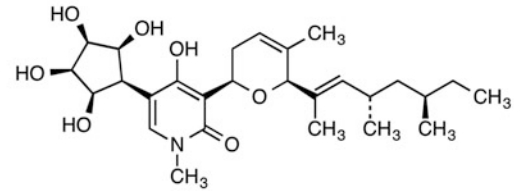
Fig. 17 Structures of atpenins and their related compounds

2. Enzyme Inhibition and Biological Activity

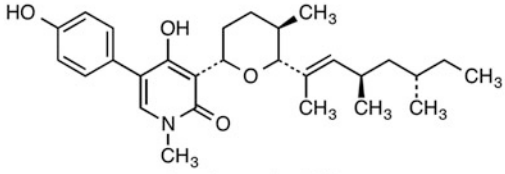
The effects of **59–61** on electron transport enzymes are shown in Table 7 (Miyadera et al. 2003). Though they inhibited NFRD as potently as nafuredin (**22**), verticipyrone (**37**), and ukulactone A (**52**), they did not inhibit complex I. However, they inhibited *Ascaris* rhodoquinol-fumarate reductase and bovine heart succinate-ubiquinone reductase. Therefore, they are specific complex II inhibitors and not just selective for helminths. The inhibition against mammalian complex II is more potent than that of helminth complex II. Atpenins inhibited complex II more potently than **59**, and **61** exhibited the most potent inhibition. Carboxin (**1**) is



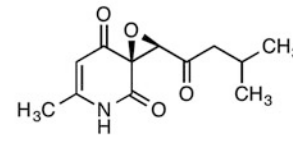
Ilicicolin H (**65**)



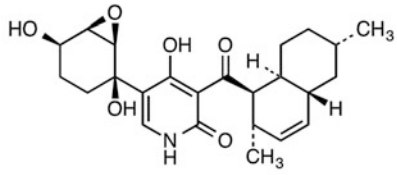
Funiculosin (**66**)



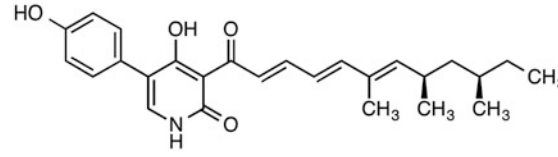
Sambutoxin (**67**)



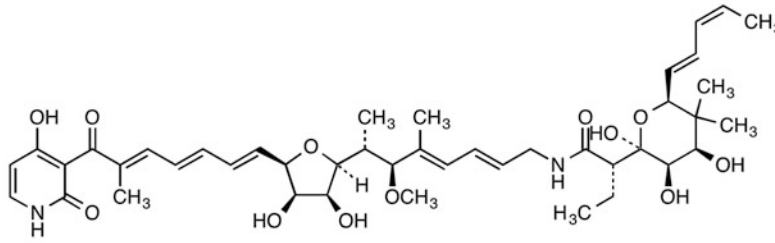
Flavipucine (**68**)



Apiosporamide (**69**)



Militarinone D (**70**)



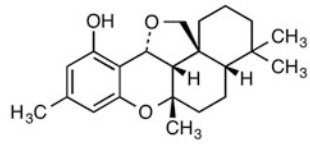
Kirromycin (**71**)

Fig. 18 Structures of 2-pyridones produced by microorganisms

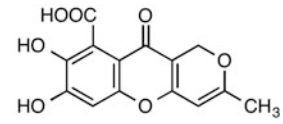
Table 7 Effects of harzianopyridone and atpenins on electron transport enzymes

Enzyme		Complex	IC_{50} (nM)		
			Harzianopyridone	Atpenin A4	Atpenin A5
Ascaris suum	NADH-fumarate reductase	I + II	1600	110	14
	NADH-quinone reductase	I	>100,000	>100,000	>100,000
	Rhodoquinol-fumarate reductase	II	360	220	12
Bovine heart	NADH–cytochrome-*c* reductase	I + III	420,000	140,000	82,000
	Succinate-ubiquinone reductase	II	17	11	3.6
	Succinate dehydrogenase	II	80	9.2	5.5

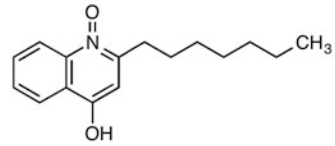
Fig. 19 Structures of complex II inhibitors produced by microorganisms



Siccanin (**12**)



Anhydrofulvic acid (**72**)



2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide (**73**)

known as a potent complex II inhibitor (Mowery et al. 1977). However, the IC_{50} value against bovine heart succinate-ubiquinone reductase was 1.1 μM, which is 300 times weaker than that for **61**. Therefore, atpenins may be useful tools for clarifying the biochemical and structural properties of complex II (Martens et al. 2005; Adebiyi et al. 2008).

X-ray crystallographic analyses of *Escherichia coli* and porcine heart complex II have been reported (Yankovskaya et al. 2003; Sun et al. 2005). Each complex is composed of four subunits, FAD-containing flavoprotein (Fp or SdhA), iron–sulfur protein (Ip or SdhB), and two membrane anchor subunits, CybL (SdhC) and CybS (SdhD). The succinate dehydrogenase catalytic portion is formed by Fp and Ip. The membrane anchor subunits are required for electron transfer to ubiquinone. Ubiquinone binds to complex II at the interface between Ip and the membrane anchor subunits. Kinetic analyses of atpenins revealed that they exhibited mixed inhibition with ubiquinone (K_i = 1.0 nM, K_i' = 5.9 nM for **61**; Miyadera et al. 2003). This indicated that atpenins may block electron transfer between the enzyme and ubiquinone by binding to a region that partly overlaps with the physiological ubiquinone-binding site (Q-site). This can explain the observation that atpenins also affected the succinate dehydrogenase activity of bovine heart complex II (Table 7).

Compound **61** was co-crystallized with *E. coli* succinate-ubiquinone reductase and analyzed in detail by X-ray crystallography (Horsefield et al. 2006). Yankovskaya et al. (2003) observed that the interaction between ubiquinone at the Q-site appeared to be mediated solely by hydrogen bonding between the carbonyl oxygen (O1) of ubiquinone and the hydroxy group of tyrosine 83 in SdhD (Q_1-site). The co-crystallization study of complex II and **61** showed that **61** existed in the same hydrophobic pocket as ubiquinone but deeper within the pocket (Q_2-site). The protein–ligand docking model of complex II and ubiquinone was analyzed in silico, and it revealed that ubiquinone docked at the Q_2-site of complex II. At the Q_2-site, ubiquinone can interact with complex II via additional hydrogen bonds between carbonyl oxygen (O4) and the hydroxy group of serine 27 in SdhC and between 4-methoxy group and the imidazole of histidine 207 in SdhB. These interactions were observed in the co-crystallization result of complex II and **61**. The above results support the proposition that the Q_1-site may be the initial binding site and the Q_2-site may be the catalytic site.

B. Other Complex II Inhibitors

Only a few complex II inhibitors have been found in microbial metabolites. Siccanin (Fig. 19, **12**), produced by the fungus *Helminthosporium siccans*, showed antifungal activity, and it is used clinically for dermatophytosis as an ointment (Ishibashi 1962;

Ishibashi et al. 1970). The structure can be regarded as derived from a *cis*-fused drimane condensed with orcinol. It showed 66% inhibition against the succinate dehydrogenase of a fungus, *Trichophyton mentagrophytes*, at 0.09 μM (Ishibashi et al. 1970; Nose and Endo 1971). Siccanin is a species-selective succinate dehydrogenase inhibitor, and it was effective against succinate dehydrogenases of *Pseudomonas aeruginosa*, *P. putida*, and rat and mouse mitochondria but ineffective or less effective against those of *E. coli*, *Corynebacterium glutamicum*, and porcine mitochondria (Mogi et al. 2009). Recently, it was reported that siccanin selectively inhibited complex II of helminths and protozoa compared to porcine complex II (Kita et al. 2015). For example, IC_{50} values against *Ascaris suum* and *Plasmodium falciparum* complex II were 0.0057 and 0.098 μM, respectively, and that against porcine complex II was 861 μM. Anhydrofulvic acid (Fig. 19, **72**) was produced by *Penicillium* spp. and showed antifungal activity (Wrigley et al. 1994; Fujita et al. 1999). It exhibited 67% inhibition against succinate oxidase (complexes II + III + IV) of a fungus, *Candida utilis*, at 1.3 μM, but inhibited NADH oxidase (complexes I + III + IV) only weakly (18%) at 86 μM, which suggests **72** may be a complex II inhibitor (Fujita et al. 1999).

As for bacteria, 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO; Fig. 19, **73**) was isolated from the culture broth of *P. aeruginosa* (Hays et al. 1945) and reported to inhibit bacterial complex II. It inhibited succinate-menaquinone reductase from *Bacillus subtilis* at the K_i value of 0.2 μM (Smirnova et al. 1995). Compound **73** is well known as a complex III inhibitor (Izzo et al. 1978).

V. Other Electron Transport Inhibitors

A. Inhibitors of Complex III

Complex III (ubiquinol–cytochrome-*c* reductase, cytochrome bc_1 complex) accepts electrons from ubiquinol and passes them to cytochrome *c*, thereby transferring protons from mitochondrial matrix to intermembrane space. Mammalian complex III is a dimer with a molecular mass of 490 kDa, and each monomer consists of 11 subunits containing cytochrome *b*, cytochrome c_1, and an iron–sulfur protein. Cytochrome *b* has two quinone-binding sites, an ubiquinol oxidation site, Q_o site (Q_P site), and a ubiquinone reduction site, Q_i site (Q_N site). Most complex III inhibitors bind to either site.

The most famous Q_i site inhibitor is antimycin A_{3a} (**11**) produced by *Streptomyces* spp. As for fungal complex III inhibitors, funiculosin (Fig. 20, **66**) produced by *Penicillium funiculosum* (Ando et al. 1969) and ilicicolin H (Fig. 20, **65**) produced by *Cylindrocladium ilicicola* (Hayakawa et al. 1971) are Q_i site inhibitors (Rotsaert et al. 2008) and possess antifungal activities. While **66** inhibits both yeast and bovine heart complex III at IC_{50} ~10 nM (Rotsaert et al. 2008), the IC_{50} values of **65** against yeast and bovine heart complex III were 3–5 nM and 200–250 nM, respectively (Gutierrez-Cirlos et al. 2004). These results suggest a high degree of specificity in the determinants of ligand binding at the Q_i site. However, in vivo efficacy of **65** may have been limited by high plasma protein binding (Singh et al. 2012). *Capnodium* sp. produces a new antifungal antibiotic, AS2077715 (**74**), structurally related to **66** (Ohsumi et al. 2014a). It inhibited complex III of *Trichophyton mentagrophytes* with an IC_{50} value of 0.9 ng/ml (Ohsumi et al. 2014c), and in vivo efficacy of AS2077715 against tinea pedis in a guinea pig model was equal to or greater than that of terbinafine (Ohsumi et al. 2014b). Sambutoxin (Fig. 20, **67**) is structurally related to **65** and **66** and produced by *Fusarium sambucinum* (Kim et al. 1995). It was also reported to inhibit complex III (Kawai et al. 1997) and may be a Q_i site inhibitor. As shown in Section "Inhibitors of Complex III", HQNO (**73**) inhibits both complexes II and III. It is a Q_i site inhibitor, but the K_D against bovine heart mitochondria was about three orders of magnitude higher than that of antimycin (von Jagow and Link 1986).

The common Q_o site inhibitor, myxothiazol (Fig. 20, **75**), is produced by the myxobacterium *Myxococcus fulvus* (Gerth et al. 1980). It has an *E*-β-methoxyacrylamide moiety. Similar *E*-β-methoxyacrylate moieties are found in some fungal metabolites, such as strobilurin A

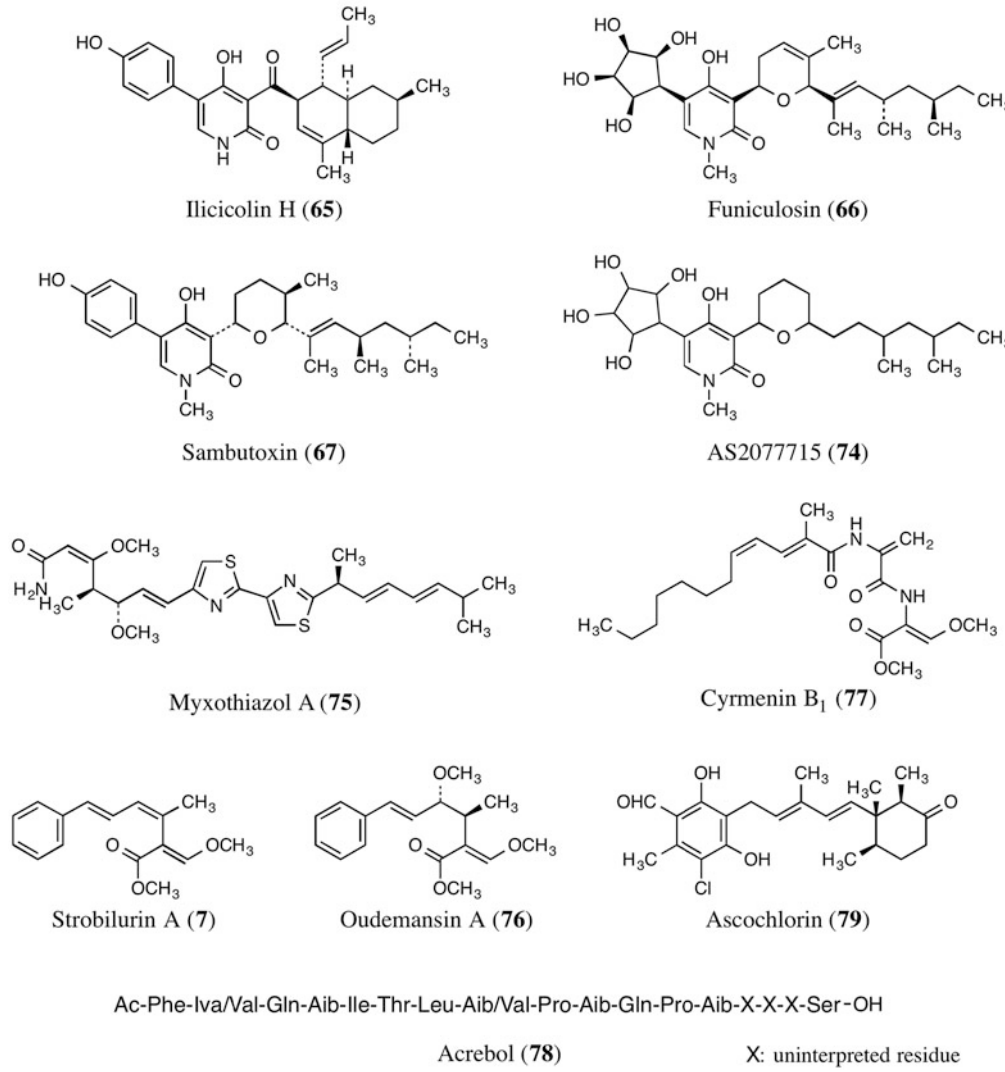


Fig. 20 Structures of complex III inhibitors produced by microorganisms

(mucidin, **7**) and oudemansin A (Fig. 20, **76**) produced by basidiomycetes. Compound **7** was originally isolated from culture broths of *Oudemansiella mucida* (Musilek et al. 1969) and *Strobilurus tenacellus* (Anke et al. 1977) and **76** from *Oudemansiella mucida* (Anke et al. 1979). All proved to be antifungal antibiotics. Their production and properties have been reviewed by Anke and Erkel (2002) in *The Mycota, Vol. X*. Compounds **7** and **76** inhibited bovine heart complex III at IC_{50} values of 65 nM and 290 nM, respectively (Brandt et al. 1988), and their targets are also Q_o site (von Jagow and Link 1986). While **7** inhibits both mammalian and fungal complex III, its toxicity against mammals is very weak. Therefore, many analogs of **7** have been synthesized, and some of them, such as azoxystrobin (**2**), are commercially used for crop protection against phytopathogenic fungi (Sauter et al. 1999). Compounds having the same α-substituted methyl (*E*)-β-methoxyacrylate moiety as **7** and **76** were isolated from the myxobacteria *Cystobacter armeniaca* and *Archangium gephyra* in 2003 (Sasse et al. 2003). They are named cyrmenins, and cyrmenin B_1 (Fig. 20, **77**) was shown to inhibit complex III. A mycotoxin, acrebol (Fig. 20, **78**), produced by *Acremonium exu-*

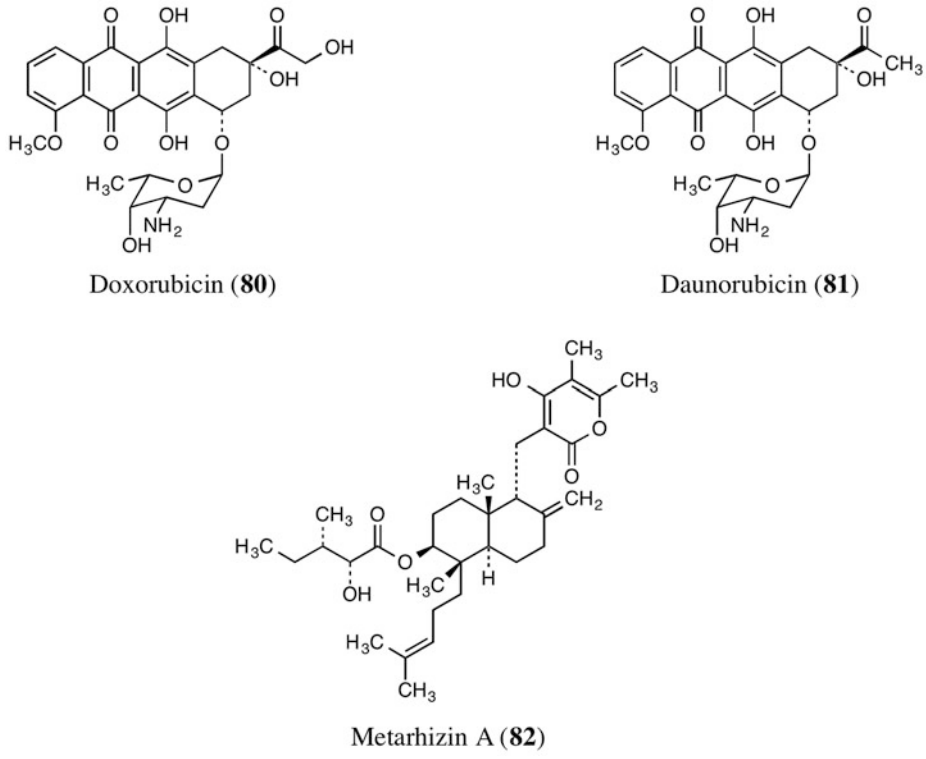


Fig. 21 Structures of complex IV inhibitors produced by microorganisms

viarum, consists of two closely similar peptaibols (1726 and 1740 Da) (Andersson et al. 2009). It is a Q_o site inhibitor and inhibited complex III of isolated rat liver mitochondria, with an IC_{50} value of ~50 nM, after a short preincubation (Kruglov et al. 2009).

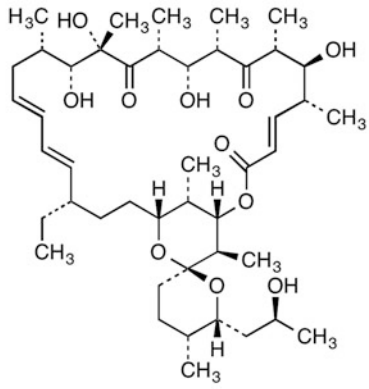
As described in Section "Inhibitors of Complex I", some inhibitors targeting quinone-binding sites of complex I inhibit complex III. Compound **7** inhibited bovine heart complex I weakly (15.5% inhibition at 3.3 μM) although the inhibition of **75** was more potent (84.0% inhibition at 3.0 μM). However, 2.9 μM of **66** showed no inhibition against complex I, and Q_o site inhibitors may affect complex I more potently than Q_i site inhibitors (Degli Esposti et al. 1993).

An antiviral and antifungal antibiotic, ascochlorin (Fig. 20, **79**) was isolated from the culture broth of *Ascochyta viciae* (Tamura et al. 1968). Ascochlorin inhibited O_2 uptake of yeast (*Pichia anomala*) and rat liver mitochondria in a manner comparable to inhibition by **11** and **75**. The crystal structure of chicken complex III soaked with ascochlorin revealed that ascochlorin bound at both the Q_i and Q_o sites (Berry et al. 2010).

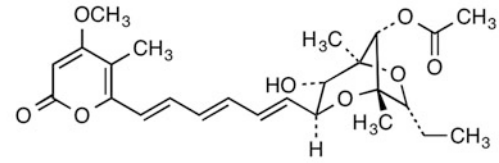
B. Inhibitors of Complex IV

Complex IV (cytochrome-*c* oxidase) is a terminal oxidase of cell respiration, and it reduces molecular oxygen coupling with proton pumping. Mammalian complex IV is a dimer with a molecular mass of 442 kDa, and each monomer consists of 13 subunits containing hemes *a* and a_3 and two copper atoms.

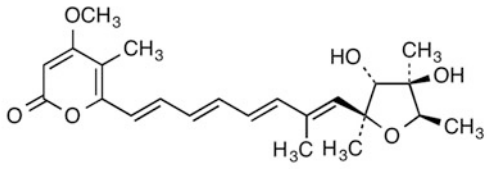
Only a few natural products having complex IV inhibitory activity are known. Anthracyclines, such as doxorubicin (adriamycin) (Fig. 21, **80**) and daunorubicin (daunomycin) (Fig. 21, **81**), are clinically used antitumor antibiotics produced by *Streptomyces* spp.



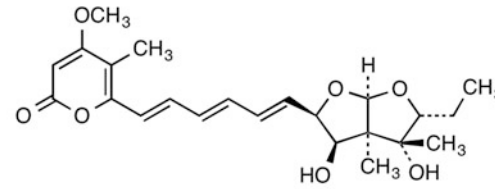
Oligomycin A (**83**)



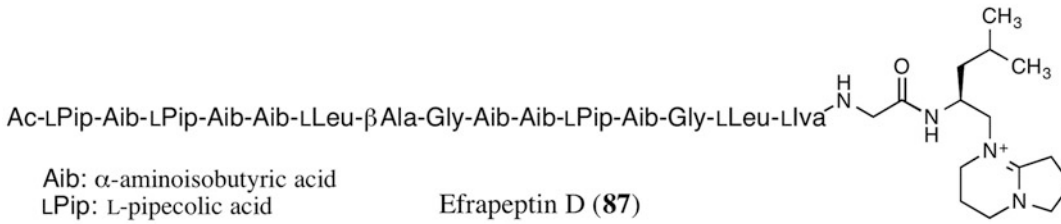
Aurovertin B (**84**)



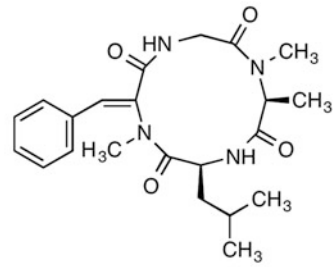
Citreoviridin (**85**)



Asteltoxin (**86**)



Efrapeptin D (**87**)



Tentoxin (**88**)

Fig. 22 Structures of complex V inhibitors produced by microorganisms

However, their uses are limited due to potent cardiotoxicity. The primary mechanism for cardiotoxicity is suggested to be mitochondrial dysfunction. Complex IV inhibition of doxorubicin–iron(III) complex or daunorubicin may be, in part, responsible for the mitochondrial dysfunction (Hasinoff and Davey 1988; Papadopoulou and Tsiftsoglou 1993).

Metarhizin A (Fig. 21, **82**), isolated from the mycelia of the culture broth of an entomopathogenic fungus, *Metarhizium flavoviride* (Kikuchi et al. 2009), showed cytotoxicity and

inhibited complex IV of saponin-permeabilized MCF-7 cells at an IC_{50} ~5 μM (Katou et al. 2014).

C. Inhibitors of Complex V

The electrochemical proton gradients produced by the electron transport chain drive complex V (ATP synthase, F_OF_1-ATPase) to produce ATP in the critical process of oxidative phosphorylation. Mammalian complex V is suggested to be a dimeric protein, with each 600-kDa monomer consisting of 15 different protein subunits (Wittig and Schägger 2008). The monomer can be separated into the F_O domain and F_1 domain. The F_O domain is inserted in the membrane and translocates protons, while the F_1 domain protrudes into the matrix and synthesizes ATP. The F_1 domain is also called F_1-ATPase because it can hydrolyze ATP to ADP as the reverse reaction of ATP synthesis.

The name of F_O is derived from the oligomycin-sensitive factor (Racker 1963). Oligomycin A (Fig. 22, **83**), produced by *Streptomyces diastatochromogenes*, inhibits proton transport through the F_O domain and is suggested to bind subunits *a* and *c* of the F_O domain (Devenish et al. 2000).

The subunit composition of the F_1 domain is $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$, and X-ray analysis of the bovine heart mitochondrial F_1 domain revealed its structure (Abrahams et al. 1994). Three β-subunits are catalytic. An ADP binds to the first subunit (β_{DP} subunit), and an ATP binds to the second subunit (β_{TP} subunit). No nucleotide binds to the third subunit (β_E subunit), and the three catalytic subunits interconvert through the cycle of conformations.

As for F_1-ATPase inhibitors, at least five inhibitory sites have been identified: nonhydrolysable NTP analog-binding site (catalytic site), aurovertin B-binding site, efrapeptin-binding site, natural inhibitor protein IF1-binding site and rhodamine 6G–binding site (Gledhill and Walker 2005). A mycotoxin, aurovertin B (Fig. 22, **84**), has been isolated from culture mycelia of the ascomycete *Calcarisporium arbuscula* (Osselton et al. 1974) and the basidiomycete *Albatrellus confluens* (Wang et al. 2005). The K_D value of **84** against bovine heart-soluble ATPase was 0.10 μM (Linnett and Beechey 1979). The co-crystallization study of F_1-ATPase and **84** revealed that **84** binds to bovine F_1 at two equivalent sites in the β_{TP} and β_E subunits in a cleft between the nucleotide binding and C-terminal domains (van Raaij et al. 1996).

Compound **84** has a 4-methoxy-5-methyl-2-pyrone with a triene side chain at C-6. The same moiety is found in two other mycotoxins: citreoviridin (Fig. 22, **85**), produced by the fungus *Penicillium citreoviride* (Sakabe et al. 1964), and asteltoxin (Fig. 22, **86**), produced by the fungus *Aspergillus stellatus* (Kruger et al. 1979). The K_D value of **85** against bovine heart soluble ATPase was 3.1 μM (Linnett and Beechey 1979). The IC_{50} value of **86** against rat liver F_1-ATPase was about 0.5 μM (Kawai et al. 1985).

Efrapeptin D (Fig. 22, **87**) is produced by the fungus *Tolypocladium inflatum* (Jackson et al. 1979). Efrapeptins are α-aminoisobutyric acid (α-Aib)-rich peptides with acetylated N-terminus (Gupta et al. 1992). They are similar to fungal peptaibols (Whitmore and Wallace 2004), but their C-terminus is different. Instead of the amino alcohol C-terminus of peptaibols, efrapeptins have pyrrolo[1,2-*a*]pyrimidine moiety at the C-terminus. Efrapeptins are mycotoxins and their antifungal, insecticidal, and antimalarial activities have been reported (Krasnoff et al. 1991; Nagaraj et al. 2001). Efrapeptins are potent F_1-ATPase inhibitors. The K_D value of efrapeptins (major components were **87** and efrapeptins E, F, and G) against bovine heart ATPase was 0.014 μM (Cross and Kohlbrenner 1978). The co-crystallization study of F_1-ATPase and efrapeptins revealed that efrapeptins made hydrophobic contact with the α-helical structure in the γ-subunit, which traversed the cavity, and with the β_E subunit and the two adjacent α subunits (Abrahams et al. 1996).

Tentoxin (Fig. 22, **88**) was isolated from a still culture broth of the fungus *Alternaria tenuis* as a chlorosis-inducing toxin (Saad et al. 1970). It is a cyclic tetrapeptide composed of

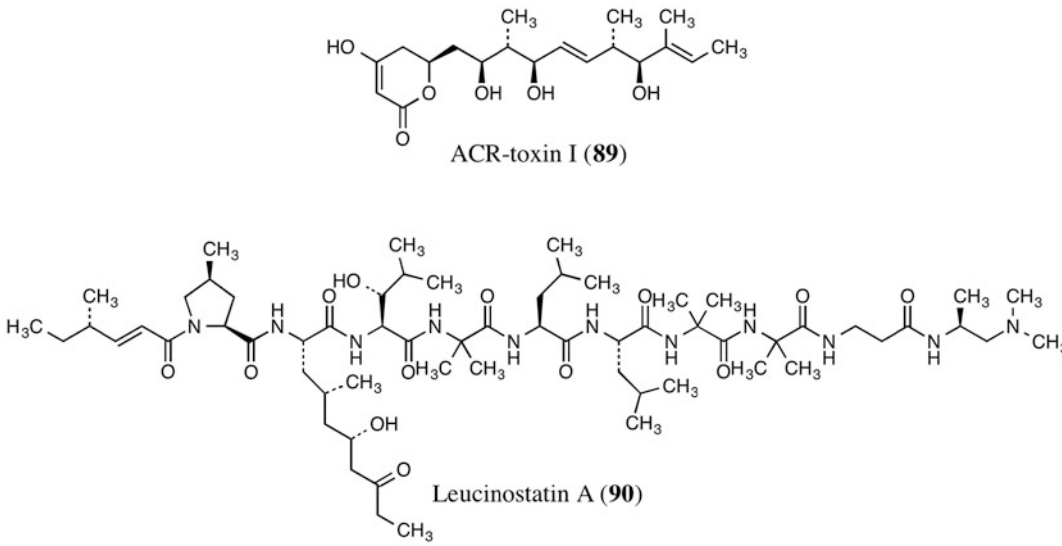


Fig. 23 Structures of uncouplers produced by fungi

N-methyl-L-alanine, L-leucine, (α*Z*)-α,β-didehydro-*N*-methylphenylalanine, and glycine. It inhibits chloroplast F_1-ATPases (CF_1s) in sensitive species (e.g., lettuce) but not in insensitive species, such as radish (Linnett and Beechey 1979). It has no effect on bacterial and mitochondrial F_1-ATPases. The K_D value of **88** against lettuce CF_1 was 3–5 nM. The co-crystallization study of spinach CF_1 and **88** revealed that **88** bound to the αβ interface of the CF_1 in a cleft (Groth 2002). Single-molecule studies of the $\alpha_3\beta_3\gamma$ complex of a cyanobacterium, *Thermosynechococcus elongatus*, with beads attached on the γ subunit suggested that **88** inhibited ATPase reaction after substrate binding to the β_E subunit by keeping the catalytic site in a closed conformation with bound **88** (Meiss et al. 2008).

D. Uncouplers

Respiration-dependent ATP synthesis is abolished by 2,4-dinitrophenol without inhibiting respiration itself. Compounds with this ability are called "uncouplers," and they selectively prevent utilization of electrochemical proton gradients derived from respiratory electron transport for net phosphorylation of ADP to ATP (Heytler 1979).

To date, some fungal uncouplers have been reported. ACR-toxin I (ACRL toxin I, Fig. 23, **89**) is a phytotoxin produced by *Alternaria citri* (Gardner et al. 1985) and *A. alternata* (Kohmoto et al. 1985). It is a δ-lactone, like nafuredin (**22**), and 1 μg/ml (2.8 μM) of **89** causes uncoupling of oxidative phosphorylation and changes in membrane potential in mitochondria from leaves of the susceptible rough lemon (*Citrus jambhiri* Lush., Akimitsu et al. 1989). Ohtani et al. (2002) discovered the ACR-toxin sensitivity gene (ACRS) in rough lemon mitochondrial DNA. Though ACRS was present in the genome of both toxin-sensitive and toxin-insensitive citrus, the ACRS transcripts of insensitive plants were shorter than those of sensitive plants. It is suggested that the gene product of ACRS may be a pore-forming transmembrane receptor of **89** which leads to uncoupling.

Leucinostatin A (Fig. 23, **90**) is a nonapeptide produced by *Penicillium lilacinum* (Arai et al. 1973) and some other fungi. It showed an uncoupling effect against rat liver mitochondria at concentrations above 0.3 μM (Shima

et al. 1990). However, **90** also inhibits ATPase activity at a lower concentration (0.2 μM). It facilitates the transport of monovalent and divalent cations with a half-maximal effect concentration in the range of 0.2–0.8 μM (Csermely et al. 1994). This ionophoric property of **90** may cause its uncoupling effect.

VI. Conclusions

Many inhibitors of electron transport and oxidative phosphorylation enzymes have been isolated from fungal cultures. Some of them, or their analogs, are used as medicines or agrochemicals. The inhibitors are also highly valuable for elucidating the mechanism of electron transport and oxidative phosphorylation systems. Specific inhibitors are used as important tools to study the systems. The X-ray crystallography structures of mitochondrial complexes II, III, and IV and the F_1 domain of complex V have been reported. Co-crystallization studies of the complexes and inhibitors clarified the mechanisms of both inhibition and electron transport. As for mitochondrial complex I, Zickermann et al. (2015) reported the crystal structure of the central subunits from a yeast, *Yarrowia lipolytica*.

Over the past few decades, mitochondria have attracted interest due to their relationship with various diseases: Leigh syndrome, paraganglioma, Parkinson's disease, Huntington's disease, Alzheimer's disease, and so on (Eng et al. 2003; Wallace 2005). Oxidative phosphorylation generates reactive oxygen species as toxic by-products, and they are suggested to cause a wide range of age-related disorders and various forms of cancer. The electron transport and oxidative phosphorylation inhibitors are extremely valuable tools for use in studies of mitochondria-associated diseases.

Recent research on the respiratory chain of the helminth *Ascaris suum* has shown that the mitochondrial NADH-fumarate reductase system has an important role in the anaerobic energy metabolism of adult parasites. Nafuredin (**22**) is a potent and selective inhibitor of complex I in this system, and it showed anthelmintic activity against *Haemonchus contortus* in vivo study. A verticipyrone analog (**42**) was effective against *Echinococcus multilocularis* in vitro. Such helminth-specific complex I inhibitors may be good lead compounds for anthelmintic drugs. A crystal of adult *A. suum* complex II (fumarate reductase), another component of the NADH-fumarate reductase system, was obtained (Shimizu et al. 2007), and analysis of parasite-specific factors in the enzyme is now in progress. This information and the co-crystallization study of the enzyme with atpenin A5 (**61**) may clarify the interaction of **61** and helminth complex II and give a clue to the design of helminth-specific analogs of **61**. The enantioselective total synthesis of atpenin A5 (**61**) has been already achieved (Ohtawa et al. 2009). Thus helminth-specific inhibitors of electron transport enzymes are good candidates for the treatment of helminthic diseases.

References

Abrahams JP, Leslie AG, Lutter R, Walker JE (1994) Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. Nature 370:621–628

Abrahams JP, Buchanan SK, van Raaij MJ, Fearnley IM, Leslie AGW, Walker JE (1996) The structure of bovine F_1-ATPase complexed with the peptide antibiotic efrapeptin. Proc Natl Acad Sci U S A 93:9420–9424

Addor RW, Babcock TJ, Black BC, Brown DG, Diehl RE, Furch JA, Kameswaran V, Kamhi VM, Kremer KA, Kuhn DG, Lovell JB, Lowen GT, Miller TP, Peevey RM, Siddens JK, Treacy MF, Trotto SH, Wright DP (1992) Insecticidal pyrroles. Discovery and overview. ACS Symp Ser 504:283–297

Adebiyi A, McNally EM, Jaggar JH (2008) Sulfonylurea receptor-dependent and -independent pathways mediate vasodilation induced by ATP-sensitive K^+ channel openers. Mol Pharmacol 74:736–743

Akimitsu K, Kohmoto K, Otani H, Nishimura S (1989) Host-specific effects of toxin from the rough lemon pathotype of *Alternaria alternata* on mitochondria. Plant Physiol 89:925–931

Alfatafta AA, Gloer JB, Scott JA, Malloch D (1994) Apiosporamide, a new antifungal agent from the coprophilous fungus *Apiospora montagnei*. J Nat Prod 57:1696–1702

Andersson MA, Mikkola R, Raulio M, Kredics L, Maijala P, Salkinoja-Salonen MS (2009) Acrebol, a novel toxic peptaibol produced by an *Acremonium*

exuviarum indoor isolate. J Appl Microbiol 106: 909–923

Ando K, Suzuki S, Saeki T, Tamura G, Arima K (1969) Funiculosin, a new antibiotic. I. Isolation, biological and chemical properties. J Antibiot 22: 189–194

Anke T, Erkel G (2002) Non-β-lactam antibiotics. In: Osiewacz HD (ed) Industrial applications. The Mycota X. Springer, Heidelberg, pp 93–108

Anke T, Oberwinkler F, Steglich W, Schramm G (1977) The strobilurins—new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (Pers. ex Fr.) Sing. J Antibiot 30:806–810

Anke T, Hecht HJ, Schramm G, Steglich W (1979) Antibiotics from basidiomycetes. IX. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (Schrader ex Fr.) Hoehnel (Agaricales). J Antibiot 32:1112–1117

Arai T, Mikami Y, Fukushima K, Utsumi T, Yazawa K (1973) New antibiotic, leucinostatin, derived from *Penicillium lilacinum*. J Antibiot 26:157–161

Arai M, Tomoda H, Okuda T, Wang H, Tabata N, Masuma R, Yamaguchi Y, Omura S (2002) Funicone-related compounds, potentiators of antifungal miconazole activity, produced by *Talaromyces flavus* FKI-0076. J Antibiot 55:172–180

Berry EA, Huang L, Lee D-W, Daldal F, Nagai K, Minagawa N (2010) Ascochlorin is a novel, specific inhibitor of the mitochondrial cytochrome bc_1 complex. Biochim Biophys Acta 1797:360–370

Brandt U, Schägger H, von Jagow G (1988) Characterization of binding of the methoxyacrylate inhibitors to mitochondrial cytochrome *c* reductase. Eur J Biochem 173:499–506

Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, Walker JE (2006) Bovine complex I is a complex of 45 different subunits. J Biol Chem 281: 32724–32727

Carruthers JR, Cerrini S, Fedeli W, Casinovi CG, Galeffi C, Vaccaro AMT, Scala A (1971) Structures of cochlioquinones A and B, new metabolites of *Cochliobolus miyabeanus*: chemical and X-ray crystallographic determination. J Chem Soc Chem Commun 164–166

Cassinelli G, Grein A, Orezzi P, Pennella P, Sanfilippo A (1966) New antibiotics produced by *Streptoverticillium orinoci*. Arch Mikrobiol 55:358–368

Cross RL, Kohlbrenner WE (1978) The mode of inhibition of oxidative phosphorylation by efrapeptin (A23871). Evidence for an alternating site mechanism for ATP synthesis. J Biol Chem 253: 4865–4873

Csermely P, Radics L, Rossi C, Szamel M, Ricci M, Mihály K, Somogyi J (1994) The nonapeptide leucinostatin A acts as a weak ionophore and as an immunosuppressant on T lymphocytes. Biochim Biophys Acta 1221:125–132

Cutler HG, Jacyno JM (1991) Biological activity of (−)-harzianopyridone isolated from *Trichoderma harzianum*. Agric Biol Chem 55:2629–2631

Degli Esposti M (1998) Inhibitors of NADH-ubiquinone reductase: an overview. Biochim Biophys Acta 1364:222–235

Degli Esposti M, Ghelli A (1999) Ubiquinone and inhibitor sites in complex I: one, two or three? Biochem Soc Trans 27:606–609

Degli Esposti M, Ghelli A, Crimi M, Estornell E, Fato R, Lenaz G (1993) Complex I and complex III of mitochondria have common inhibitors acting as ubiquinone antagonists. Biochem Biophys Res Commun 190:1090–1096

Desai K, Sullards MC, Allegood J, Wang E, Schmelz EM, Hartl M, Humpf H-U, Liotta DC, Peng Q, Merrill AH Jr (2002) Fumonisins and fumonisin analogs as inhibitors of ceramide synthase and inducers of apoptosis. Biochim Biophys Acta 1585:188–192

Devenish RJ, Prescott M, Boyle GM, Nagley P (2000) The oligomycin axis of mitochondrial ATP synthase: OSCP and the proton channel. J Bioenerg Biomembr 32:507–515

Dickinson JM, Hanson JR, Hitchcock PB, Claydon N (1989) Structure and biosynthesis of harzianopyridone, an antifungal metabolite of *Trichoderma harzianum*. J Chem Soc Perkin Trans 1:1885–1887

Dong Y, Lin J, Lu X, Zheng Z, Ren X, Zhang H, He J, Yang J (2009) Cathepsin B inhibitory tetraene lactones from the fungus *Talaromyces wortmannii*. Helv Chim Acta 92:567–574

Eng C, Kiuru M, Fernandez MJ, Aaltonen LA (2003) A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer 3:193–202

Engler M, Anke T, Sterner O, Brandt U (1997) Pterulinic acid and pterulone, two novel inhibitors of NADH:ubiquinone oxidoreductase (complex I) produced by a *Pterula* species. I. Production, isolation and biological activities. J Antibiot 50: 325–329

Findlay JA, Radics L (1972) Flavipucine [3′-isovaleryl-6-methylpyridine-3-spiro-2′-oxirane-2(1*H*),4(3*H*)-dione], an antibiotic from *Aspergillus flavipes*. J Chem Soc Perkin Trans 1:2071–2074

Friedrich T, van Heek P, Leif H, Ohnishi T, Forche E, Kunze B, Jansen R, Trowitzsch-Kienast W, Höfle G, Reichenbach H, Weiss H (1994) Two binding sites of inhibitors in NADH:ubiquinone oxidoreductase (complex I). Relationship of one site with the ubiquinone-binding site of bacterial glucose:ubiquinone oxidoreductase. Eur J Biochem 219:691–698

Frisvad JC (2015) Taxonomy, chemodiversity, and chemoconsistency of *Aspergillus*, *Penicillium*, and *Talaromyces* species. Front Microbiol 5:article 773

Frisvad JC, Filtenborg O, Samson RA, Stolk AC (1990) Chemotaxonomy of the genus *Talaromyces*. Antonie Van Leeuwenhoek 57:179–189

Fujita K, Nagamine Y, Ping X, Taniguchi M (1999) Mode of action of anhydrofulvic acid against *Candida utilis* ATCC 42402 under acidic condition. J Antibiot 52:628–634

Fuska J, Fuskova A, Nemec P (1979) Vermistatin, an antibiotic with cytotoxic effects, produced by *Penicillium vermiculatum*. Biologia 34:735–739

Gardner JM, Kono Y, Tatum JH, Suzuki Y, Takeuchi S (1985) Structure of the major component of ACRL-toxins, host-specific pathotoxic compounds produced by *Alternaria citri*. Agric Biol Chem 49:1235–1238

Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, Kriek NPJ (1988) Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol 54:1806–1811

Gerth K, Irschik H, Reichenbach H, Trowitzsch W (1980) Myxothiazol, an antibiotic from *Myxococcus fulvus* (Myxobacterales). I. Cultivation, isolation, physicochemical and biological properties. J Antibiot 33:1474–1479

Gledhill JR, Walker JE (2005) Inhibition sites in F_1-ATPase from bovine heart mitochondria. Biochem J 386:591–598

Groth G (2002) Structure of spinach chloroplast F_1-ATPase complexed with the phytopathogenic inhibitor tentoxin. Proc Natl Acad Sci U S A 99:3464–3468

Gupta S, Krasnoff SB, Roberts DW, Renwick JAA, Brinen LS, Clardy J (1992) Structure of efrapeptins from the fungus *Tolypocladium niveum*: peptide inhibitors of mitochondrial ATPase. J Org Chem 57:2306–2313

Gutierrez-Cirlos EB, Merbitz-Zahradnik T, Trumpower BL (2004) Inhibition of the yeast cytochrome bc_1 complex by ilicicolin H, a novel inhibitor that acts at the Qn site of the bc_1 complex. J Biol Chem 279:8708–8714

Hamamoto T, Gunji S, Tsuji H, Beppu T (1983) Leptomycins A and B, new antifungal antibiotics. I. Taxonomy of the producing strain and their fermentation, purification and characterization. J Antibiot 36:639–645

Hasinoff BB, Davey JP (1988) The iron(III)-adriamycin complex inhibits cytochrome *c* oxidase before its inactivation. Biochem J 250:827–834

Hayakawa S, Minato H, Katagiri K (1971) Ilicicolins, antibiotics from *Cylindrocladium ilicicola*. J Antibiot 24:653–654

Hays EE, Wells IC, Katzman PA, Cain CK, Jacobs FA, Thayer SA, Doisy EA, Gaby WL, Roberts EC, Muir RD, Carroll CJ, Jones LR, Wade NJ (1945) Antibiotic substances produced by *Pseudomonas aeruginosa*. J Biol Chem 159:725–750

Heytler PG (1979) Uncouplers of oxydative phosphorylation. In: Fleischer S, Packer L (eds) Methods in enzymology. Biomembranes part F: bioenergetics—oxidative phosphorylation, vol 55. Academic, New York, pp 462–472

Horsefield R, Yankovskaya V, Sexton G, Whittingham W, Shiomi K, Ōmura S, Byrne B, Cecchini G, Iwata S (2006) Structural and computational analysis of the quinone-binding site of complex II (succinate-ubiquinone oxidoreductase): a mechanism of electron transfer and proton conduction during ubiquinone reduction. J Biol Chem 281:7309–7316

Houbraken J, Samson RA (2011) Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. Stud Mycol 70:1–51

Ishibashi K (1962) Studies on antibiotics from *Helminthosporium* sp. fungi. VII. Siccanin, a new antifungal antibiotic produced by *Helminthosporium siccans*. J Antibiot Ser A 15:161–167

Ishibashi K, Hirai K, Arai M, Sugawara S, Endo A, Yasumura A, Masuda H, Muramatsu T (1970) Siccanin, a new antifungal antibiotic. Ann Sankyo Res Lab 22:1–33

Izzo G, Guerrieri F, Papa S (1978) On the mechanism of inhibition of the respiratory chain by 2-heptyl-4-hydroxyquinoline-*N*-oxide. FEBS Lett 93:320–322

Jackson CG, Linnett PE, Beechey RB, Henderson PJF (1979) Purification and preliminary structure analysis of the efrapeptins, a group of antibiotics that inhibit the mitochondrial adenosine triphosphatase. Biochem Soc Trans 7:224–226

Jayasuriya H, Guan Z, Dombrowski AW, Bills GF, Polishook JD, Jenkins RG, Koch L, Crumley T, Tamas T, Dubois M, Misura A, Darkin-Rattray SJ, Gregory L, Singh SB (2007) Isolation, structure, and coccidiostat activity of coccidiostatin A. J Nat Prod 70:1364–1367

Kaifuchi S, Mori M, Nonaka K, Masuma R, Ōmura S, Shiomi K (2015) Ukulactone C, a new NADH-fumarate reductase inhibitor produced by *Talaromyces* sp. FKI-6713. J Gen Appl Microbiol 61: 57–62

Katou Y, Yu J, Kikuchi H, Oshima Y, Endo N, Suzuki T, Homma Y (2014) Metarhizin A suppresses cell proliferation by inhibiting cytochrome *c* oxidase activity. Life Sci 103:1–7

Kawai K, Fukushima H, Nozawa Y (1985) Inhibition of mitochondrial respiration by asteltoxin, a respiratory toxin from *Emericella variecolor*. Toxicol Lett 28:73–77

Kawai K, Suzuki T, Kitagawa A, Kim J-C, Lee Y-W (1997) A novel respiratory chain inhibitor, sambutoxin from *Fusarium sambucinum*. Cereal Res Commun 25:325–326

Kepler RM, Humber RA, Bischoff JF, Rehner SA (2014) Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia 106:811–829

Kikuchi H, Hoshi T, Kitayama M, Sekiya M, Katou Y, Ueda K, Kubohara Y, Sato H, Shimazu M, Kurata S, Oshima Y (2009) New diterpene pyrone-type compounds, metarhizins A and B, isolated from entomopathogenic fungus, *Metarhizium flavoviride* and their inhibitory effects on cellular proliferation. Tetrahedron 65:469–477

Kim J-C, Lee Y-W, Tamura H, Yoshizawa T (1995) Sambutoxin: a new mycotoxin isolated from *Fusarium sambucinum*. Tetrahedron Lett 36:1047–1050

Kita K, Miyadera H, Saruta F, Miyoshi H (2001) Parasite mitochondria as a target for chemotherapy. J Health Sci 47:219–239

Kita K, Shiomi K, Ōmura S (2007) Parasitology in Japan. Advances in drug discovery and biochemical studies. Trends Parasitol 23:223–229

Kita K, Inaoka KD, Yamamoto M (2015) Antiparasitic agent. Patent Appl WO2015/056768

Kobayashi S, Tsuchiya K, Harada T, Nishide M, Kurokawa T, Nakagawa T, Shimada N, Kobayashi K (1994) Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK 10958. I. Taxonomy, production, isolation and preliminary characterization. J Antibiot 47:697–702

Kohmoto K, Kohguchi T, Kondoh Y, Otani H, Nishimura S, Nakatsuka S, Goto T (1985) The mitochondrion: the prime site for a host-selective toxin (ACR-toxin I) produced by *Alternaria alternata* pathogenic to rough lemon. Proc Jpn Acad, Ser B 61:269–272

Komuniecki R, Tielens AGM (2003) Carbohydrate and energy metabolism in parasitic helminths. In: Marr JJ, Nielsen TW, Komuniecki RW (eds) Molecular medical parasitology. Academic, London, pp 339–358

Kondoh M, Usui T, Nishikiori T, Mayumi T, Osada H (1999) Apoptosis induction via microtubule disassembly by an antitumor compound, pironetin. Biochem J 340:411–416

Krasnoff SB, Gupta S, St. Leger RJ, Renwick JAA, Roberts DW (1991) Antifungal and insecticidal properties of the efrapeptins: metabolites of the fungus *Tolypocladium niveum*. J Invertebr Pathol 58:180–188

Kruger GJ, Steyn PS, Vleggaar R, Rabie CJ (1979) X-ray crystal structure of asteltoxin, a novel mycotoxin from *Aspergillus stellatus* Curzi. J Chem Soc Chem Commun 1979:441–442

Kruglov AG, Andersson MA, Mikkola R, Roivainen M, Kredics L, Saris N-EL, Salkinoja-Salonen MS (2009) Novel mycotoxin from *Acremonium exuviarum* is a powerful inhibitor of the mitochondrial respiratory chain complex III. Chem Res Toxicol 22:565–573

Kumagai H, Nishida H, Imamura N, Tomoda H, Ōmura S, Bordner J (1990) The structures of atpenins A4, A5 and B, new antifungal antibiotics produced by *Penicillium* sp. J Antibiot 43:1553–1558

Kunze B, Jansen R, Pridzun L, Jurkiewicz E, Hunsmann G, Höfle G, Reichenbach H (1992) Phenoxan, a new oxazole-pyrone from myxobacteria: production, antimicrobial activity and its inhibition of the electron transport in complex I of the respiratory chain. J Antibiot 45:1549–1552

Lang G, Wiese J, Schmaljohann R, Imhoff JF (2007) New pentaenes from the sponge-derived marine fungus *Penicillium rugulosum*: structure determination and biosynthetic studies. Tetrahedron 63:11844–11849

Lardy HA (1980) Antibiotic inhibitors of mitochondrial energy transfer. Pharmacol Ther 11:649–660

Li D-H, Cai S-X, Tian L, Lin Z-J, Zhu T-J, Fang Y-C, Liu P-P, Gu Q-Q, Zhu W-M (2007) Two new metabolites with cytotoxicities from deep-sea fungus, *Aspergillus sydowi* YH11-2. Arch Pharm Res 30: 1051–1054

Lim C-H, Ueno H, Miyoshi H, Miyagawa H, Iwamura H, Ueno T (1996) Phytotoxic compounds cochlioquinones are inhibitors of mitochondrial NADH-ubiquinone reductase. J Pestic Sci 21:213–215

Linnett PE, Beechey RB (1979) Inhibitors of the ATP synthetase system. In: Fleischer S, Packer L (eds) Methods in enzymology. Biomembranes part F: Bioenergetics—oxidative phosphorylation, vol 55. Academic, New York, pp 472–518

Martens GA, Cai Y, Hinke S, Stange G, Van de Casteele M, Pipeleers D (2005) Glucose suppresses superoxide generation in metabolically responsive pancreatic β cells. J Biol Chem 280:20389–20396

Masuma R, Yamaguchi Y, Noumi M, Ōmura S, Namikoshi M (2001) Effect of sea water on hyphal growth and antimicrobial metabolite production in a marine fungi. Mycoscience 42:455–459

Matsumoto J, Sakamoto K, Shinjyo N, Kido Y, Yamamoto N, Yagi K, Miyoshi H, Nonaka N, Katakura K, Kita K, Oku Y (2008) Anaerobic NADH-fumarate reductase system is predominant in the respiratory chain of *Echinococcus multilocularis*, providing a novel target for the chemotherapy of alveolar echinococcosis. Antimicrob Agents Chemother 52:164–170

Meiss E, Konno H, Groth G, Hisabori T (2008) Molecular processes of inhibition and stimulation of ATP synthase caused by the phytotoxin tentoxin. J Biol Chem 283:24594–24599

Merlini L, Nasini G, Selva A (1970) Structure of funicone, a new metabolite from *Penicillium funiculosum*. Tetrahedron 26:2739–2749

Miyadera H, Shiomi K, Ui H, Yamaguchi Y, Masuma R, Tomoda H, Miyoshi H, Osanai A, Kita K, Ōmura S (2003) Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). Proc Natl Acad Sci U S A 100: 473–477

Mogi T, Kawakami T, Arai H, Igarashi Y, Matsushita K, Mori M, Shiomi K, Ōmura S, Harada S, Kita K (2009) Siccanin rediscovered as a species-selective succinate dehydrogenase inhibitor. J Biochem 146: 383–387

Mori M, Morimoto H, Kim YP, Ui H, Nonaka K, Masuma R, Sakamoto K, Kita K, Tomoda H, Shiomi K, Ōmura S (2011) Ukulactones A and B, new NADH-fumarate reductase inhibitors produced by *Penicillium* sp. FKI-3389. Tetrahedron 67:6582–6586

Mowery PC, Steenkamp DJ, Ackrell AC, Singer TP, White GA (1977) Inhibition of mammalian succinate dehydrogenase by carboxins. Arch Biochem Biophys 178:495–506

Musilek V, Cerna J, Sasek V, Semerdzieva M, Vondracek M (1969) Antifungal antibiotic of the basidiomycete, *Oudemansiella mucida*. I. Isolation and cultivation of a producing strain. Folia Microbiol 14:377–387

Nagamitsu T, Takano D, Shiomi K, Ui H, Yamaguchi Y, Masuma R, Harigaya Y, Kuwajima I, Ōmura S (2003) Total synthesis of nafuredin-γ, a γ-lactone related to nafuredin with selective inhibitory activity against NADH-fumarate reductase. Tetrahedron Lett 44:6441–6444

Nagamitsu T, Takano D, Seki M, Arima S, Ohtawa M, Shiomi K, Harigaya Y, Ōmura S (2008) The total synthesis and biological evaluation of nafuredin-γ and its analogues. Tetrahedron 64:8117–8127

Nagaraj G, Uma MV, Shivayogi MS, Balaram H (2001) Antimalarial activities of peptide antibiotics isolated from fungi. Antimicrob Agents Chemother 45:145–149

Nago H, Matsumoto M, Nakai S (1993) Degradation pathway of 2-deceno-δ-lactone by the lactone-producing fungus, *Fusarium solani*. Biosci Biotechnol Biochem 57:2111–2115

Nonaka K, Ōmura S, Masuma R (2013) Three new *Pochonia* taxa (Clavicipitaceae) from soils in Japan. Mycologia 105:1202–1218

Nose K, Endo A (1971) Mode of action of the antibiotic siccanin on intact cells and mitochondria of *Trichophyton mentagrophytes*. J Bacteriol 105: 176–184

Ohsumi K, Masaki T, Takase S, Watanabe M, Fujie A (2014a) AS2077715: a novel antifungal antibiotic produced by *Capnodium* sp. 339855. J Antibiot 67: 707–711

Ohsumi K, Murai H, Nakamura I, Watanabe M, Fujie A (2014b) Therapeutic efficacy of AS2077715 against experimental tinea pedis in guinea pigs in comparison with terbinafine. J Antibiot 67:717–719

Ohsumi K, Watanabe M, Fujie A (2014c) AS2077715 is a selective inhibitor of fungal mitochondrial cytochrome bc_1 complex. J Antibiot 67:713–716

Ohtani K, Yamamoto H, Akimitsu K (2002) Sensitivity to *Alternaria alternata* toxin in citrus because of altered mitochondrial RNA processing. Proc Natl Acad Sci U S A 99:2439–2444

Ohtawa M, Ogihara S, Sugiyama K, Shiomi K, Harigaya Y, Nagamitsu T, Ōmura S (2009) Enantioselective total synthesis of atpenin A5. J Antibiot 62: 289–294

Okun JG, Lummen P, Brandt U (1999) Three classes of inhibitors share a common binding domain in mitochondrial complex I (NADH:ubiquinone oxidoreductase). J Biol Chem 274:2625–2630

Ōmura S, Shiomi K (2007) Discovery, chemistry, and chemical biology of microbial products. Pure Appl Chem 79:581–591

Ōmura S, Tomoda H, Kimura K, Zhen DZ, Kumagai H, Igarashi K, Imamura N, Takahashi Y, Tanaka Y, Iwai Y (1988) Atpenins, new antifungal antibiotics produced by *Penicillium* sp. Production, isolation, physicochemical and biological properties. J Antibiot 41:1769–1773

Ōmura S, Miyadera H, Ui H, Shiomi K, Yamaguchi Y, Masuma R, Nagamitsu T, Takano D, Sunazuka T, Harder A, Kölbl H, Namikoshi M, Miyoshi H, Sakamoto K, Kita K (2001) An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Proc Natl Acad Sci U S A 98:60–62

Ōmura S, Shiomi K, Masuma R (2007) Novel FKI-3389 substance and method for producing the same. Patent Appl WO2007/148412

Oshino K, Kumagai H, Tomoda H, Ōmura S (1990) Mechanism of action of atpenin B on Raji cells. J Antibiot 43:1064–1068

Osselton MD, Baum H, Beechey RB (1974) Isolation, purification, and characterization of aurovertin B. Biochem Soc Trans 2:200–202

Otsuka T, Takase S, Terano H, Okuhara M (1992) New angiogenesis inhibitors, WF-16775 A_1 and A_2. J Antibiot 45:1970–1973

Papadopoulou LC, Tsiftsoglou AS (1993) Mitochondrial cytochrome *c* oxidase as a target site for daunomycin in K-562 cells and heart tissue. Cancer Res 53:1072–1078

Parmeggiani A, Nissen P (2006) Elongation factor Tu-targeted antibiotics: four different structures, two mechanisms of action. FEBS Lett 580:4576–4581

Racker E (1963) A mitochondrial factor conferring oligomycin sensitivity on soluble mitochondrial ATPase. Biochem Biophys Res Commun 10:435–439

Roberge M, Tudan C, Hung SMF, Harder KW, Jirik FR, Anderson H (1994) Antitumor drug fostriecin inhibits the mitotic entry checkpoint and protein phosphatases 1 and 2A. Cancer Res 54:6115–6121

Rotsaert FAJ, Ding MG, Trumpower BL (2008) Differential efficacy of inhibition of mitochondrial and bacterial cytochrome bc_1 complexes by center N inhibitors antimycin, ilicicolin H and funiculosin. Biochim Biophys Acta 1777:211–219

Saad SM, Halloin JM, Hagedorn DJ (1970) Production, purification, and bioassay of tentoxin. Phytopathology 60:415–418

Sakabe N, Goto T, Hirata Y (1964) Structure of citreoviridin, a toxic compound produced by *Penicillium citreoviride* on rice. Tetrahedron Lett:1825–1830

Samson RA, Yilmaz N, Houbraken J, Spierenburg H, Seifert KA, Peterson SW, Varga J, Frisvad JC (2011) Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. Stud Mycol 70: 159–183

Saraste M (1999) Oxidative phosphorylation at the *fin de siècle*. Science 283:1488–1493

Sassa T, Nukina M, Suzuki Y (1991) Deoxyfunicone, a new γ-pyrone metabolite from a resorcylide-producing fungus (*Penicillium* sp.) Agric Biol Chem 55:2415–2416

Sasse F, Leibold T, Kunze B, Höfle G, Reichenbach H (2003) Cyrmenins, new β-methoxyacrylate inhibitors of the electron transport. Production, isolation, physico-chemical and biological properties. J Antibiot 56:827–831

Sauter H, Steglich W, Anke T (1999) Strobilurins: evolution of a new class of active substances. Angew Chem Int Ed 38:1329–1349

Schaeffer JM, Frazier EG, Bergstrom AR, Williamson JM, Liesch JM, Goetz MA (1990) Cochlioquinone A, a nematocidal agent which competes for specific [^{3}H]ivermectin binding sites. J Antibiot 43:1179–1182

Schmidt K, Riese U, Li Z, Hamburger M (2003) Novel tetramic acids and pyridone alkaloids, militarinones B, C, and D, from the insect pathogenic fungus *Paecilomyces militaris*. J Nat Prod 66:378–383

Shima A, Fukushima K, Arai T, Terada H (1990) Dual inhibitory effects of the peptide antibiotics leucinostatins on oxidative phosphorylation in mitochondria. Cell Struct Funct 15:53–58

Shimamura H, Sunazuka T, Izuhara T, Hirose T, Shiomi K, Ōmura S (2007) Total synthesis and biological evaluation of verticipyrone and analogues. Org Lett 9:65–67

Shimizu H, Harada S, Osanai A, Inaoka DK, Otani H, Sakamoto K, Kita K (2007) Crystal structure analysis of *Ascaris suum* adult mitochondrial complex II. In: 2nd International symposium on diffraction structural biology, P015

Shimomura Y, Kawada T, Suzuki M (1989) Capsaicin and its analogs inhibit the activity of NADH-coenzyme Q oxidoreductase of the mitochondrial respiratory chain. Arch Biochem Biophys 270: 573–577

Shiomi K, Ōmura S (2004) Antiparasitic agents produced by microorganisms. Proc Jpn Acad Ser B 80B:245–258

Shiomi K, Ui H, Suzuki H, Hatano H, Nagamitsu T, Takano D, Miyadera H, Yamashita T, Kita K, Miyoshi H, Harder A, Tomoda H, Ōmura S (2005) A γ-lactone form nafuredin, nafuredin-γ, also inhibits helminth complex I. J Antibiot 58:50–55

Singh SB, Liu W, Li X, Chen T, Shafiee A, Card D, Abruzzo G, Flattery A, Gill C, Thompson JR, Rosenbach M, Dreikorn S, Hornak V, Meinz M, Kurtz M, Kelly R, Onishi JC (2012) Antifungal spectrum, in vivo efficacy, and structure-activity relationship of ilicicolin H. ACS Med Chem Lett 3:814–817

Smirnova IA, Hägerhäll C, Konstantinov AA, Hederstedt L (1995) HOQNO interaction with cytochrome *b* in succinate:menaquinone oxidoreductase from *Bacillus subtilis*. FEBS Lett 359:23–26

Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, Rao Z (2005) Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121:1043–1057

Takano D, Nagamitsu T, Ui H, Shiomi K, Yamaguchi Y, Masuma R, Kuwajima I, Ōmura S (2001a) Absolute configuration of nafuredin, a new specific NADH-fumarate reductase inhibitor. Tetrahedron Lett 42:3017–3020

Takano D, Nagamitsu T, Ui H, Shiomi K, Yamaguchi Y, Masuma R, Kuwajima I, Ōmura S (2001b) Total synthesis of nafuredin, a selective NADH-fumarate reductase inhibitor. Org Lett 3:2289–2291

Tamura G, Suzuki S, Takatsuki A, Ando K, Arima K (1968) Ascochlorin, a new antibiotic, found by the paper-disc agar-diffusion method. I. Isolation, biological and chemical properties of ascochlorin (Studies on antiviral and antitumor antibiotics. I). J Antibiot 21:539–544

Tan AK, Ramsay RR, Singer TP, Miyoshi H (1993) Comparison of the structures of the quinone-binding sites in beef heart mitochondria. J Biol Chem 268:19328–19333

Trowitzsch-Kienast W, Forche E, Wray V, Reichenbach H, Jurkiewicz E, Hunsmann G, Höfle G (1992) Antibiotika aus Gleitenden Bakterien, 45. Phenalamide, neue HIV-1-Inhibitoren aus *Myxococcus stipitatus* Mx s40. Liebigs Ann Chem:659–664

Tsukamoto S, Hirota H, Imachi M, Fujimuro M, Onuki H, Ohta T, Yokosawa H (2005) Himeic acid A: a new ubiquitin-activating enzyme inhibitor isolated from a marine-derived fungus, *Aspergillus* sp. Bioorg Med Chem Lett 15:191–194

Tunac JB, Graham BD, Dobson WE (1983) Novel antitumor agents CI-920, PD 113,270 and PD 113,271. I. Taxonomy, fermentation and biological properties. J Antibiot 36:1595–1600

Ueki M, Machida K, Taniguchi M (2000) Antifungal inhibitors of mitochondrial respiration: discovery and prospects for development. Curr Opin Anti-Infect Invest Drugs 2:387–398

Ui H, Shiomi K, Yamaguchi Y, Masuma R, Nagamitsu T, Takano D, Sunazuka T, Namikoshi M, Ōmura S (2001) Nafuredin, a novel inhibitor of NADH-fumarate reductase, produced by *Aspergillus niger* FT-0554. J Antibiot 54:234–238

Ui H, Shiomi K, Suzuki H, Hatano H, Morimoto H, Yamaguchi Y, Masuma R, Sakamoto K, Kita K, Miyoshi H, Tomoda H, Tanaka H, Ōmura S (2006a) Paecilaminol, a new NADH-fumarate reductase inhibitor, produced by *Paecilomyces* sp. FKI-0550. J Antibiot 59:591–596

Ui H, Shiomi K, Suzuki H, Hatano H, Morimoto H, Yamaguchi Y, Masuma R, Sunazuka T, Shimamura H, Sakamoto K, Kita K, Miyoshi H, Tomoda H, Ōmura S (2006b) Verticipyrone, a new NADH-fumarate reductase inhibitor, produced by *Verticillium* sp. FKI-1083. J Antibiot 59:785–790

Umezawa I, Komiyama K, Oka H, Okada K, Tomisaka S, Miyano T, Takano S (1984) A new

antitumor antibiotic, kazusamycin. J Antibiot 37: 706–711

Van Hellemond JJ, Klockiewicz M, Gaasenbeek CPH, Roos MH, Tielens AGM (1995) Rhodoquinone and complex II of the electron transport chain in anaerobically functioning eukaryotes. J Biol Chem 270:31065–31070

van Raaij MJ, Abrahams JP, Leslie AGW, Walker JE (1996) The structure of bovine F_1-ATPase complexed with the antibiotic inhibitor aurovertin B. Proc Natl Acad Sci U S A 93:6913–6917

von Jagow G, Link TA (1986) Use of specific inhibitors on the mitochondrial bc_1 complex. In: Fleischer S, Fleischer B (eds) Methods in enzymology, vol 126. Biomembranes, part N. Transport in bacteria, mitochondria, and chloroplasts: protonmotive force. Academic, London, pp 253–271

Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 39:359–407

Wang F, Luo D-Q, Liu J-K (2005) Aurovertin E, a new polyene pyrone from the basidiomycete *Albatrellus confluens*. J Antibiot 58:412–415

Washizu F, Umezawa H, Sugiyama N (1954) Chemical studies on a toxic product of *Streptomyces thioluteus*, aureothin. J Antibiot Ser A 7:60

Wei H, Itoh T, Kinoshita M, Kotoku N, Aoki S, Kobayashi M (2005) Shimalactone A, a novel polyketide, from marine-derived fungus *Emericella variecolor* GF10. Tetrahedron 61:8054–8058

Wessely F, Schönol K, Isemann W (1936) Zur Kenntnis der Bitterstoffe der Colombowurzel III. Über das Palmarin. Monats Chem 68:21–28

Whitmore L, Wallace BA (2004) The peptaibol database: a database for sequences and structures of naturally occurring peptaibols. Nucleic Acids Res 32:D593–D594

Wittig I, Schägger H (2008) Structural organization of mitochondrial ATP synthase. Biochim Biophys Acta 1777:592–598

Wolf H, Zähner H (1972) Metabolic products of microorganisms. 99. Kirromycin. Arch Mikrobiol 83: 147–154

Wolff B, Sangliere J-J, Wang Y (1997) Leptomycin B is an inhibitor of nuclear export: inhibition of nucleocytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. Chem Biol 4:139–147

Wrigley SK, Latif MA, Gibson TM, Chicarelli-Robinson MI, Williams DH (1994) Structure elucidation of xanthone derivatives with CD4-binding activity from *Penicillium glabrum* (Wehmer) Westling. Pure Appl Chem 66:2383–2386

Yagi T (1990) Inhibition by capsaicin of NADH-quinone oxidoreductases is correlated with the presence of energy-coupling site 1 in various organisms. Arch Biochem Biophys 281: 305–311

Yankovskaya V, Horsefield R, Toernroth S, Luna-Chavez C, Miyoshi H, Leger C, Byrne B, Cecchini G, Iwata S (2003) Architecture of succinate dehydrogenase and reactive oxygen species generation. Science 299:700–704

Yilmaz N, Visagie CM, Houbraken J, Frisvad JC, Samson RA (2014) Polyphasic taxonomy of the genus *Talaromyces*. Stud Mycol 78:175–341

Yilmaz N, Visagie CM, Frisvad JC, Houbraken J, Jakobs K, Samson RA (2016) Taxonomic re-evaluation of species in *Talaromyces* section *Islandici*, using a polyphasic approach. Persoonia 36:37–56

Yonemitsu M, Fukuda N, Kimura T, Komori T, Lindner HJ, Habermehl G (1989) Crystal structure and NMR spectrometric analysis of palmarin. Liebigs Ann Chem:485–487

Zhang Y, Zhu T, Fang Y, Liu H, Gu Q, Zhu W (2007) Carbonarones A and B, new bioactive γ-pyrone and α-pyridone derivatives from the marine-derived fungus *Aspergillus carbonarius*. J Antibiot 60:153–157

Zickermann V, Wirth C, Nasiri H, Siegmund K, Schwalbe H, Hunte C, Brandt U (2015) Mechanistic insight from the crystal structure of mitochondrial complex I. Science 347:44–49

Cyclic Peptides and Depsipeptides from Fungi

Heidrun Anke[1], Hartmut Laatsch[2]

CONTENTS

I. Introduction

Cyclic peptides and depsipeptides are widely distributed in nature. They are found in plants (Gournelis et al. 1998; Tan and Zhou 2006), sponges and other lower sea animals (Bertram and Pattenden 2007), cyanobacteria (Welker and von Döhren 2006), bacteria, and fungi alike, and their bioactivities range from antimicrobial, insecticidal, nematicidal, antiviral, hepatotoxic, cytotoxic/cytostatic to immunosuppressive and other pharmacological properties (Kleinkauf and von Döhren 1997; Pomilio et al. 2006).

Some of the peptides and depsipeptides produced by fungi have gained entrance into the pharmaceutical market like cyclosporins (Kürnsteiner et al. 2002), ergopeptides (Keller and Tudzynski 2002), penicillins (Demain and Elander 1999), and cephalosporins (Schmidt 2002) or are currently undergoing clinical trials like the candines, promising antifungal drugs against aspergillosis and candidiasis (Denning 2002; Johnson and Perfect 2003; Pasqualotto and Denning 2008). Caspofungins derived from pneumocandin and micafungin derived from FR901379 are examples of those novel drugs targeting fungal cell wall synthesis, e.g., biosynthesis of 1,3-β-glucan (Odds et al. 2003; Butler 2004). Emodepsin, a semisynthetic depsipeptide, is used in veterinary medicine against helminths (von Samson-Himmelstjerna et al. 2005). The drug is derived from PF1022A, a metabolite of an endophytic fungus from *Camellia japonica* (Sasaki et al. 1992; Scherkenbeck et al. 2002). As these groups of compounds are well covered in the literature, they will not be addressed here in detail.

The biosynthesis of cyclic peptides and depsipeptides has attracted the interest of biochemists since the mid-1960s (Gevers et al. 1968). Today, the focus has shifted from enzymology to genetics, e.g., the biosynthetic genes and their regulation; see Chap. 12. A condensation-like domain has been found to be responsible for the macrocyclization reaction in fungal non-ribosomal peptide synthetases (Gao et al. 2012). Recently biosynthetic investigations have revealed the ribosomal origin of some peptides including cyclic peptides in fungi, bacteria, and archaea (Arnison et al. 2013). These ribosomally synthesized and posttranslationally modified

[1] Institute for Biotechnology and Drug Research, IBWF gGmbH, Kaiserslautern, Germany; e-mail: anke@rhrk.uni-kl.de
[2] University of Goettingen, Institute of Organic and Biomolecular Chemistry, Goettingen, Germany; e-mail: hlaatsc@gwdg.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

peptides called RiPPs may be found in ascomycetes, for example, *Phomopsis leptostromiformis*, *Ustilaginoidea virens*, or *Aspergillus flavus*, (Ding et al. 2016; Nagano et al. 2016; Tsukui et al. 2015) as well as basidiomycetes like *Amanita* species (Walton et al. 2010, 2012).

A special group of cyclopeptides are the diketopiperazines which consist of two amino acids linked by two peptide bonds. In the related epipolythiodioxopiperazines, the 6-ring is bridged by one to four sulfur atoms. The structural diversity of diketopiperazines, more than 500 different compounds are known from fungi (Buckingham 2008; Laatsch 2017), is matched by their biological activities. Recently published reviews are available (Cole and Schweikert 2003; Gardiner et al. 2005). Interestingly, for some of these compounds functions for the producing organisms have been detected, gliotoxin and related compounds, for example, play a role as virulence factors in invasive aspergillosis (Sugui et al. 2007) and coprogens in host invasion of plant pathogenic fungi (Oide et al. 2006; Hof et al. 2007). The reported biological activities of gliotoxin are very broad and diverse. Antibacterial, antifungal, antiviral, amoebicidal, and immunosuppressive properties have been described (see below). Most of these activities are based on interactions with essential thiol groups in proteins (Waring and Beaver 1996). Iron chelators like dimerumic acid, rhodotorulic acid, and coprogen and its derivatives are involved in iron uptake (Winkelmann and Drechsel 1997; Renshaw et al. 2002; Antelo et al. 2006), while other siderophores, e.g., the hexapeptides ferrichrome or ferricrocin, in addition to iron transport or storage functions, act as virulence factors in some human and plant pathogens similar to coprogens (Howard 1999; Haas et al. 2008). Therefore, iron acquisition is discussed as target for the treatment of fungal infections (Leal et al. 2013; Schrettl and Haas 2011).

The group of peptaibiotics, a constantly growing family of linear α-aminobutyric acid (Aib) containing linear peptides, has been enlarged by a small group of cyclic peptides also containing Aib now called cyclopeptaibiotics. Whereas the linear group comprises more than 800 compounds, only nine cyclic compounds have been reported to date. These are seven tetrapeptides structurally related to chlamydocin (Degenkolb et al. 2008) and the scytalidamides, two heptapeptides containing Aib residues (Tan et al. 2003). Readers interested in these compounds are referred to the peptaibiotics database (Stoppacher et al. 2013).

Whereas the detection of novel secondary metabolites was originally driven by biological activities, in recent years the isolation and identification of metabolites by HPLC-UV or MS and NMR profiles has been successfully employed by many groups (Isaka et al. 2011; Peng et al. 2014). Especially the investigation in fungal strains already known to produce interesting metabolites seems rewarding.

II. Occurrence of Cyclic Peptides and Depsipeptides Within the Kingdom *Eumycota* (True Fungi)

A. Siderophores

The occurrence and distribution of siderophores among the taxonomic groups of fungi are very well covered by the reviews of Renshaw et al. (2002) and Haas et al. (2008). Zygomycetes very rarely produce cyclic peptide or depsipeptide siderophores. Up to now the hexapeptide ferrichrysin seems to be the only example. It is produced by *Cunninghamella blakesleeana* (Patil et al. 1995). The production of diketopiperazine and hexapeptide siderophores is common among asco- and basidiomycetes (Renshaw et al. 2002). The fact that members of some orders have not yet been reported to produce siderophores rather reflects a lack of investigation than of presence. There are a few fungi, however, which do not produce siderophores: the ascomycetous yeasts *Saccharomyces cerevisiae* and *Candida albicans* or *Geotrichum candidum* and the basidiomycete *Cryptococcus neoformans* (teleomorph *Filobasidiella*) (Howard 1999; Haas et al. 2008). The investigation of basidiomycetes is difficult because iron-free media, which upregulate the biosynthesis of siderophores, often hardly support mycelial growth, requiring incubation times of 8 to 10 weeks (Welzel et al. 2005). Nevertheless, the complete ferrichrome A biosynthetic pathway in

Ustilago maydis has been elucidated (Winterberg et al. 2010). On the other hand, modern analytical techniques like HPLC-MS^n are sensitive enough to allow the detection and characterization of very small amounts of μg/litre of culture. In addition, as more fungal genomes and NRPS genes and products become available, it becomes clear that siderophores and iron metabolism are important virulence determinants (Eichhorn et al. 2006; Oide et al. 2006; Haas et al. 2008).

It is remarkable that extracellular and intracellular siderophores are not identical and that the synthesis of intracellular siderophores is often not iron-dependent.

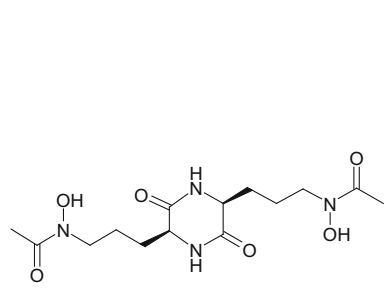
As an example, most *Trichoderma* species excrete coprogen-type siderophores and ferricrocin for capture and transport of iron and use palmitoylcoprogen located within the mycelia as storage compound. In *T. pseudokoningii* and *T. longibrachiatum*, however, palmitoylcoprogen was not detected, but these two species excreted fusigen-type siderophores in addition to coprogen and fericrocin (Anke et al. 1991). *Magnaporthe grisea* uses intracellular ferricrocin for iron storage and under iron deprivation excretes four coprogen derivatives (Hof et al. 2007). In other plant pathogenic fungi like *Fusarium graminearum*, *F. culmorum*, *F. pseudograminearum*, *Cochliobolus heterostrophus*, and *Gibberella zeae*, ferricrocin has also been reported as intracellular siderophore (Oide et al. 2007; Tobiasen et al. 2007). The situation in the human pathogen *A. fumigatus* is similar. Ferricrocin is located in the mycelia, a hydroxylated derivative in the conidia, and triacetylfusigen is excreted (Schrettl et al. 2007).

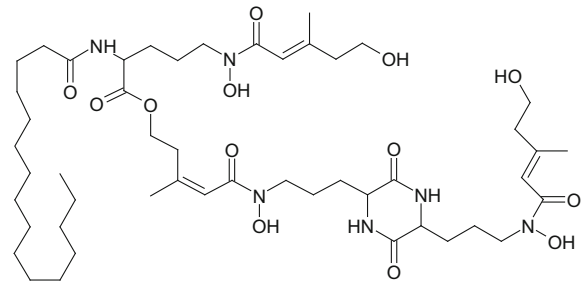
A review on siderophore metabolism, its regulation, and functions has recently appeared (Haas 2014). Interestingly, in *A. fumigatus* and *Neurospora crassa*, several siderophore biosynthetic enzymes are localized in peroxisomes (Gruendlinger et al. 2013), while others are cytosolic (Blatzer et al. 2011). The structures of several iron-free siderophores, e.g., rhodotorulic acid, 2-*N*-methylcoprogen, palmitoylcoprogen, ferricrocin, and ferrichrome, are given in Fig. 1.

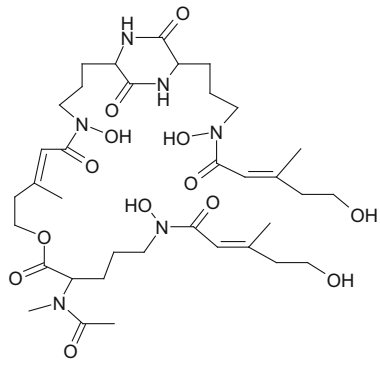
B. Diketopiperazines

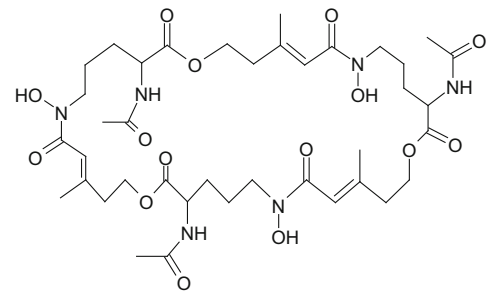
Simple diketopiperazines may be detected in fermentations of many fungi. Sometimes it is difficult to decide whether these are degradation products of proteins and peptides, formed by heating the nutrient broth or synthesized de novo (Prasad 1995). In the future, this problem might be solved by molecular genetics, since the presence of the relevant biosynthetic genes can be proof of de novo synthesis (Eisfeld 2009). The recently demonstrated behavioral effects and occurrence in humans of cyclo (His-Pro) have stimulated the research on such compounds which are easily accessible by chemical synthesis. However, cyclo(His-Pro) has not yet been reported from fungi. This may be due to the fact that its bioactivities, e.g., inhibition of food intake and inhibition of prolactin secretion or modulation of pain perception (Prasad 1995), are not suited for a screening of microbial cultures. Usually these compounds are detected during the isolation of other metabolites and described as sideproducts. A recent example is L-alanyl-L-tryptophan anhydride isolated together with golmaenone, a radical scavenger compound, and neoechinulin from a marine *Aspergillus* species (Li et al. 2004). As in many other cases, the simple diketopiperazine is the biogenetic precursor of the other two compounds. With antimicrobial, cytotoxic, phytotoxic, insecticidal, and other test systems which have been extensively used in screenings for bioactive natural products, simple diketopiperazines are less frequently detected. One example is the fungistatic mactanamide from a marine *Aspergillus* species (Lorenz et al. 1998). Simple diketopiperazines have been described from hetero- and homobasidiomycetes, for example, *Ustilago cynodontis*, *Entoloma haastii*, and *Stereum hirsutum* (Turner and Aldridge 1983) and ascomycetes like *Rosellinia necatrix*; *Claviceps* species, *Eurotium*, *Torrubiella*, and *Emericella* species; *Leptosphaeria* species including their anamorphs; and *Aspergillus*, *Paecilomyces*, *Phoma*, and *Coniothyrium* species (Turner and Aldridge 1983; Cole and Schweikert 2003; Blunt et al. 2006; Isaka et al. 2007a). To the many bioactivities described for diketopiperazines, biofilm-inhibiting properties have been added (de Garvalho and Abraham 2012).

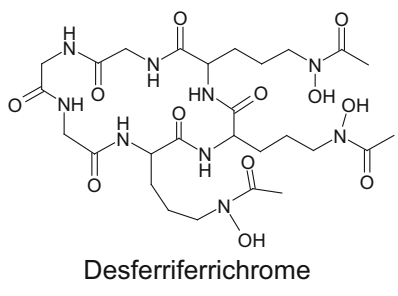
Aspergillus and *Penicillium* species are very prolific producers of cyclic dipeptide-derived mycotoxins like fumitremorgins, verruculogens, or roquefortine C, while sporidesmins, mycotoxins that cause facial











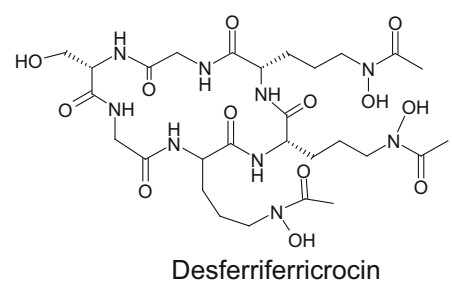


Fig. 1 Structures of some intracellular and extracellular siderophores produced by fungi

eczema in grasing sheep, are produced by *Pithomyces chartarum* (Betina 1989). From several *Penicillium* species, mycelianamide, one of the very "old" diketopiperazines, is known since 1931. This compound has been detected during the early screenings after the discovery of penicillin G. The recently described sulfur-containinggliovictin was obtained from an endophytic *Penicillium janczewskii* (Gunatilaka 2006) and diketopiperazine-derived rostratins from a marine *Exserohilum rostratum* (Tan et al. 2004). To the long list of *Penicillium* species producing diketopiperazines, *P. dipodomyis*, *P. nalgiovense*, *P. fellutanum*, *P. simplicissimum* (Lewis 2002), *P. corylophilum*, and a fungicolous strain of *P. lanosum* were recently added (Hwang et al. 2016). *Perisporiopsis melioloides*, an ascomycete isolated from soil, produced cytotoxic diketopiperazine siderophores identical or similar to fragments known from hydrolytic cleavage of isotriornicin, a siderophore from *Epicoccus purpurascens* (Kawada et al. 2010).

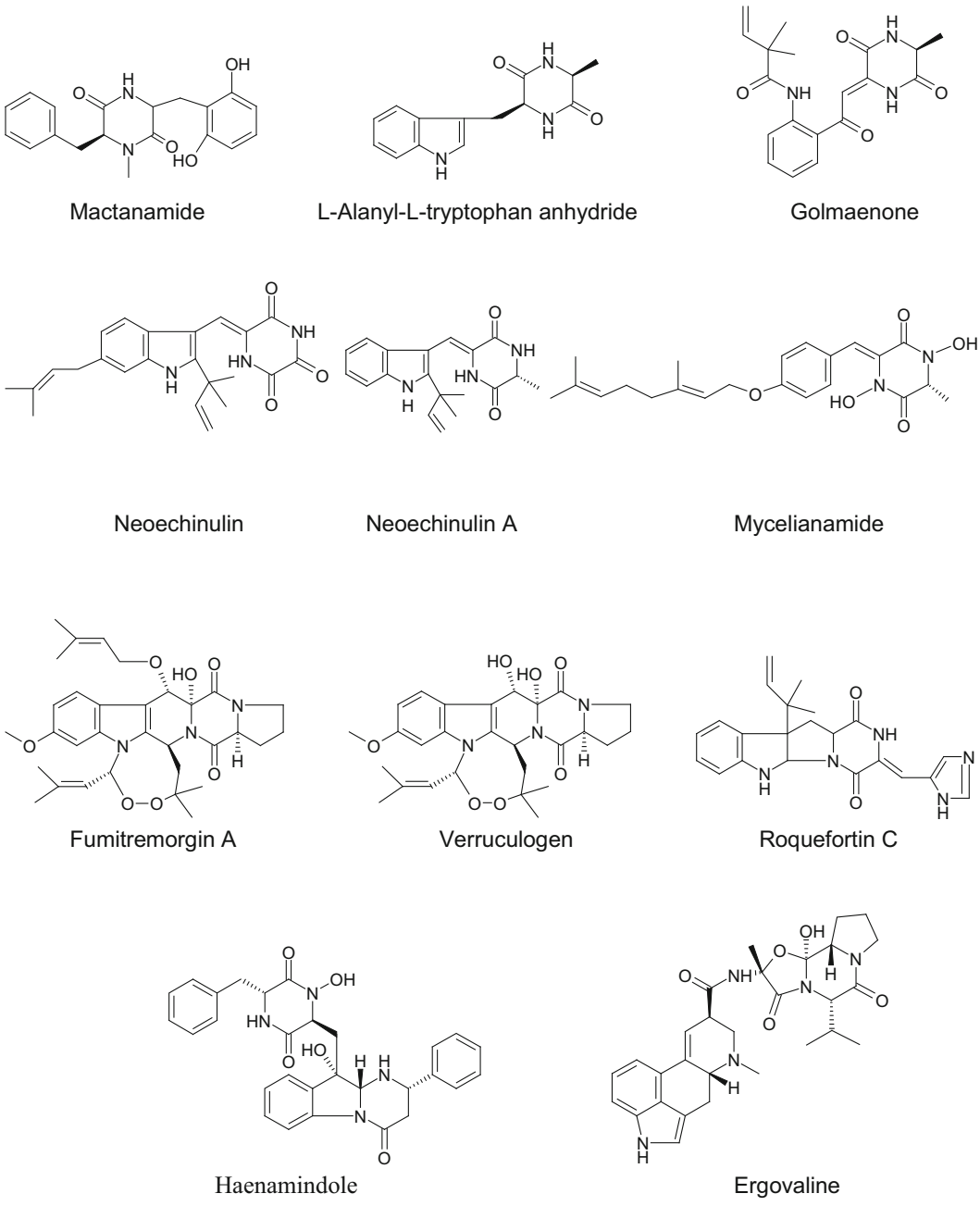


Fig. 2 Structures of some diketopiperazines

Examples for structures of simple and complex diketopiperazines are found in Fig. 2.

From cultures of a number of fungi producing cyclic depsipeptides, e.g., *Beauveria bassiana*, dipeptides composed of the amino acids occurring in the depsipeptides have been isolated. Other insect pathogens like *Verticillium* species and *Metarhizium anisopliae* as well as plant pathogenic fungi, e.g., *Colletotrichum gloeosporioides*, *Exserohilum holmi*, *Gliocladium deliquenscens*, *Alternaria*, and *Trichoderma*, produce dipeptides. An unidentified endophyte

from mangrove leaf produces two cyclic depsipeptides and three diketopiperazines (Huang et al. 2007). The role of the compounds, dipeptides, and depsipeptides, in insect and plant pathogenicity, has not yet been completely elucidated. As molecular tools become more easily available, this question might be addressed, resp., answered in the near future especially since the elucidation of the ecological function of secondary metabolites for the producers becomes more interesting (see below).

Epipolythiopiperazines with more than 80 members, gliotoxin being the most prominent, are widely distributed in nature. Their producers are mainly found among the ascomycete genera *Aspergillus*, *Penicillium*, *Gliocladium*, *Verticillium*, *Chaetomium*, *Emericella*, *Acrostalagmus* (syn. *Verticillium*), *Pithomyces*, *Bionectria*, *Leptosphaeria*, *Hyalodendron*, *Trichoderma*, *Sirodesmium* (syn. *Coniosporium*), *Epicoccum, Arachniotus*, and *Pseudallescheria* (Turner and Aldridge 1983; Takahashi et al. 1994; Betina 1989; Gardiner et al. 2005; Li et al. 2006; Zheng et al. 2007). Recently an endophytic *Phoma* species isolated from the fruit of a mangrove has been identified as a prolific producer of complex gliotoxin derivatives (Kong et al. 2014). There is one report on the occurrence of an epipolythiopiperazine in lichens, e.g., *Xanthoparmelia scabrosa* (Ernst-Russell et al. 1999). As is true for many lichen metabolites, it may be also in this case the ascomycetous fungal partner which is responsible for the production of scabrosin. The production of epicorazine C by *Stereum hirsutum*, a basidiomycete, seems a bit questionable since related epicorazines are produced by *Epicoccum nigrum* and *E. purpurascens* (Kleinwachter et al. 2001). Overlaps between metabolites from basidiomycetes and ascomycetes are fairly rare but do occur occasionally. Other examples may be beauvericin and chlamydocin (see below). The structures of gliotoxin, epicorazines, scabrosin, vertihemiptellide A, and other epipolythiopiperazines are given in Fig. 3, while Fig. 4 shows the diversity of metabolites produced by the endophyte *Lasiodiplodia pseudotheobromae* F2 (Wei et al. 2014).

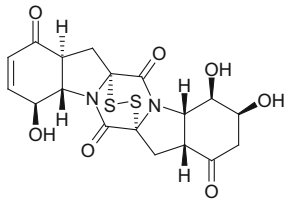
C. Cyclic Peptides

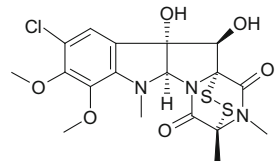
Cyclic peptides are mainly produced by ascomycetes and their anamorphs. Among cyclic peptides, the immunomodulating cyclosporins constitute the largest group with 46 members. The producing organisms are found mainly in the ascomycetous families *Hypocreaceae* and *Clavicipitaceae* and their anamorphs *Tolypocladium inflatum*, *T. tundrense*, and *T. terricola*. In addition, three soilborne insect pathogens, *Neocosmospora vasinfecta*, *Acremonium luzulae*, a *Cyclindrotrichum* species, *Stachybotrys chartarum*, *Trichoderma viride*, a *Leptostroma* anamorph of *Hypoderma eucalyptii*, *Chaunopycnis alba*, and an unidentified mycelium sterilium have been reported to produce cyclosporins (Matha et al. 1992; Traber and Dreyfuss 1996). The structure of cyclosporin A is found in Fig. 5, whereas Fig. 6 shows examples of more simple cyclopeptides.

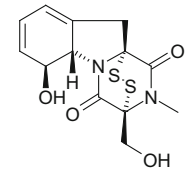
A review on metabolites (among these cyclic peptides and depsipeptides) of entomophatogenic hypocrealan fungi has been published (Molnar et al. 2010). *Xylaria* species are prolific producers of secondary metabolites inclusive of cyclopeptides (Li et al. 2011). From cultures of *Tuber indicum*, a cyclic octapeptide has been isolated (Li et al. 2013).

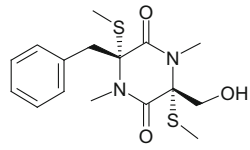
The malformins, a group of ten phytotoxic compounds, are only found within the *Aspergillus niger* group (Kobbe et al. 1977). Some authors classify the compounds as mycotoxins even so they are rarely found in food or feed stuff.

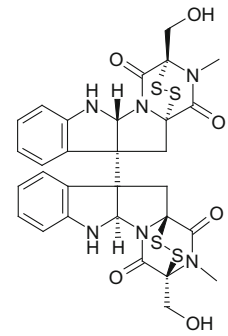
The antifungal echinocandins comprising different compounds (aculeacin A; echinocandin B; pneumocandins; mulundocandins; FR901379; WF11899 A, B, and C; FR227673; FR190293; and others) have been reported from several Aspergilli, *Coleophoma empetri*, *C. crateriformis*, *Chalara* species, *Tolypocladium parasiticum*, and *Zalerion arboricola* (Iwamoto et al. 1994b; Iwamoto et al. 1994a, b; Anke and Erkel 2002; Denning 2002; Kanasaki et al. 2006a, b, c). The *Zalerion* strain producing echinocandin B was later reclassified as *Glarea lozoyensis*, a new anamorph genus and species within the *Leotiales* (Bills et al. 1999). The fungus producing

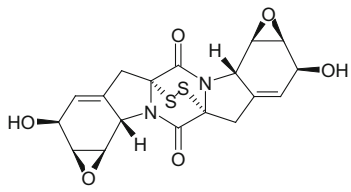


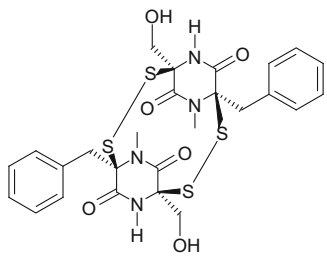












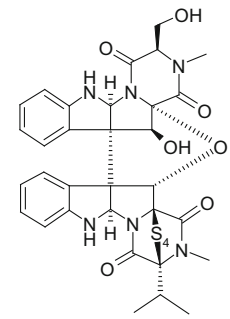


Fig. 3 Structures of some epipolythiopiperazines

arborcandins (Ohyama et al. 2000) has not been identified. The structures of some of these compounds can be found in Fig. 7. A review addressing the different aspects of the biosynthesis, the involved genes, as well as the biological functions of echinocandins and related compounds has appeared (Bills et al. 2014).

Producers of various cyclic peptides are found in many other families and genera, for example, *Diheterospora*, *Gliocladium*, *Cylindrocarpon*, *Clonostachys*, *Cochliobolus*, *Fusarium*, (Lewis 2002; Adachi et al. 2005; Weber et al. 2006; Degenkolb et al. 2008), as well as *Eurotiales* like *Talaromyces* species (Bara et al. 2013) and

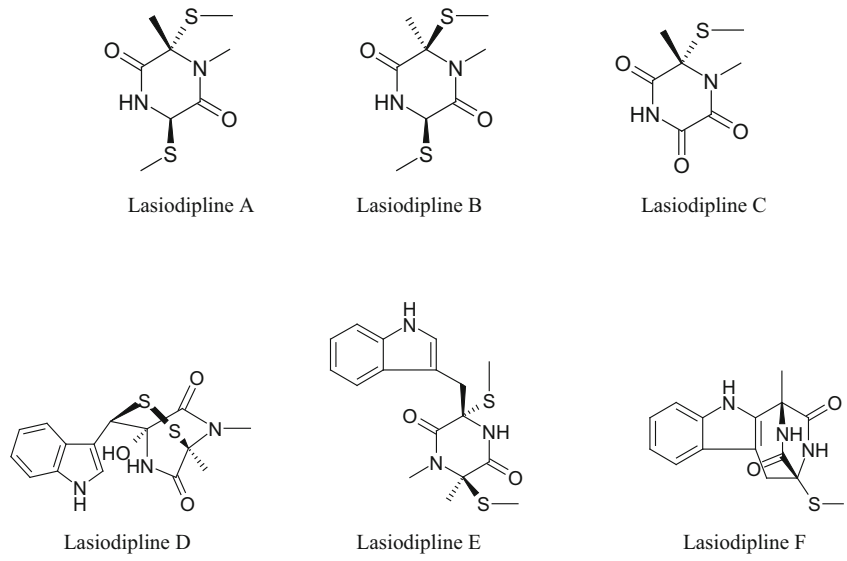


Fig. 4 Structures of metabolites produced by *Lasiodiplodia pseudotheobromae* F2

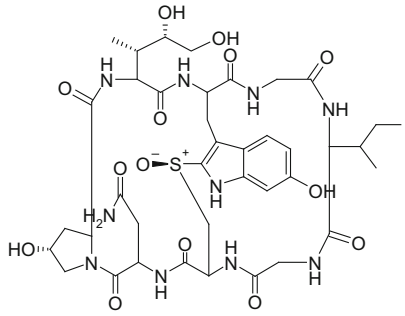
their anamorphs, for example, *Penicillium* species (Kawahara et al. 2016) and *Onychocola sclerotica* belonging to the new order *Arachnomycetales* (Perez-Victoria et al. 2012). Species of the genus *Cordyceps* not only produce cyclodepsipeptides (see below) but also cyclopeptides (Rukachaisirikul et al. 2006; Isaka et al. 2007b).

As endophytic fungi have recently come into focus as producers of bioactive natural compounds, it is not astonishing that also novel cyclic peptides have been reported from these fungi.

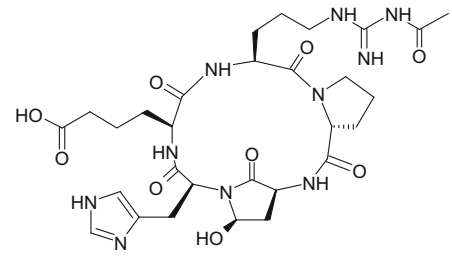
A pentapeptide was isolated from an unidentified endophyte from the seed of *Avicennia marina* (Gunatilaka 2006), other cyclopeptides from endophytic *Fusarium* species (Shiono et al. 2007), *Epichloe typhina* (Seto et al. 2007), or endophyte "2221" from *Castaniopsis fissa* (Yin et al. 2005). More than 450 cyclic peptides are known from plants (Tan and Zhou 2006); some of these actually may be produced by endophytic fungi in plants. The list of endophytic fungi producing cyclopeptides has been enlarged by *Trichoderma asperellum* (Ding et al. 2012), *Bionectria ochroleuca* (Ebrahim et al. 2012), *F. decemcellulare* (Li et al. 2016), *Aspergillus tamarii* (Ma et al. 2016), *A. tubingensis* (Tan et al. 2015), *Myrothecium verrucaria* (Zou et al. 2011) or an endolichenic *Xylaria* species (Wu et al. 2011), and an unguisin F producing *Mucor irregularis* (Akone et al. 2016). Interestingly, hexacyclopeptides from *F. solani* N06 act as cross talk molecules in *Narcissus tazetta* (Wang et al. 2015). In some cases it is not absolutely clear whether the producing fungi are true endophytes or only plant-derived.

In recent years, marine habitats have drawn much attention as ecological niches for producers of novel bioactive metabolites. In the meantime more than thousand metabolites have been described by marine-derived fungi, but only a small fraction of these were produced by true (obligate) marine fungi (Overy et al. 2014). A review on antitumor compounds from marine-derived fungi has appeared (Pejin et al. 2013).

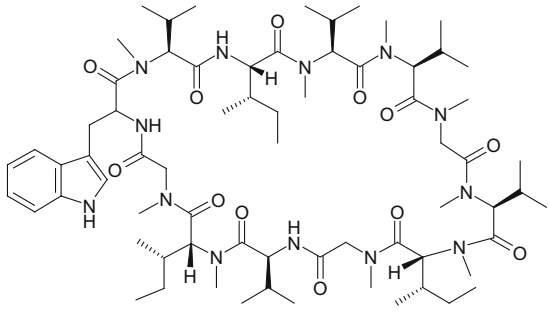
The unguisins were isolated from a marine-derived strain of *Emericella unguis* (Malmstrom et al. 2002); unguisin E (structure Fig. 6) was recently obtained from a marine *Aspergillus* species (Liu and Shen 2011), cordyhepta-peptides from *Acremonium persicinum* (Chen et al. 2012), endolides (for structures see Fig. 6) from sponge-derived*Stachylidium* sp. (Almeida et al. 2016), asperpeptide A from a gorgonian-derived*Aspergillus* species (Chen et al. 2014) and cyclic tetrapeptides from another gorgonian-derived*Aspergillus* species (Bao et al. 2013), psychrophilins E–H from *A. versicolor* (Peng et al. 2014), nine psychrophilins (A–I) from *Penicillium rivulum* (Zhao et al. 2016), cyclic peptides from a coral-associated*A. versicolor* (Zhuang et al. 2011), cyclic tri- and hexapeptides from a marine-derived and halotolerant *A. sclerotiorum* (Zheng et al. 2009,



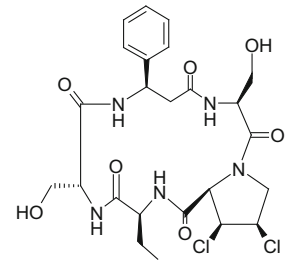
α-Amanitin



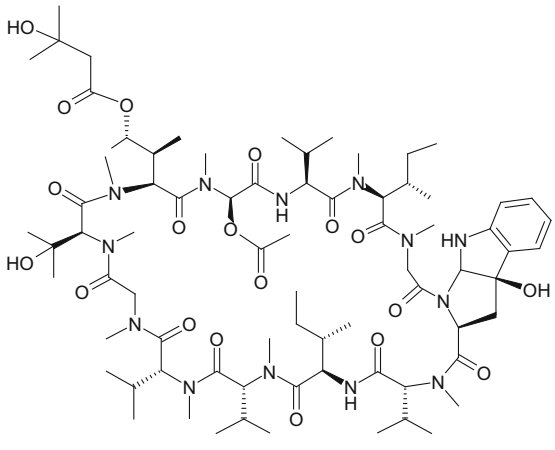
Argadin



Omphalotin A



Cyclochlorotine

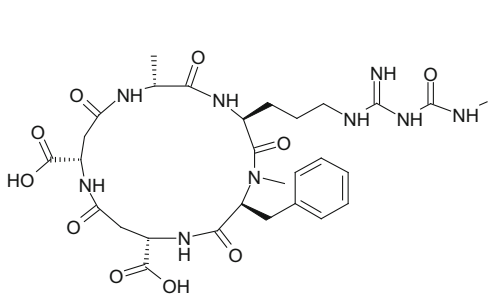


Omphalotin C

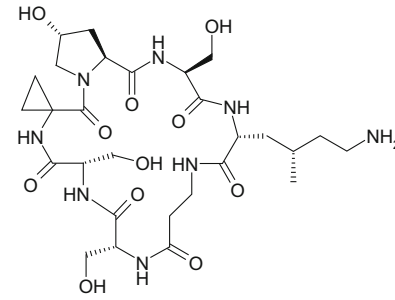
Fig. 5 Structures of some complex cyclopeptides

2010), a tetrapeptide from *A. flavipes* isolated from the gut of *Ligia oceanica* (Xu et al. 2014), novel cyclic peptides from a *Penicillium* species isolated from the mangrove *Bruguiera gymnorrhiza* (Zhou et al. 2014), and cyclic tetrapeptide from a jellyfish-derived *Phoma* species (Kim et al. 2012).

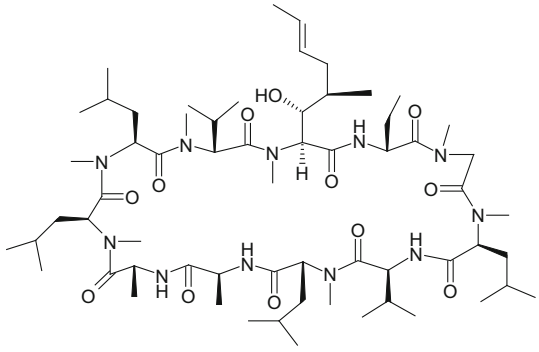
Among cyclic peptides from obligate marine ascomycetes are the highly cytotoxic trapoxin A produced by *Corollospora intermedia* (Daferner 2000) or scytalidamides from a *Scytalidium* species from a marine alga (Tan



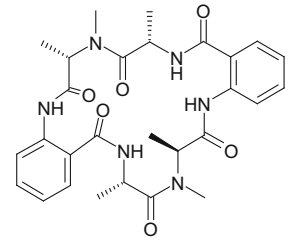
Argifin



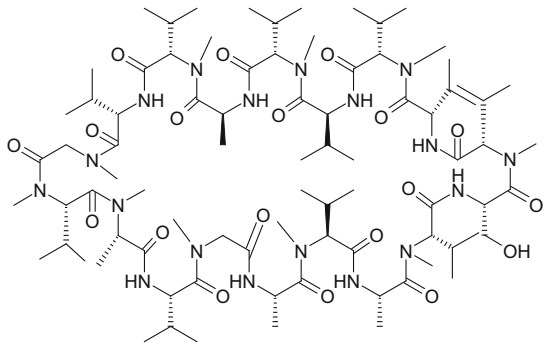
Serinocyclin B



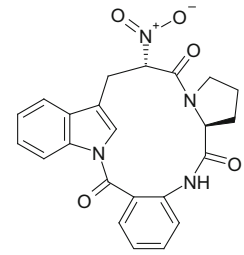
Cyclosporin A



Versicotide C



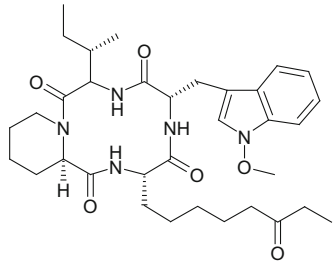
Gymnopeptide A



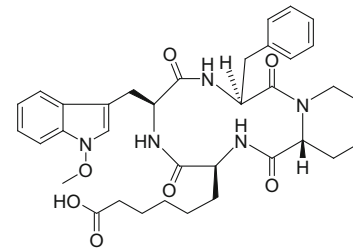
Psychrophilin A

Fig. 5 (continued)

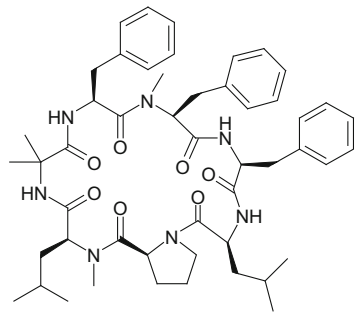
et al. 2003). JM47, structurally related to HC-toxins and trapoxin, was isolated together with enniatin from a marine-derived *Fusarium* species (Jiang et al. 2002). Trapoxins are also known from terrestrial fungi, e.g., *Helicoma ambiens*, the anamorph of *Thaxteriella pezicula* (Itazaki et al. 1990), and structurally related metabolites have been described from the phytopathogenic *Cyclindrocladium scorparium* (teleomorph *Calonectria morganii*), *Cochliobolus carbonum* (Degenkolb et al. 2008), and a sweet water-derived *Tolypocladium* species



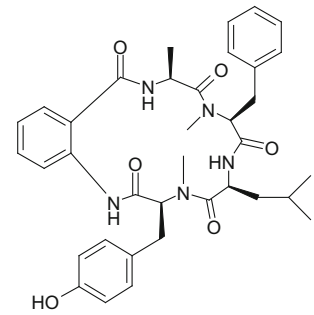
Apicidin



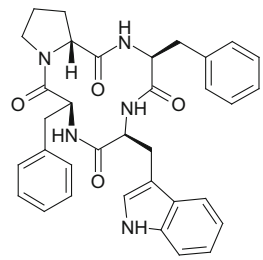
Apicidin F



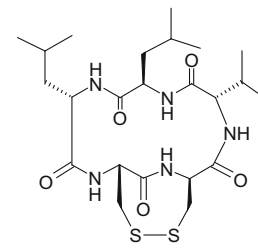
Scytalidamide A



Cycloaspeptide A



CJ 15,208

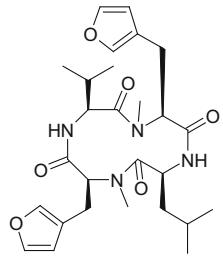


Malformin C

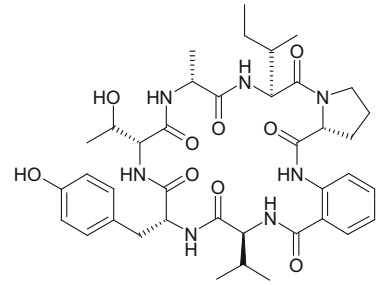
Fig. 6 Structures of some simple cyclopeptides

(Du et al. 2014a). For structures see Fig. 8. *Asteromyces cruciatus*, an anamorphic ascomycete, produced a cyclic pentapeptide (Gulder et al. 2012). In an interesting approach to find novel secondary metabolites, two marine-derived *Aspergillus* species (Ebada et al. 2014) or the mangrove-derived *Phomopsis* sp. K38 and *Alternaria* sp. E33 (Huang et al. 2014; Li et al. 2014) were successfully cocultivated.

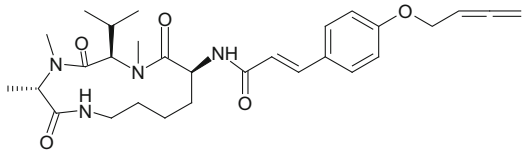
The only cyclopeptides, besides the siderophores, known from submerged cultures of basidiomycetes are the omphalotins from *Omphalotus olearius* (Büchel et al. 1998a, b), amanitins from *Amanita exitialis* (Zhang et al. 2005), and chlamydocins from a *Peniophora* strain isolated from soil (Tani et al. 2001). The chlamydocins are tetrapeptides with Aib and the unusual 2-amino-decanoic acid. Most of



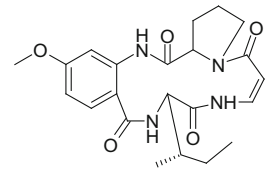
Endolide A



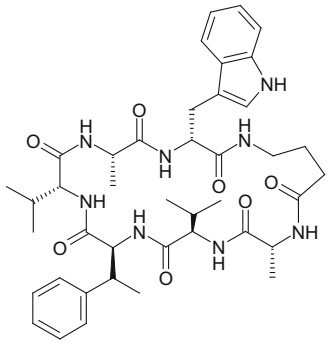
Talaromin A



Xyloallenoide



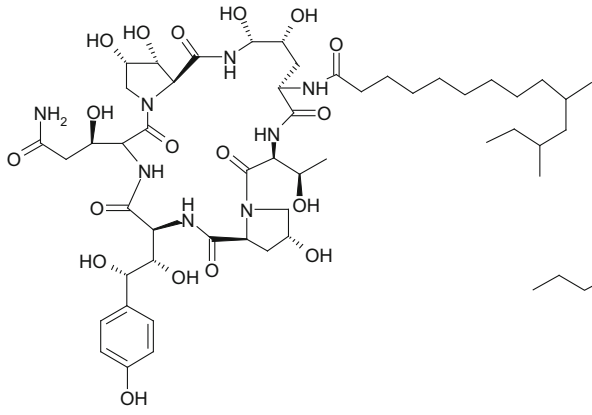
Cyclic peptide [Xu et al. 2014]



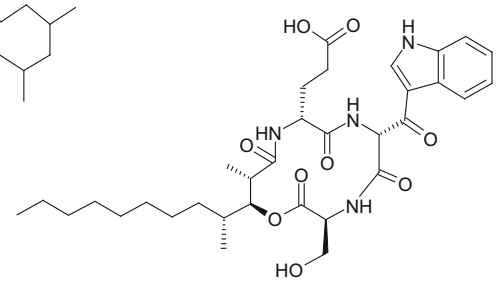
Unguisin E

Fig. 6 (continued)

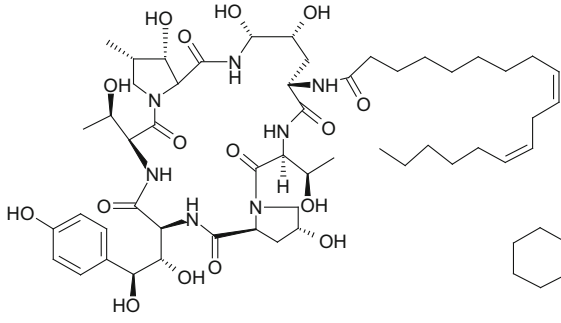
these are produced by ascomycetes, e.g., *Diheterospora chlamyydosporia* (Closse and Huguenin 1974) and *V. coccosporum* (Gupta et al. 1994). Interestingly, the omphalotins produced by a monokaryotic strain differ from those found in the dikaryotic parental strain (Liermann et al. 2009). However, all *O. olearius* strains irrespective of their geographical origin produce omphalotin derivatives (Anke et al. unpublished data). In fruiting bodies omphalotins could not be detected, contrary to *Amanita exitialis* carpophores which contained tenfold more α- and β-amanitin as compared to the slow-growing mycelial cultures (Zhang et al. 2005). For recent surveys of *Amanita* toxins from fruiting bodies, see Li and Oberlies (2005), Liu (2005), and Pomilio et al. (2006). Structures of omphalotins and α-amanitin are found in Fig. 5. Ternatin was isolated from fruiting bodies of *Coriolus versicolor* (Shimokawa et al. 2006). From *Gymnopus fusipes* basidiocarps, two cyclic octadecapeptides have



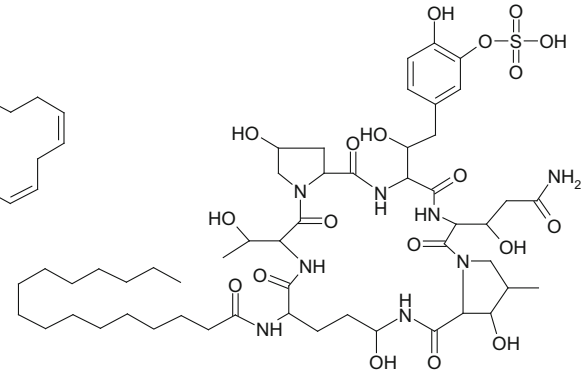
Pneumocandin D_0



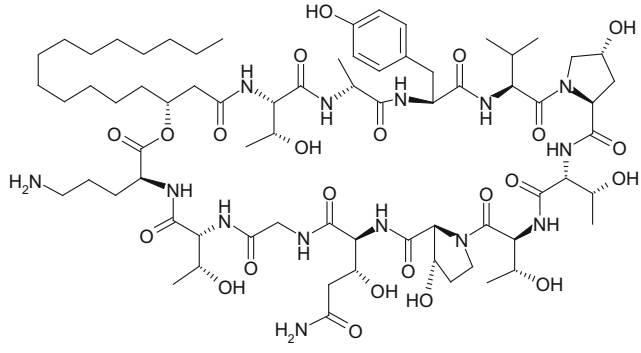
Arthrichitin, LL15G256γ



Echinocandin B

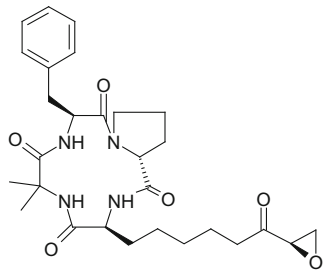


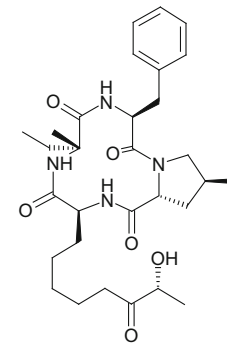
WF11899C

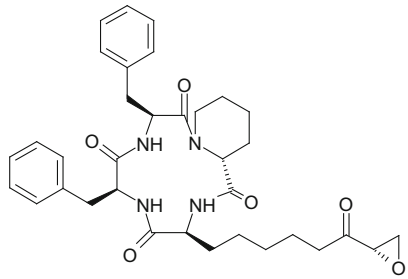


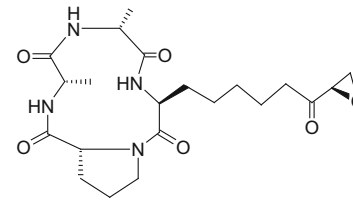
FR 901469

Fig. 7 Structures of some 1,3-β-glucan synthase inhibitors









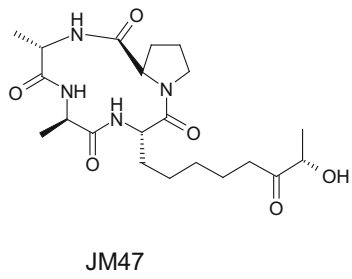


Fig. 8 Structures of some histone deacetylase inhibitors

been described. The compounds are highly cytotoxic (Ványolós et al. 2016).

D. Cyclic Depsipeptides

Most depsipeptides are metabolites from ascomycetes and their anamorphs. They are widespread in phytopathogens (e.g., *Cochliobolus* with anamorphs *Helminthosporium* and *Bipolaris, Calonectria* and its anamorph *Cyclindrocladium*, as well as *Fusarium* and *Alternaria*), endophytic fungi like *Phomopsis glabrae* (Verekar et al. 2014), *Phaeosphaeria* sp. (Singh et al. 2013), insect pathogens (*Aschersonia*, *Beauveria*, *Cordyceps*, *Diheterospora*, *Fusarium*, *Hirsutella*, *Isaria*, *Metarhizium*, *Paecilomyces*, *Verticillium*), and others (Zimmermann 2007a, b; Buckingham 2008). For a compilation of beauvericins and enniatins produced by *Cordyceps* species and their anamorphs as well as other insect pathogens, see Isaka et al. (2005a, b). Recently *Cordyceps cardinalis* (Umeyama et al. 2014) and *Ophiocordyceps communis*

(Haritakun et al. 2010) were found to produce cyclodepsipeptides. Verlamelins A and B from *Lecanicillium* sp. have been reported, and their biosynthetic genes have been identified (Ishidoh et al. 2014a, b). A review on non-ribosomal peptides from entomogenous fungi has appeared (Hu and Dong 2015). *Conoideocrella tenuis*, a pathogen of scale insects, produced among many compounds the cyclic conoideocrellide A and its linear derivatives (Isaka et al. 2011). Conidia of *Metarhizium acridum*, a commercial product against grasshoppers, contain metacridamides A and B (Krasnoff et al. 2012). The structures of some cyclodepsipeptides are given in Figs. 9 and 10. Among fungi isolated from marine habitats, several were found to produce cyclodepsipeptides, for example, *Alternaria* sp. SF-5016 (Kim et al. 2009), *Aspergillus clavatus* (Jiang et al. 2013), *A. clavatus* from a crab in a metal-rich hydrothermal vent (Ye et al. 2014), and *Beauveria felina* (Du et al. 2014b, c). Besides the production of cyclic peptides that could be induced by co-culturing fungal strains (see above), depsipeptide synthesis was also induced by the co-culturing of *Fusarium* species (Wang et al. 2013). The co-culturing of an *Emericella* species with a marine actinomycete allowed the isolation of emericellamides A and B (see Fig. 10), since the production was increased a 100-fold by the presence of the bacterium (Oh et al. 2007).

Up to now the pteratides (Fig. 10) are the only depsipeptides reported from basidiomycetes, namely, from the fruiting bodies of a *Pterula* species (Chen et al. 2006). From zygomycetes none have been described. One report on the production of beauvericin by *Laetiporus sulphureus* (Badan et al. 1978) could not be confirmed by other groups. In our cultures from *L. sulphureus* from different locations, we could only detect laetiporic acid and its derivatives (Davoli et al. 2005).

Since the review of Anke and Sterner (2002), additional producers of bioactive depsipeptides have been reported such as marine-derived strains of *Beauveria fellina* (Lira et al. 2006; Du et al. 2014c, b), *Acremonium* sp. BCC 2629 (Bunyapaiboonsri et al. 2012), *Verticillium* sp. FKI-1033 (Monma et al. 2006), *Aspergillus carneus* (Capon et al. 2003), *Torrubiella luteorostrata* and its anamorph *Paecilomyces cinnamomeus* both isolated from a scale insect (Isaka et al. 2007a, b), *Verticillium hemipterigenum* (Supothina et al. 2004), an *Aureobasidium* species from the tropical rain forest (Boros et al. 2006), an unidentified endophytic fungus (Huang et al. 2007), a soilborne *Phoma* species (Aoyagi et al. 2007), and an endophytic *Phomopsis glabrae* (Verekar et al. 2014). Pseudodestruxins have been reported from *Nigrosabulum globosum* (Che et al. 2001), and reviews on destruxins and the producing organisms have been published by Pedras et al. (2002) and Zimmermann (2007b).

The endophyte-producing PF1022A and related anthelmintic cyclooctadepsipeptides which has been isolated from leaves of a camelia have been identified based on its 18S rRNA gene sequence as a member of the *Xylariaceae* close to *Xylaria polymorpha* and *Rosellinia necatrix* (Miyado et al. 2000).

One of the few fungi from sweet water investigated for secondary metabolite production is *Clavariopsis aquatica* from which the antifungal clavariopsins A and B were isolated (Kaida et al. 2001).

Among the coprophilous fungi isolated from elephant dung, an unidentified fungus belonging to the family *Pleosporaceae* was found to produce several cyclodepsipeptides (Isaka et al. 2014). Coprophilous fungi still seem to be an underexplored group with respect to secondary metabolism and its function (Bills et al. 2013).

Analogues of the lipopeptides with 1,3-β-glucan synthase inhibitory activity are the lipodepsipeptides FR901469 or LL15G256γ (see Fig. 7). The former is produced by an unidentified fungus and the latter (identical to arthrichitin from *Arthrinium phaeospermum;* Vijayakumar et al. 1996) by *Hypoxylon oceanicum* (Abbanat et al. 1998; Fujie et al. 2000). Recently, *Colispora cavincola* was found to produce antifungal lipopeptides (Ortíz-López et al. 2015).

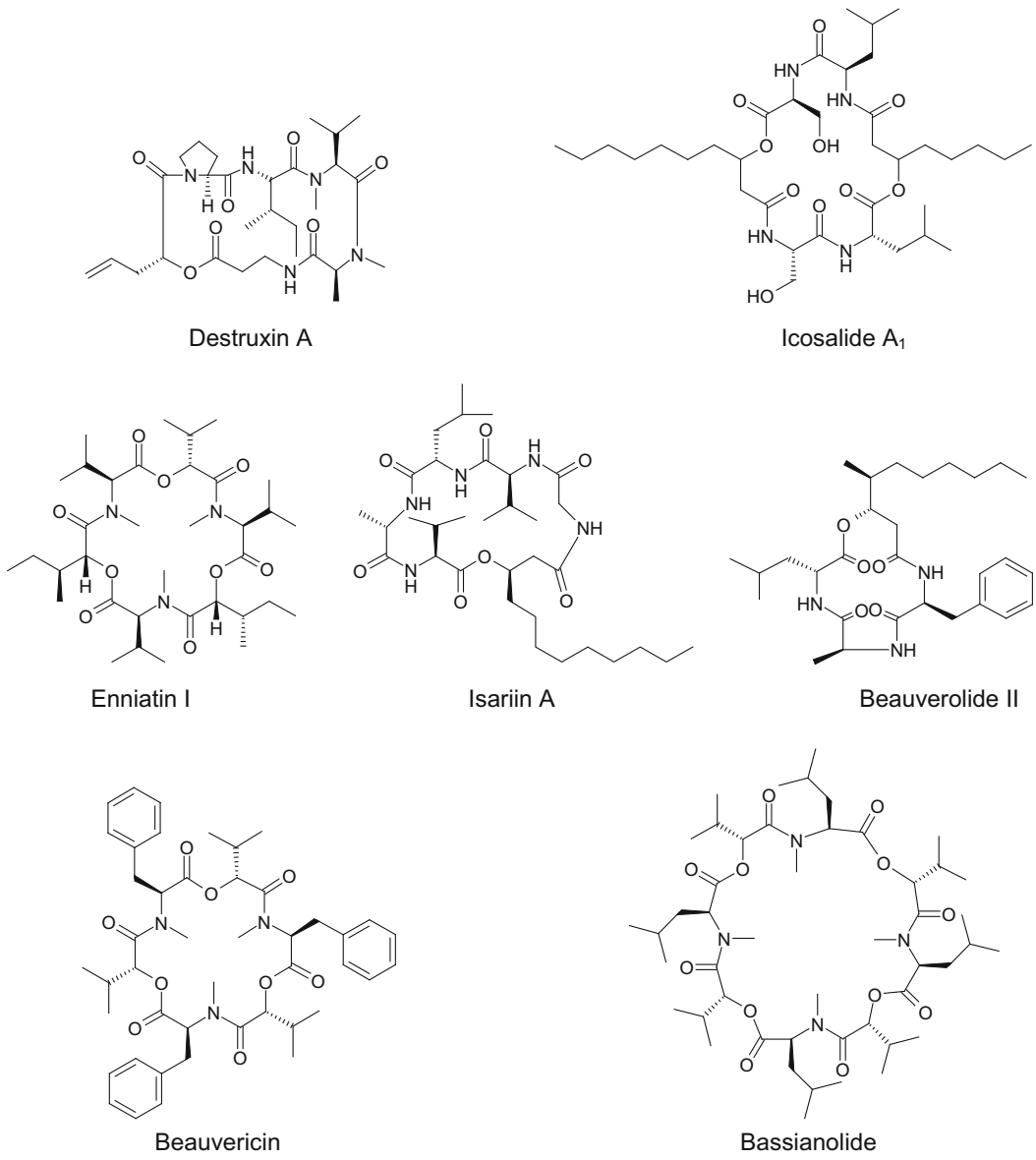
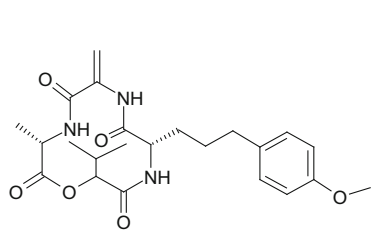


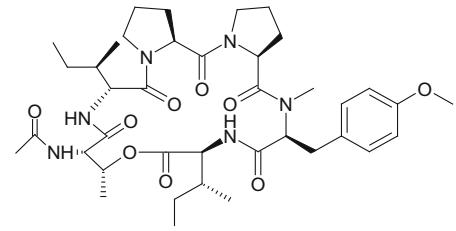
Fig. 9 Structures of some simple cyclodepsipeptide

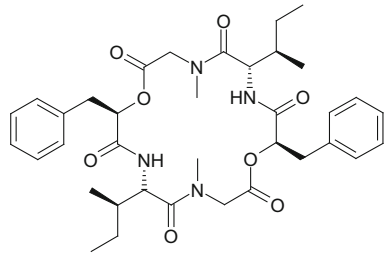
III. Chemical and Biological Diversity of Cyclic Peptides and Depsipeptides

A. Diversity of the Building Blocks

Cyclic peptides and depsipeptides constitute a class of natural compounds with an enormous structural diversity. This diversity is brought upon by the different building blocks in the ring: proteinogenic amino acids including their D-isomers, nonproteinogenic AAs, branched or unbranched lipoamino acids, and hydroxylated short-, medium-, and long-chain fatty acids. The diversity of the building blocks can be deduced from Tables 1 and 2, and 3 in which a compilation of unusual building blocks (Table 1 unusual amino acids, Table 2 unusual fatty acids) and various modifications (Table 3) is found.







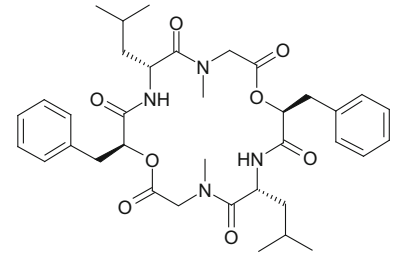
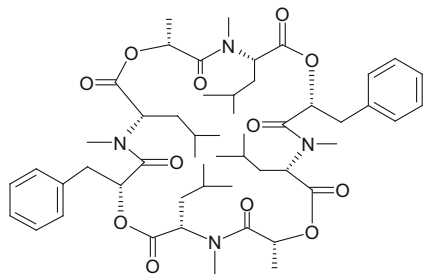


Fig. 9 (continued)

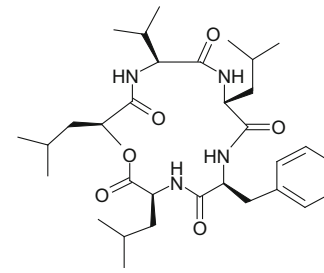
B. Diversity of the Structures

Additional variations are due to the different numbers of building blocks; their arrangement, e.g., sequence in the ring; and their linkage, e.g., amide and ester bonds. Some depsipeptides like the enniatins, beauvericins, bassianolide, or verticilide show a symmetric arrangement in the ring. The majority, however, are asymmetric like the destruxins, beauverolides, isariins, or *Alternaria* toxins (Figs. 9 and 10).

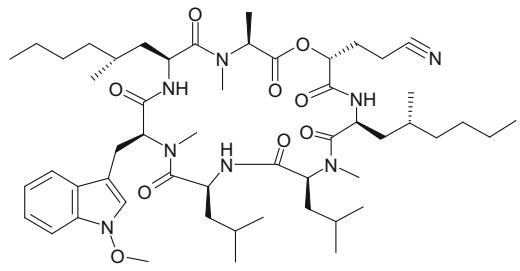
Cyclic peptides including the cylosporins are asymmetric as are the echinocandins. The number of building blocks in cyclic peptides varies from 2 in the diketopiperazines, some of which are symmetric if composed of 2 residues of the same AA, to 12 in the omphalotins, which are at present the largest cyclopeptides known from submerged cultures of fungi and to 18 in the gymnopeptides (for structures see Fig. 5) from basidiocarps of *Gymnopus fusipes*, which are the largest cyclic peptides known from basidiomycetes. Ten out of 18 amino acids are methylated (see Fig. 5) (Ványolós et al. 2016). In addition, the omphalotins are an example of modifications after ring closure. Omphalotins B, C, and D are derived from omphalotin A by hydroxylation followed by acylation to the corresponding esters and formation of additional ring structures (Büchel et al. 1998a, b). Recently from a monokaryotic strain, novel omphalotins have been isolated. The elucidation of their structures was greatly hampered by their instability (Liermann et al. 2009). These omphalotins bear additional hydroxyl groups, thus bringing the number of known cyclic peptides from *O. olearius* to 11. A second hydroxylation at the tryptophan leads to a novel ring system (Fig. 5). HPLC-MS spectra of enriched extracts indicate the presence of additional members of the group. Psychrophilins A–E are nitropeptides with unusual structures (Fig. 6). The compounds are produced by several psychrotolerant *Penicillium* species (Dalsgaard et al. 2004a, b, 2005). The oxidation of an amino group by a P450 cytochrome monooxygenase to form the nitro group has recently been elucidated by Zhao et al. (2016). During these biosynthetic investigations, the substrate of the P450 enzyme and direct precursor of psychrophilin B, psychrophilin I, was identified. Cyclochlorotine, a mycotoxin from *P. islandicum* contains a dichloroprolyl residue (Betina 1989).



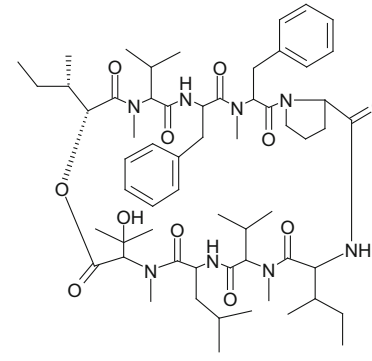
PF1022A



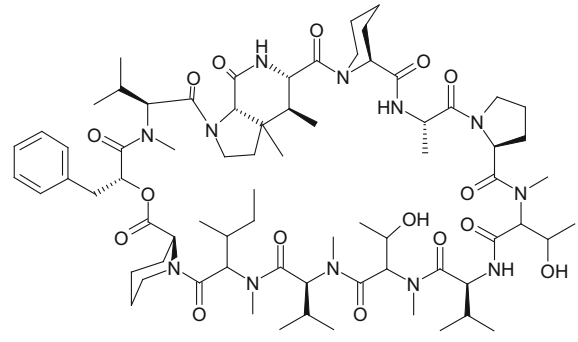
Sansalvamide A



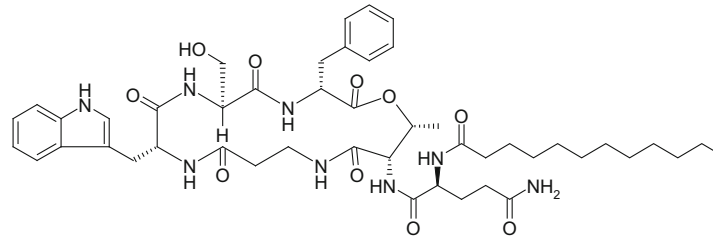
Pestahivin



Aureobasidin A

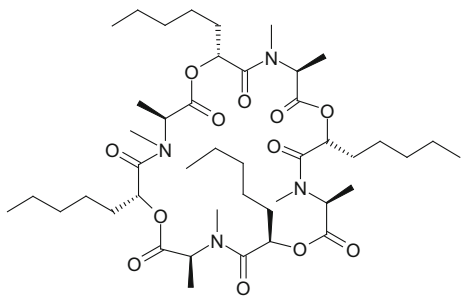


Petriellin A

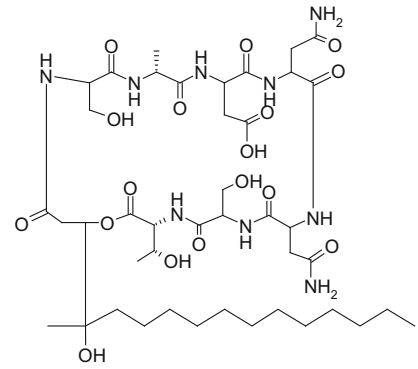


Colisporifungin

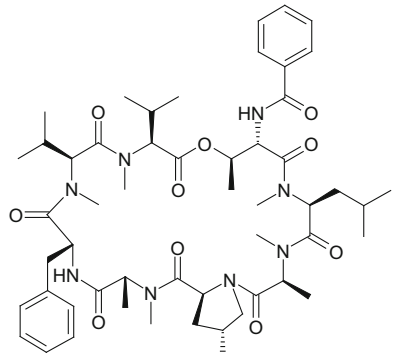
Fig. 10 Structures of some complex cyclodepsipeptides



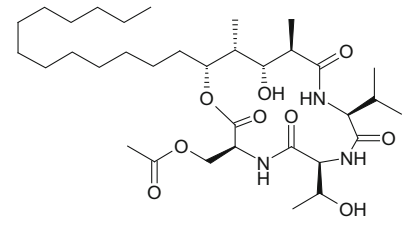
Verticilide



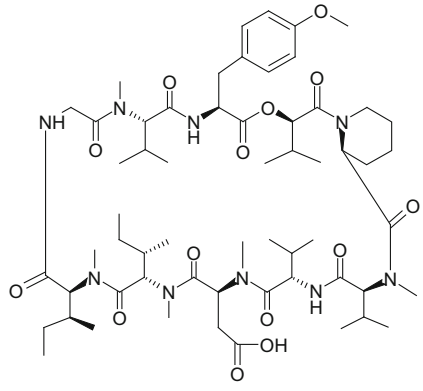
Glomosporin



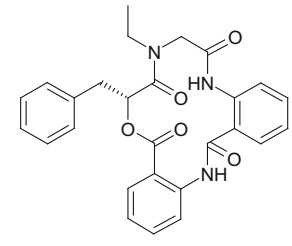
Pteratide I



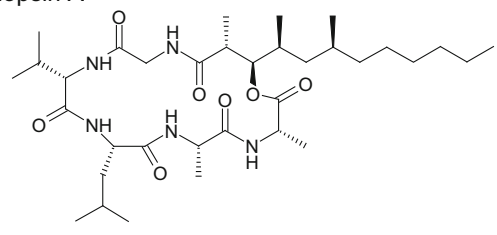
Stevastelin B



Clavariopsin A



Clavatustide B



Emericellamide B

Fig. 10 (continued)

Table 1 Diversity of amino acid building blocks in cyclic peptides and depsipeptides

Amino acid	Example	Fig.	Reference
α-Aminoadipic acid, Prolyl-homoserine	Argadin	5	Arai et al. (2000)
D-Allo-isoleucine	Aspergillicin F	9	Kikuchi et al. (2015)
β-Keto tryptophan	LL15G256γ	7	Abbanat et al. (1998)
Propylleucine	Pestahivin	10	Hommel et al. (1996)
Dehydroalanine, α-Amino-*p*-methoxyphenylvaleric acid	AM-toxin I	9	Ueno et al. (1975)
N-Methoxytryptophan, 2-Aminooctanedioic acid	Apicidin F	6	Von Bargen et al. (2013)
N^5-Hydroxyornithine	Siderophores	1	Renshaw et al. (2002)
4-Methylproline	FR-235222	8	Mori et al. (2003)
2-Butenyl-4-methylthreonine, α-Aminobutyric acid	Cyclosporin A	5	Rüegger et al. (1975)
3-Aminoacrylic acid, 5-Methoxyanthranilic acid	Cyclic peptide	6	Xu et al. (2014)
3-Hydroxyhomotyrosine, 5-Hydroxyornithine	WF-11899C	7	Iwamoto et al. (1994a)
2-Amino-8-oxo-9-hydroxydecanoic acid	JM47	8	Jiang et al. (2002)
Dichloro-proline, β-Phenyl-β-aminopropionic acid	Cyclochlorotine	5	Yoshioka et al. (1973)
3-(3-Furyl)-alanine	Enolides A and B	6	Almeida et al. (2016)
β-Alanine	Destruxin A	9	Rees et al. (1996)
β-Aspartic acid	Argifin	5	Arai et al. 2000
α-Aminoisobutyric acid	Chlamydocin	8	Closse and Huguenin (1974)
Isovaline	FR-235222	8	Mori et al. (2003)
1-Aminocyclopropane-1-carboxylic acid	Serinocyclin A	5	Krasnoff et al. (2007)
Pipecolinic acid	Trapoxin A	8	Itazaki et al. (1990)
Anthranilic acid	Psychrophilin D	5	Dalsgaard et al. (2005)
	Versicotide C	5	Peng et al. (2014)
	Talaromin B	6	Bara et al. (2013)
	Clavatustide B	10	Ye et al. (2014)
γ-Aminobutyric acid	Unguisin E	6	Liu and Shen (2011)
2-Amino-9,10-epoxy-8-oxodecanoic acid	HC-toxin I	8	Gross et al. (1982)

Table 2 Diversity of hydroxy acids building blocks in cyclic depsipeptides

Acid	Example	Fig.	Reference
2-Hydroxyisovaleric acid	Clavariopsin A	10	Kaida et al. (2001)
3,4-Dihydroxy-4-ethylhexadecanoic acid	Glomosporin	10	Ishiyama et al. (2000)
2-Hydroxy-3-methylpentanoic acid	Enniatin I	9	Nilanonta et al. (2003)
	Pleosporin A		Isaka et al. (2014)
2-Hydroxyheptanoic acid	Verticilide	10	Monma et al. (2006)
2-Hydroxy-4-metylpentanoic acid	Sansalvamide A	10	Belofsky et al. (1999)
Phenyllactic acid (2-Hydroxy-3 phenylpropanoic acid)	PF1022A	10	Sasaki et al. (1992)
	Cardinalisamide A	9	Umeyama et al. (2014)
	Hirsutellide A	9	Vongvanich et al. (2002)
Lactic acid	PF1022A	10	Sasaki et al. (1992)
3-Hydroxydodecanoic acid	Isariin A	9	Wolstenholme and Vining (1966)
Dodecanoic acid	Colisporifungin	10	Ortíz-López et al. (2015)
3-Hydroxy-4-methyldecanoic acid	Beauverolide II	9	Mochizuki et al. (1993)
3-Hydroxydecanoic acid	Icosalide A_1	9	Boros et al. (2006)
3-Hydroxy-2,4-dimethyldecanoic acid	Emericellamide A	10	Oh et al. (2007)
3-Hydroxy-2,4,6 trimethyldodecanoic acid	Emericellamide B		Oh et al. (2007)
3,5-Dihydroxy-2,4-dimethylstearic acid	Stevastelin B	10	Morino et al. (1994)
2-Hydroxy-4-cyanobutyric acid	Pestahivin	10	Hommel et al. (1996)
2-Hydroxy-4-enoylpentanoic acid	Destruxin A	9	Rees et al. (1996)
2,4-Dimethyl-3-hydroxydodecanoicacid	LL15G256γ	7	Abbanat et al. (1998)

Table 3 Modifications in cyclic peptide and depsipeptides

Modification/substitution	Example	Fig.	Reference
O-methyl	Clavariopsin A	10	Kaida et al. (2001)
N-methyl	Omphalotin A	5	Sterner et al. (1997)
Methoxy	Pestahivin	10	Hommel et al. (1996)
Acetyl	Omphalotin C	5	Büchel et al. (1998a)
3-Hydroxy-methylbutanoyl	Omphalotin C	5	Büchel et al. (1998a)
Palmitic acid	WF-11899C	7	Iwamoto et al. (1994a)
3-Hydroxypalmitic acid	FR 901469	7	Fujie et al. (2000)
Linoleic acid	Echinocandin B	7	Keller-Juslen et al. (1976)
3-[4-(buta-2,3-dienyl-1-oxy)-phenyl]prop-2-enoic acid	Xyloallenoide A	6	Wang et al. (2012)
Sulfate	WF-11899C	7	Iwamoto et al. (1994a)
Nitro	Psychrophilin A	5	Dalsgaard et al. (2005) Zhao et al. (2016)
Halogenation	Sporidesmin A	3	Fridrichsons and Mathieson (1962)
Isoprenyl	Roquefortine C	2	Scott et al. (1979)
Prenyl	Fumitremorgin A	2	Eickman et al. (1975)
Geranyl	Mycelianamide	2	Birch et al. (1956)
N-Methylcarbamoyl	Argifin	5	Arai et al. (2000)
Hydroxylation			
3-Hydroxyvaline	Omphalotin C	5	Büchel et al. (1998a)
4,5-Dihydroxyornithine	Echinocandin B	7	Keller-Juslen et al. (1976)
3,4-Dihydroxyhomotyrosine	Echinocandin B	7	Keller-Juslen et al. (1976)
2,6-Dihydroxyphenylalanine	Mactanamide	2	Lorenz et al. (1998)
3,4-Dihydroxyproline	Pneumocandin D_0	7	Morris et al. (1994)

The depsipeptides start with 4 building blocks (angolide, beauverolides) up to 12 in the antibiotic FR901469, a member of the 1,3-β-glucan synthase inhibitors (Fujie et al. 2001), and 13 in petriellin A (Lee et al. 1995). The latter contains β-phenyllactic acid, a building block not often found in cyclopeptides and cyclodepsipeptides. Further modifications of cyclic peptides and depsipeptides include N-methylation, hydroxylations, acylation, isoprenylation, and introduction of sulfate, chloro, or cyano groups. These modifications can occur at the beginning of the biosynthesis like the N-methylations or after the cyclization, e.g., C- and N-hydroxylations followed by an acylation (Glinski et al. 2001; Eisfeld 2009). In many cases, however, it is not clear at which step the modifications occur. The low substrate specificity of the NRPS enzymes allows the incorporation of modified ring components. In fact, Zocher and his group have made use of this to produce novel enniatin derivatives in vitro (Feifel et al. 2007) and new derivatives of PF1022A (Müller et al. 2009). Scrambled beauvericins were produced by combinatorial mutasynthesis with a *Beauveria bassiana* mutant (Xu et al. 2009). An unusual side chain is found in xyloallenoide A, produced by a *Xylaria* species isolated from a mangrove (Wang et al. 2012). The structure is found in Fig. 6.

C. Diversity of the Biological Activities

The structural diversity of diketopiperazines, cyclopeptides, and cyclodepsipeptides is matched by the diversity of their biological activities. To list all activities and compounds would be beyond of the scope of this chapter. An overview on biological activities of diketopiperazines is given by Martins and Carvalho (2007); cyclic depsipeptides and their biological activities have been reviewed by Sarabia et al. (2004)

and insecticidal and other biological activities of destruxins, isariins, enniatins, and beauverolides by Anke and Sterner (2002) and by Zimmermann (2007a, b). Some of the compounds exhibit rather selective activities like the antifungal 1,3-β-glucan synthesis inhibitors (see below), whereas others like gliotoxin show a broad spectrum of activities. While the former due to fewer side effects generally have a higher potential to be developed into drugs or pesticides, the latter might be of interest as biochemical tools or chemical building blocks. In the following, we attempt to give an overview on the different biological activities exhibited by fungal cyclopeptides and cyclodepsipeptides.

Gliotoxin, already isolated in 1932, has recently regained interest not only due to its immunosuppressive and apoptosis-inducing activities (Waring et al. 1988) but moreover due to its occurrence in the blood of aspergillosis patients and its effects on various human cells among them an inhibition of cell adherence in macrophages (Amitani et al. 1995; Kamei and Watanabe 2005). The plethora of biological activities is evident from the number of papers published on gliotoxin and related epipolythiodioxopiperazines (Waring and Beaver 1996; Hume et al. 2002; Gardiner et al. 2005). Haenamindole (see Fig. 2) and fumiquinazoline analogues selectively reduced the growth rate of the army worm, *Spodoptera frugiperda* (Hwang et al. 2016). The vertihemiptellides A and B and their S-methylated monomers exhibit antimycobacterial and cytotoxic effects (Isaka et al. 2005b). Sirodesmin PL produced by *Leptosphaeria maculans* has phytotoxic, antibacterial, and insecticidal properties (Rouxel et al. 1988; Boudart 1989), and the leptosins inhibited the proliferation of P388 lymphocytic leukemia cells with an ED_{50} between 1.1 and 1.3 μg/ml (Takahashi et al. 1994).

The HC-toxins, host-specific toxins from *Cochliobolus carbonum* (anamorph *Helminthosporium carbonum*), are cyto- and phytotoxic and inhibitors of histone deacetylase (Taunton et al. 1996). Structurally related tetrapeptides (Fig. 8) like apicidin from a *Fusarium* species (Darkin-Rattray et al. 1996; Singh et al. 2002), JM47 from a marine *Fusarium* species (Jiang et al. 2002), FR235222 from an *Acremonium* species (Mori et al. 2003), or the chlamydocins from *Diheterospora chlamydosporia* (Closse and Huguenin 1974) and *Peniophora* sp. (Tani et al. 2001) have been reported to exhibit antiprotozoal activity, to induce apoptosis, to have immunosuppressive effects, or to retard plant growth (de Schepper et al. 2003). Apicidin F (see Fig. 6), a newly isolated derivative of apicidin, also showed antimalarial activity (von Bargen et al. 2013), and 1-alaninechlamydocin induces G2/M cell cycle arrest and apoptosis in MIA PaCa-2 cells (Du et al. 2014a).

Due to their toxic effects in animal and humans and their occurrence in food and feedstuff, fumitremorgens, verruculogens, roquefortines C and D, sporidesmins, chaetocin, cyclochlorotine, and malformins were classified as mycotoxins (Betina 1989). For their different biological activities, the reader is referred to the vast online literature on this group of fungal products.

Malformin C (Fig. 6), despite its antibacterial, plant-deforming, and fibrinolytic activities, has recently aroused some interest due to its inhibitory effects on bleomycin-induced G2 arrest, thus potentiating its DNA-damaging action, a mode of action that might be useful for the treatment of cancer (Hagimori et al. 2007).

Cyclosporins are not the only immunomodulating fungal metabolites. Many epipolythiodioxopiperazines, in addition to other biological activities, are immunosuppressants.

Aspergillicin F (see Fig. 9) is a depsipeptide with innate immunomodulating activity (Kikuchi et al. 2015). Stevastelins, cyclodepsipeptides with a lipophilic side chain, from a *Penicillium* species blocked human T-cell activation in vitro and showed low acute toxicity in mice (Morino et al. 1994). HUN-7293 acts as inhibitor of cytokine-induced expression of vascular cell adhesion molecule-1 on human endothelial cells (Hommel et al. 1996). It is structurally identical to pestahivin.

When novel targets for potential antitumor drugs are included into screening assays, cyclic peptides, depsipeptides, and diketopiperazines

are often detected like the siderophore-type compounds from *Perisporiopsis melioloides* (Kawada 2016; Kawada et al. 2010). An overview on fungal metabolites as potential pharmaceuticals has been compiled by Beekman and Barrow (2014).

Ternatin from *Trametes versicolor* fruit bodies and its derivatives suppressed hyperglycemia and hepatic fatty acid synthesis in mice (Kobayashi et al. 2012). The high cytotoxic activities of the gymnopeptides are interesting as omphalotins and cyclosporins exhibit no or low cytotoxicity (Ványolós et al. 2016).

The depsipeptide aureobasidin A has an interesting mode of action, the inositol phosphoceramide synthase (IPS). The fungal enzyme is considered to be an attractive target for novel fungicides. Further development of aureobasidin A was hampered by its inhibitory effects on ABC transporters in yeasts and humans (Fostel and Lartey 2000). The pleofungins from a *Phoma* species showed antifungal activity toward *Candida albicans, Cryptococcus neoformans*, and *A. fumigatus* with minimal inhibitory concentrations in the range of 1 μg/ml or lower (Yano et al. 2007). The compounds inhibited the *A. fumigatus* IPS with IC_{50} values of 1 ng/ml (Aoyagi et al. 2007).

Neoechinulin A has protective activity in PC12 cells against lethal effects of peroxynitrite and 1-methyl-4-phenylpyridine, a neurotoxin capable of inducing neurodegeneration in humans (Kajimura et al. 2008). The cyclic tetrapeptide CJ-15208 is a kappa opioid receptor antagonist (Saito et al. 2002), four depsipeptides were reported to be selective and competitive human tachykinin receptor antagonists (Hedge et al. 2001), and the enolides interfere with the vasopressin and serotonin receptor (Almeida et al. 2016), whereas alternaramide inhibited the inflammatory mediator expression in lipopolysaccharide-stimulated BV2 cells (Ko et al. 2016).

Among nine beauverolides tested for acyl-CoA:cholesterol acyltransferase (ACAT) inhibitory activity in CHO cells expressing ACAT1 or ACAT2, beauverolides I and III inhibited ACAT1 rather selectively, without antimicrobial or cytotoxic activities, while beauvericin was cytotoxic (Matsuda et al. 2004; Ohshiro et al. 2007). ACAT is discussed as a target for new antiatherosclerotic agents (Roth 1998; Namatame et al. 2004).

The outstanding anthelmintic activity of PF1022A combined with its mode of action, e.g., binding to the latrophilin-like receptor of *Haemonchus contortus* (Conder et al. 1995; Saeger et al. 2001), and low toxicity have led to the development of emodepsin, a novel drug used in animal health.

Antiparasitic properties have been reported for cycloaspeptide A and D (Dalsgaard et al. 2004b). Verticilide, a cyclic depsipeptide isolated from the culture broth of *Verticillium* sp. FKI-1033, inhibits the binding of ryanodine to the receptor (RyR) and has insecticidal activity (Monma et al. 2006). Serinocyclin A isolated from *M. anisopliae* condia produced a sublethal locomotory defect in mosquito larvae (Krasnoff et al. 2007). Argifin and argadin, two cyclopentapeptides from a *Gliocladium* and a *Clonostachys* species, are potent inhibitors of chitinase B from *Serratia marcescens* (Houston et al. 2002). When injected into cockroach larvae, the molt was arrested. Besides cyclopeptides and cyclodepsipeptides, fungi also produce other peptides with insecticidal activities; recent examples are the neoefrapeptins from *Geotrichum candidium* (Fredenhagen et al. 2006). Selective nematicidal properties have been reported only for the omphalotins with high inhibitory activity toward *Meloidogyne incognita* and low activity toward *Caenorhabditis elegans* (Mayer et al. 1997, 1999; Sterner et al. 1997). The nematicidal properties of the hydroxylated omphalotins are higher than those of the parent compound, but unfortunately they are not stable (Büchel et al. 1998a; Liermann et al. 2009).

Antiviral properties have been reported of sansalvamide A, a cyclodepsipeptide from a marine *Fusarium*, which inhibited viral topoisomerase-catalyzed DNA relaxation (Hwang et al. 1999).

The clavariopsins, cyclic depsipeptides from *Clavariopsis aquatica*, showed selective antifungal activity, bacteria were not affected, and mice tolerated 100 mg/kg of clavariopsin A. As mode of action, an inhibition of cell components was proposed (Kaida et al. 2001). A lipophilic depsipeptide with antifungal activity is glomosporin from a *Glomospora* species (Sato

et al. 2000). Whether this compound also inhibits cell wall synthesis was not reported. Antifungal and cytotoxic activities were reported for petriellin A (Lee et al. 1995). Cytotoxic activities are exhibited by many cyclopeptides and cyclodepsipeptides. The destruxins have been intensively investigated (Vey et al. 2002; Skrobek and Butt 2005). Psychrophilin D was weakly cytotoxic toward P388 murine leukemia cells with an IC_{50} value of 10 μg/ml (Dalsgaard et al. 2005), while the icosalides inhibited the replication of MDCK cells with LD_{50} between 5 and 10 μg/ml (Boros et al. 2006). The aspergillicins were weakly cytotoxic with LD_{99} between 25 and 50 μg/ml (Capon et al. 2003).

As inhibitors of 1,3-β-glucan synthesis have high potential as antimycotic drugs (Fostel and Lartey 2000), fungi have been intensively screened for the production of inhibitors of cell wall synthesis and cyclic peptides, and cyclic depsipeptides have been found.

The antimycotic drugs already on the market (caspofungin, micafungin, and anidulafungin) are derived from lipopeptides (Butler 2004; Morrison 2006). Their spectrum of activity is mainly restricted to *Candida* and *Aspergillus* species. *Cryptococcus neoformans*, *Trichosporon* and *Fusarium* species, or Zygomycetes are not affected (Denning 2003), although the glucan synthase from *C. neoformans* is sensitive to echinocandins (Maligie and Selitrennikoff 2005).

IV. Ecological Role

Many secondary metabolites play a crucial role for fungi in their natural habitats. Endophytic fungi of grasses belonging to genera of *Neotyphodium/Epichloe* confer protection from mammalian and insect herbivors or enhanced resistance to nematodes and phytopathogenic fungi (Schardl et al. 2004; Panaccione et al. 2006). Some of these beneficial effects are due to NRPS products. Ergovaline has been identified among the fungal metabolites in the plant host. Malformins have been detected in onion scales after infection with *A. niger* (Curtis et al. 1974). An interesting review addressing the multiple interactions of endophytic fungi and endophytic and endofungal bacteria with their hosts has appeared (Scherlach et al. 2013).

The role of siderophores in plant and human pathogens is currently elucidated by many research groups (for review see Haas et al. 2008). Additional functions of siderophores for the producing organism are acquisition and storage of iron as well as regulation of asexual and sexual development and protection against oxidative stress (Eisendle et al. 2006). Nonproducing organisms like *Saccharomyces cerevisiae* are able to use, e.g., transport iron-siderophore complexes; thus the compounds might also play a role in fungal–fungal interactions.

In plant pathogenic fungi, cyclic peptides, like HC-toxins in *Cochliobolus carbonum*, AM-toxins in *Alternaria alternata*, sirodesmin PL in *Leptosphaeria maculans* (anamorph *Phoma lingam*), or enniatins in *Fusarium* species, act as putative virulence factors. In some cases this has already been proven, when gene deletions resulted in apathogenic strains or strains with reduced virulence (Ahn and Walton 1998; Pedley and Walton 2001; Elliott et al. 2007). Likewise the insecticidal depsipeptides of insect pathogens have the same function. Investigation on the role of destruxins in the pathogenicity of *Metarhizium anisopliae* against three species of insects revealed a direct relationship between the titer of destruxins produced by the strains in vitro and their destructive action (Kershaw et al. 1999). In the plant pathogenic *Alternaria brassicae*, destruxin B is a host-specific toxin. In three *Brassica* species, the degree of their sensitivity to destruxin B positively correlated with their degree of susceptibility (Pedras et al. 2002). Beauvericin may be of chemotaxonomic value as marker in the *Isaria tenuipes* complex (Luangsa-Ard et al. 2009).

The function of shearamide A, an insecticidal cyclopeptide isolated from the ascostromata of *Eupenicillium shearii* (Belofsky et al. 1998), may be in protecting the fungi against insects, similar to ergopeptides in sclerotia of *Claviceps* species (Leistner and Steiner 2017).

As many entomopathogenic fungi have been or are developed as commercial products in insect control, the role played by the

depsipeptides in respect to virulence and efficacy of the product is still not fully known.

V. Conclusions

The capability to produce secondary metabolites derived from amino acids by NRPS is widespread among the higher fungi and not dependent on the ecological niches inhabited by them. There are no special habitats from which highly prolific secondary metabolite producers are isolated.

Cyclopeptides and cyclodepsipeptides constitute an interesting class of secondary metabolites with great potential not only in medicine but also in agriculture. This can easily be grasped by the wide array of biological activities exhibited by these compounds. Their chemical diversity is enhanced by the possibility of producing an array of related compounds by precursor-supplemented fermentations of the correspondent fungus. This readily facilitates investigations on structure-activity relationship.

In agriculture, fungal-derived pesticides offer ecological advantages, and strains with enhanced production of bioactive compounds might be developed as biopesticides. For both, agriculture and pharmacology bioactive natural compounds may lead to novel targets and serve as lead structures.

Acknowledgments The work in the IBWF was supported by the state of Rhineland-Palatinate, the BASF SE, the Bayer AG, the BMBF, the DAAD, and the DFG.

References

Abbanat D, Leighton M, Maiese W, Jones EBG, Pearce C, Greenstein M (1998) Cell wall active compounds produced by the marine fungus *Hypoxylon oceanicum* LL-15G56. J Antibiot 51:296–302

Adachi K, Kanoh K, Wisespong P, Nishijima M, Shizuri Y (2005) Clonostachysins A and B, new antidinoflagellate cyclic peptides from a marine-derived fungus. J Antibiot 58:145–150

Ahn JH, Walton JD (1998) Regulation of cyclic peptide biosynthesis and pathogenicity in *Cochliobolus carbonum* by TOXEP, a novel protein with a bZIP basic DNA-binding motif and four ankyrin repeats. Mol Gen Genet 260:462–469

Akone SH, Daletos G, Lin W, Proksch P (2016) Unguisin F, a new cyclic peptide from the endophytic fungus *Mucor irregularis*. Z Naturforsch 71:15–19

Almeida C, El Maddah F, Kehraus S, Schnakenburg S, Koenig GM (2016) Endolides A and B, vasopressin and serotonin receptor interacting N-methylated peptides from the sponge-derived fungus *Stachylidium* sp. Org Lett 18:528–531

Amitani R, Taylor G, Elezis EN, Liewellyn-Jones C, Mitchell J, Kuze F, Cole PJ, Wilson R (1995) Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. Infect Immun 63:3266–3271

Anke T, Erkel O (2002) Non β-lactam antibiotics. In: Osiewacz HD (ed) Industrial applications (Mycota, X). Springer, Heidelberg, pp 93–108

Anke H, Sterner O (2002) Insecticidal and nematicidal metabolites from fungi. In: Osiewacz HD (ed) Industrial applications (Mycota, X). Springer, Heidelberg, pp 109–127

Anke H, Kinn J, Bergquist KE, Sterner O (1991) Production of siderophores by strains of the genus *Trichoderma*: isolation and characterization of the new lipophilic coprogen derivative, palmitoylcoprogen. Biol Met 4:176–180

Antelo L, Hof C, Eisfeld K, Sterner O, Anke H (2006) Siderophores produced by *Magnaporthe grisea* in the presence and absence of iron. Z Naturforsch 61c:461–464

Aoyagi A, Yano T, Kozuma S, Takatsu T (2007) Pleofungins, novel inositol phosphorylceramide synthase inhibitors, from *Phoma* sp. SANK 13899. J Antibiot 60:143–152

Arai N, Shiomi K, Iwai Y, Omura S (2000) Argifin, a new chitinase inhibitor, produced by *Gliocladium* sp. FTD-0668. II. Isolation, physico-chemical properties, and structure elucidation. J Antibiot 53:609–614

Arnison PG, Bibb MJ, Bierbaum G Bowers AA, Bugni TS, Bulaj G, Camarero JA, Campopiano DJ, Challis GL, Clardy J, C otter PD, Craik DJ, Dawson M, Dittmann E, Donadio S, Dorrestein PC, Entian K-D, Fischbach MA, Garavelli JS, Göransson U, Gruber CW, Haft DH, Hemscheidt TK, Hertweck C, Hill C, Horswill AR, Jaspars M, Kelly WL, Klinman JP, Kuipers OP, Link AJ, Liu W, Marahiel MA, Mitchell DA, Moll GN, Moore BS, Müller R, Nair SK, Nes IF, Norris GE, Olivera BM, Onaka H, Patchett ML, Piel J, Reaney MJT, Rebuffat S, Ross RP, Sahl H-G, Schmidt EW, Selsted ME, Severinov K, Shen B, Sivonen K, Smith L, Stein T, Süssmuth RD, Tagg JR, Tang G-L, Truman AW, Vederas JC, Walsh CT, Walton JD, Wenzel SC, Willey JM, van der Donk WA (2013) Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for an universal nomenclature. Nat Prod Rep 30:108–160

Badan SD, Ridley DD, Singh P (1978) Isolation of cyclodepsipeptides from plant pathogenic fungi. Aust J Chem 31:1397–1399

Bao J, Zhang X-Y, Xu X-Y, He F, Nong X-H, Qi S-H (2013) New cyclic tetrapeptides and asteltoxins from gorgonian-derived fungus *Aspergillus* sp. SCSGAF 0076. Tetrahedron 69:2113–2117

Bara R, Aly AH, Wray V, Lin WH, Proksch P, Debbab A (2013) Talaromins A and B, new cyclic peptides from the endophytic fungus *Talaromyces wortmannii*. Tetrahedron Lett 54:1686–1689

Beekman AM, Barrow RA (2014) Fungal metabolites as pharmaceuticals. Aust J Chem 67:827–843

Belofsky GN, Gloer JB, Wicklow DT, Dowd PF (1998) Shearamide A: a new cyclic peptide from the ascostromata of *Eupenicillium shearii*. Tetrahedron Lett 39:5497–5500

Belofsky GN, Jensen PR, Fenical W (1999) Sansalvamide: a new cytotoxic cyclic depsipeptide produced by a marine fungus of the genus *Fusarium*. Tetrahedron Lett 40:2913–2916

Bertram A, Pattenden G (2007) Marine metabolites: metal binding and metal complexes of azole-based cyclic peptides of marine origin. Nat Prod Rep 24:18–30

Betina V (1989) Epipolythiopiperazine-3,6-diones. In: Mycotoxins, chemical, biological and environmental aspects. Elsevier, Amsterdam, pp 388–405

Bills GF, Platas G, Peláez F, Masurekar P (1999) Reclassification of a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. Mycol Res 103:179–192

Bills GF, Gloer JB, An Z (2013) Coprophilous fungi: antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. Curr Opin Microbiol 16:549–565

Bills GF, Li Y, Chen L, Yue Q, Niu XM, An Z (2014) New insights into the echinocandins and other fungal non-ribosomal peptides and peptaibiotics. Nat Prod Rep 31:1348–1375

Birch AJ, Massy-Westropp RA, Rickards RW (1956) Studies in relation to biosynthesis. Part VIII. The structure of mycelianamide. J Chem Soc:3717–3721

Blatzer M, Schrettl M, Sarg B, Lindner H, Pfaller K, Haas H (2011) SidL, an *Aspergillus fumigatus* transacetylase involved in biosynthesis of the siderophores ferricrocin and hydroxyferricrocin. Appl Environ Microbiol 77:4959–4966

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2006) Marine natural products. Nat Prod Rep 23:26–78

Boros C, Smith CJ, Vasina Y, Che Y, Dix AB, Darveaux B, Pearce C (2006) Isolation and identification of the icosalides—cyclic peptolides with selective antibiotic and cytotoxic activities. J Antibiot 59:486–494

Boudart G (1989) Antibacterial activity of sirodesmin PL phytotoxin: application to the selection of phytoxin-deficient mutants. Appl Environ Microbiol 55:1555–1559

Büchel E, Martini U, Mayer A, Anke H, Sterner O (1998a) Omphalotins B, C, and D, nematicidal cyclopeptides from *Omphalotus olearius*. Absolute configuration of omphalotin A. Tetrahedron 54:5345–5352

Büchel E, Mayer A, Martini U, Anke H, Sterner O (1998b) Structure elucidation of omphalotin, a cyclic dodecapeptide with potent nematicidal activity from *Omphalotus olearius*. Pest Sci 54:309–311

Buckingham J (ed) (2008) Dictionary of natural products on DVD, Version 17.1. Chapman and Hall, CRC, Boca Raton

Bunyapaiboonsri T, Vongvilai P, Auncharoen P, Isaka M (2012) Cyclohexadepsipeptides from the filamentous fungus *Acremonium* sp. BCC 2629. Helv Chim Acta 95:963–972

Butler MS (2004) The role of natural product chemistry in drug discovery. J Nat Prod 67:2141–2154

Capon RJ, Skene C, Stewart M, Ford J, O'Hair RAJ, Williams L, Lacey E, Gill JH, Heiland K, Friedel T (2003) Aspergillicins A-E: five novel depsipeptides from the marine-derived fungus *Aspergillus carneus*. Org Biomol Chem 1:1856–1862

Che Y, Swenson DC, Gloer JB, Koster B, Malloch D (2001) Pseudodestruxins A and B: new cyclic depsipeptides from the coprophilous fungus *Nigrosabulum globosum*. J Nat Prod 64:555–558

Chen CH, Lang G, Mitova MI, Murphy AC, Cole ALJ, Din LB, Blunt JW, Munro MHG (2006) Pteratides I-IV, new cytotoxic cyclodepsipeptides from the Malaysian basidiomycete *Pterula* sp. J Org Chem 71:7947–7951

Chen Z, Song Y, Chen Y, Huang H, Zhang W, Ju J (2012) Cyclic heptapeptides, cordyhepta-peptides C−E, from the marine-derived fungus *Acremonium persicinum* SCSIO 115 and their cytotoxic activities. J Nat Prod 75:1215–1219

Chen M, Shao C-L,X-M F, Kong C-J, She Z-G, Wang C-Y (2014) Lumazine peptides, penilumamides B−D and the cyclic pentapeptide asperpeptide A from a gorgonian-derived *Aspergillus* sp. fungus. J Nat Prod 77:1601–1606

Closse A, Huguenin R (1974) Isolierung und Strukturaufklärung von Chlamydocin. Helv Chim Acta 57:533–545

Cole RJ, Schweikert MA (2003) Diketopiperazines. In: Cole RJ, Schweikert MA (eds) Handbook of secondary fungal metabolites, vol 1. Academic, Amsterdam, pp 145–244

Conder GA, Johnson SS, Nowakowski DS, Blake TE, Dutton FE, Nelson SJ, Thomas EM, Davis JP, Thompson DP (1995) Anthelmintic profile of the cyclodepsipeptide PF1022A in in vitro and in vivo models. J Antibiot 48:820–823

Curtis RW, Stevenson WR, Tuite J (1974) Malformin in *Aspergillus niger*-infected onion bulbs (*Allium cepa*). Appl Environ Microbiol 28:362–365

Daferner M (2000) Antibiotisch aktive Sekundärstoffe aus höheren marinen Pilzen. Dissertation Universität Kaiserslautern

Dalsgaard PW, Blunt JW, Munro MHG, Larsen TO, Christophersen C (2004a) Psychrophilin B and C: cyclic nitropeptides from the psychrotolerant fungus *Penicillium rivulum*. J Nat Prod 67:1950–1952

Dalsgaard PW, Larsen TO, Frydenvang K, Christophersen C (2004b) Psychrophilin A and cycloaspeptide D, novel cyclic peptides from the psychrotolerant fungus *Penicillium ribeum*. J Nat Prod 67:878–881

Dalsgaard PW, Larsen TO, Christophersen C (2005) Bioactive cyclic peptides from the psychrotolerant fungus *Penicillium algidum*. J Antibiot 58:141–144

Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarel MA, Singh SB, Goetz MA, Dombrowski AW, Polishook ED, Schmatz DM (1996) Apicidin, a novel antiprotozoal agent that inhibits parasite histone deacetylase. Proc Natl Acad Sci U S A 93:13143–31147

Davoli P, Mucci A, Schenetti L, Weber RWS (2005) Laetiporic acids, a family of non-carotenoid polyene pigments from fruit-bodies and liquid cultures of *Laetiporus sulphureus* (Polyporales, Fungi). Phytochemistry 66:817–823

de Garvalho MP, Abraham W-R (2012) Antimicrobial and biofilm inhibiting diketopiperazines. Curr Med Chem 19:3564–3577

Degenkolb T, Gams W, Brückner H (2008) Natural cyclopeptaibiotics and related cyclic tetrapeptides: structural diversity and future prospects. Chem Biodivers 5:693–706

Demain AL, Elander RP (1999) The beta-lactam antibiotics: past, present, and future. Antonie Van Leeuwenhoek 75:5–19

Denning DW (2002) Echinocandins: a new class of antifungals. J Antimicrob Chemother 49:889–891

Denning DW (2003) Echinocandin antifungal drugs. Lancet 362:1142–1151

Ding G, Chen AJ, Lan J, Zhang H, Chen X, Liu X, Zou Z (2012) Sesquiterpenes and cyclopeptides from the endophytic fungus *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg. Chem Biodivers 9:1205–1212

Ding W, Liu W-Q, Jia Y, Li Y, van der Donk W, Zhang Q (2016) Biosynthetic investigation of phomopsins reveals a widespread pathway for ribosomal natural products in Ascomycetes. Proc Natl Acad Sci U S A 113:3521–3526

Du L, Risinger AL, King JB, Powell DR, Cichewicz RH (2014a) A potent HDAC inhibitor, 1-alaninechlamydocin, from a *Tolypocladium* sp. induces G2/M cell cycle arrest and apoptosis in MIA PaCa-2 cells. J Nat Prod 77:1753–1757

Du L, Zhang P, Li X-M, Li C-S, Cui C-M, Wang B-G (2014b) Cyclohexadepsipeptides of the isaridin class from the marine derived fungus *Beauveria felina* EN-135. J Nat Prod 77:1164–1169

Du L, Li X-M, Zhang P, Li C-S, Wang B-G (2014c) Cyclodepsipeptides and other O-containing heterocyclic metabolites from *Beauveria felina* EN-135, a marine-derived entomopathogenic fungus. Mar Drugs 12:2816–2826

Ebada SS, Fischer T, Hamacher A, Du F-Y, Roth YO, Kassack MU, Wang B-G, Roth EH (2014) Psychrophilin E, a new cyclotripeptide, from co-fermentation of two marine alga-derived fungi of the genus *Aspergillus*. Nat Prod Res 28:776–781

Ebrahim W, Kjer J, El Amrani M, Wray V, Lin W, Ebel R, Lai D, Proksch P (2012) Pullularins E and F, two new peptides from the endophytic fungus *Bionectria ochroleuca* isolated from the mangrove plant *Sonneratia caseolaris*. Mar Drugs 10:1081–1091

Eichhorn H, Lessing F, Winterberg B, Schirawski J, Kamper J, Mueller P, Kahmann R (2006) A ferroxidation/permeation iron uptake system is required for virulence in *Ustilago maydis*. Plant Cell 18:3332–3345

Eickman N, Clardy J, Cole RJ, Kirksey JW (1975) The structure of fumitremorgin A. Tetrahedron Lett 16:1051–1054

Eisendle M, Schrettl M, Kragl C, Müller D, Illmer P, Haas H (2006) The intracellular siderophore ferricrocin is involved in iron storage, oxidative-stress resistance, germination, and sexual development in *Aspergillus nidulans*. Eukaryot Cell 5:1596–1603

Eisfeld K (2009) Non-ribosomal peptide synthetases of fungi. In: Anke T, Weber D (eds) Physiology and genetics (The Mycota, vol XV). Springer, Berlin, pp 305–330

Elliott CE, Gardiner DM, Thoma G, Cozijnsen A, van de Wouw A, Howlett BJ (2007) Production of the toxin sirodesmin PL by *Leptosphaeria maculans* during infection of *Brassica napus*. Mol Plant Pathol 8:791–802

Ernst-Russell M, Chai CL, Hurne AM, Waring P, Hockless DCR, Elix JA (1999) Structure revision and cytotoxic activity of the scabrosin esters, epithiopiperazinediones from the lichen *Xanthoparmelia scabrosa*. Aust J Chem 52:279–283

Feifel SC, Schmiederer T, Hornbogen T, Berg H, Süssmuth RD, Zocher R (2007) In vitro synthesis of new enniatins: probing the α-D-hydroxy carboxylic acid binding pocket of the multienzyme enniatin synthetase. Chembiochem 8:1767–1770

Fostel JM, Lartey PA (2000) Emerging novel antifungal agents. Drug Discov Today 5:25–32

Fredenhagen A, Molleyres LP, Böhlendorf B, Laue G (2006) Structure determination of neofrapeptins A to N: peptides with insecticidal activity produced by the fungus *Geotrichum candidum*. J Antibiot 59:267–280

Fridrichsons J, Mathieson AML (1962) The structure of sporidesmin: causative agent of facial eczema in sheep. Tetrahedron Lett 3:1265–1268

Fujie A, Iwamoto T, Muramatsu H, Okudaira T, Nitta K, Nakanishi T, Sakamoto K, Hori Y, Hino M, Hashimoto S, Okuhara M (2000) FR901469, a novel

antifungal antibiotic from an unidentified fungus No 11243. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological properties. J Antibiot 53:912–919

Fujie A, Muramatsu H, Yoshimura S, Hashimoto M, Shigematsu N, Takase S (2001) FR901469, a novel antifungal antibiotic from an unidentified fungus No 112434. III. Structure determination. J Antibiot 54:588–594

Gao X, Haynes SW, Ames BD, Wang P, Vien LP, Walsh CT, Tang Y (2012) Cyclization of fungal nonribosomal peptides by a terminal condensation-like domain. Nat Chem Biol 8:823–830

Gardiner DM, Waring P, Howlett BJ (2005) The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. Microbiology 151:1021–1032

Gevers W, Kleinkauf H, Lipmann F (1968) The activation of amino acids for biosynthesis of gramicidin S. Proc Natl Acad Sci U S A 63:1335–1342

Glinski M, Hornbogen T, Zocher R (2001) Enzymatic synthesis of fungal N-methylated cyclopeptides and depsipeptides. In: Kirst H, Yeh WK, Zmijewski M (eds) Enzyme technologies for pharmaceutical and biotechnological applications. Dekker, New York, pp 471–497

Gournelis DC, Laskaris GG, Verpoorte R (1998) Cyclopeptide alkaloids. In: Herz W, Falk H, Kirby GW, Moore RE, Tamm C (eds) Fortschritte der Chemie organischer Naturstoffe, vol 75. Springer, Wien, New York, pp 1–179

Gross ML, McCrery D, Crow F, Tomer KB, Pope MR, Ciuffetti LM, Knoche HW, Daly JM, Dunkle DL (1982) The structure of the toxin from *Helminthosporium carbonum*. Tetrahedron Lett 51:5381–5384

Gruendlinger M, Yasmin S, Lechner BE, Geley S, Schrettl M, Hynes M, Haas H (2013) Fungal siderophore biosynthesis is partially localized in peroxisomes. Mol Microbiol 88:862–875

Gulder TAM, Hong H, Correa J, Egereva E, Wiese J, Imhoff JE, Gross H (2012) Isolation, structure elucidation and the total synthesis of lajollamide A from the marine fungus *Asteromyces cruciatus*. Mar Drugs 10:2912–2935

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69:509–526

Gupta S, Peiser G, Nakajima T, Hwang Y-S (1994) Characterization of a phytotoxic cyclotetrapeptide, a novel chlamydocin analogue, from *Verticillium coccosporum*. Tetrahedron Lett 35:6009–6012

Haas H (2014) Fungal siderophore metabolism with a focus on *Aspergillus fumigatus*. Nat Prod Rep 31:1266–1276

Haas H, Eisendle M, Turgeon BG (2008) Siderophores in fungal physiology and virulence. Annu Rev Phytopathol 46:149–187

Hagimori K, Fukuda T, Hasegawa Y, Omura S, Tomoda H (2007) Fungal malformins inhibit bleomycin-induced G2 checkpoint in Jurkat cells. Biol Pharm Bull 30:1379–1383

Haritakun R, Sappan M, Suvannakad R, Tasanathai K, Isaka M (2010) An antimycobacterial cyclodepsipeptide from the entomopathogenic fungus *Ophiocordyceps communis* BCC 16475. J Nat Prod 73:75–78

Hedge VR, Puar MS, Dai P, Pu H, Patel M, Anthes JC, Richard C, Terracciano J, Das PR, Gullo V (2001) A family of depsipeptide fungal metabolites, as selective and competitive human tachykinin receptor (NK2) antagonists: fermentation, isolation, physico-chemical properties, and biological activity. J Antibiot 54:125–135

Hof C, Eisfeld K, Welzel K, Antelo L, Foster AJ, Anke H (2007) Ferricrocin synthesis in *Magnaporthe grisea* and its role in pathogenicity. Mol Plant Pathol 8:163–172

Hommel U, Weber H-P, Oberer L, Naegeli HU, Oberhauser B, Foster CA (1996) The 3D-structure of a natural inhibitor of cell adhesion molecule expression. FEBS Lett 379:69–73

Houston DR, Shiomi K, Arai N, Omura S, Peter MG, Turberg A, Synstad B, Eijsink VG, van Aalten DMF (2002) High-resolution structures of a chitinase complex with natural product cyclopentapeptide inhibitors: mimicry of carbohydrate substrate. Proc Natl Acad Sci U S A 99:9127–9132

Howard DH (1999) Acquisition, transport, and storage of iron by pathogenic fungi. Clin Microbiol Rev 12:394–404

Hu Q, Dong T (2015) Non-ribosomal peptides from entomogenous fungi. Soil Biol 43:169–206

Huang H, She Z, Lin Y, Vrijmoed LLP, Lin W (2007) Cyclic peptides from an endophytic fungus obtained from a mangrove leaf (*Kandelia candel*). J Nat Prod 70:1696–1699

Huang S, Ding W, Li C, Cox DG (2014) Two new cyclopeptides from the co-culture broth of two marine mangrove fungi and their antifungal activity. Pharmacogn Mag 10:410–414

Hume AM, Chai CLL, Moermann K, Waring P (2002) Influx of calcium through a redox-sensitive plasma membrane channel in thymocytes causes early necrotic cell death induced by the epipolythiodioxopiperazine toxins. J Biol Chem 35:31631–31638

Hwang Y, Rowley D, Rhodes D, Gertsch J, Fenical W, Bushman F (1999) Mechanism of inhibition of a poxvirus topoisomerase by the marine natural product sansalvamide A. Mol Pharmacol 55:1049–1053

Hwang IH, Che Y, Swenson DC, Gloer JB, Wicklow DT (2016) Haenamindole and fumiquinazoline analogs from a fungicolous isolate of *Penicillium lanosum*. J Antibiot 69:631–636

Isaka M, Kittakoop P, Kirtikara K, Hywel-Jones NI, Thebtaranonth Y (2005a) Bioactive substances from insect pathogenic fungi. Acc Chem Res 38:813–823

Isaka M, Palasarn S, Rachtawee P, Vimuttipong S, Kongsaeree P (2005b) Unique diketopiperazine dimers from the insect pathogenic fungus *Verticillium hemipterigenum* BCC 1449. Org Lett 7:2257–2260

Isaka M, Palasarn S, Kocharin K, Hywel-Jones NI (2007a) Comparison of the bioactive secondary metabolites from the scale insect pathogens, anamorph *Paecilomyces cinnamomeus*, and teleomorph *Torrubiella luteorostrata*. J Antibiot 60:577–581

Isaka M, Srianoh U, Lartpornmatulee N, Boonruangprapa T (2007b) ES-242 derivatives and cycloheptapeptides from *Cordyceps* sp. strains BCC 16173 and BCC 16176. J Nat Prod 70:1601–1604

Isaka M, Palasarn S, Supothina S, Komwijit S, Luangsaard JJ (2011) Bioactive compounds from the scale insect pathogenic fungus *Conoideocrella tenuis* BCC 18627. J Nat Prod 74:782–789

Isaka M, Palasarn S, Komwijit S, Somrithipol S, Sommai S (2014) Pleosporin A, an antimalarial cyclodepsipeptide from an elephant dung fungus (BCC7069). Tetrahedron Lett 55:469–471

Ishidoh K, Kinoshita H, Igarashi Y, Ijara F, Nihira T (2014a) Cyclic lipodepsipeptides verlamelin A and B, isolated from entompathogenic fungus *Lecanicillium* sp. J Antibiot 67:459–463

Ishidoh K, Kinoshita H, Nihira T (2014b) Identification of a gene cluster responsible for the biosynthesis of cyclic lipopeptide verlamelin. Appl Microbiol Biotechnol 98:7501–7510

Ishiyama D, Sato T, Honda R, Senda H, Konno H, Kanazawa S (2000) Glomosporin, a novel antifungal cyclic depsipeptide from *Glomospora* sp. II. Structure elucidation. J Antibiot 53:525–631

Itazaki H, Nagashima K, Sugita K, Yoshida H, Kawamura Y, Yashuda Y, Matsumoto K, Ishii K, Uotani N, Nakai H, Terui A, Yoshimatsu S, Ikenishi Y, Nakagawa Y (1990) Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation. J Antibiot 43:1524–1532

Iwamoto T, Fujie A, Sakamota K, Tsurumi Y, Shigematsu N, Yamashita M, Hashimoto S, Okuhara M, Kohsaka M (1994a) WF11899A, B and C, novel antifungal lipopeptides I. Taxonomy, fermentation, isolation and physico-chemical properties. J Antibiot 47:1084–1091

Iwamoto T, Fujie A, Nitta K, Hashimoto S, Okuhara M, Kohsaka M (1994b) WF11899A, B and C, novel antifungal lipopeptides II. Biological properties. J Antibiot 45:1092–1097

Jiang Z, Barret MO, Boyd KG, Adams DR, Boid ASF, Burgess JG (2002) JM47, a cyclic tetrapeptide HC-toxin analogue from a marine *Fusarium* species. Phytochemistry 60:33–38

Jiang W, Ye P, Che CTA, Wang K, Liu P, He S, Wu X, Gan L, Ye Y, Wu B (2013) Two novel hepatocellular carcinoma cycle inhibitory cyclodepsipeptides from a hydrothermal vent crab-associated fungus *Aspergillus clavatus* C2WU. Mar Drugs 11:4761–4772

Johnson MD, Perfect JR (2003) Caspofungin: first approved agent in a new class of antifungals. Expert Opin Pharmacother 4:807–823

Kaida K, Fudou R, Kameyama T, Tubaki K, Suzuki Y, Ojika M, Sakagami Y (2001) New cyclic depsipeptide antibiotics, clavariopsins A and B, produced by an aquatic hyphomycete, *Clavariopsis aquatica*. J Antibiot 54:17–21

Kajimura Y, Aoki T, Kuramochi K, Kobayashi S, Sugawara F, Watanabe N, Arai T (2008) Neoechinulin A protects PC12 cells against MPP+-induced cytotoxicity. J Antibiot 61:330–333

Kamei K, Watanabe A (2005) *Aspergillus* mycotoxins and their effect on the host. Med Mycol 43(Suppl 1):95–99

Kanasaki R, Kobayashi M, Fujine K, Sato I, Hashimoto M, Takase S, Tsurumi Y, Fujie A, Hino M, Hashimoto S (2006a) FR227673 and FR190293, novel antifungal lipopeptides from *Chalara* sp. No22210 and *Tolypocladium parasiticum* No 16616. J Antibiot 59:158–167

Kanasaki R, Sakamota K, Hashimoto M, Takase S, Tsurumi Y, Fujie A, Hino M, Hashimoto S, Hori Y (2006b) FR209602 and related compounds, novel antifungal lipopeptides from *Coleophoma crateriformis* No. 738. J Antibiot 59:137–144

Kanasaki R, Abe F, Kobayashi M, Katsuoka M, Hashimoto M, Takase S, Tsurumi Y, Fujie A, Hino M, Hashimoto S, Hori Y (2006c) FR220897 and FR220899, novel antifungal lipopeptides from *Coleophoma empetri* No. 14573. J Antibiot 59:149–157

Kawada M (2016) Small molecules modulating tumor-stromal cell interactions: new candidates for anti-tumor drugs. J Antibiot 69:411–414

Kawada M, Someno T, Inoue H, Ohba S, Masuda T, Kato T, Ikeda D (2010) NBRI16716A, a new anti-tumor compound against human prostate cancer cells, produced by *Perisporiopsis melioloides* Mer-f16716. J Antibiot 63:319–323

Kawahara T, Itoh M, Lozone I, Izumikawa M, Sakata N, Tsuchida T, Shin-ya K (2016) MBJ-0110, a novel cyclopeptide isolated from the fungus *Penicillium* sp. f25267. J Antibiot 69:66–68

Keller U, Tudzynski P (2002) Ergot alkaloids. In: Osiewacz HD (ed) Industrial applications (Mycota X). Springer, Berlin, pp 157–181

Keller-Juslen C, Kuhn M, Loosli HR, Petcher TJ, Weber HP, von Wartburg A (1976) Struktur des Cyclopeptid-Antibiotikums SL 7810 (= Echinocandin B). Tetrahedron Lett 17:4147–4150

Kershaw M, Moorhouse ER, Bateman R, Reynolds SE, Charnley AK (1999) The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insects. J Invertebr Pathol 74:213–223

Kikuchi H, Hoshikawa T, Fujimura S, Sakata N, Kurata S, Katou Y, Oshima Y (2015) Isolation of a cyclic

depsipetide, aspergillicin F, and synthesis of aspergillicins with innate immune-modulating activity. J Nat Prod 78:1949–1956

Kim M-Y, Sohn JH, Ahn JS, Oh H (2009) Alternaramide, a cyclic depsipeptide from the marine-derived fungus *Alternaria* sp. SF-5016. J Nat Prod 72:2065–2068

Kim EL, Li JL, Xuao B, Hong J, Yoo ES, Yoon WD, Jung H (2012) A new cyclic tetrapeptide from the jellyfish-derived fungus *Phoma* sp. Chem Pharm Bull 60:1590–1593

Kleinkauf H, von Döhren H (1997) Peptide antibiotics. In: Kleinkauf H, von Döhren H (eds) Products of secondary metabolism (Biotechnology, 7) VCH, Weinheim, pp 277–322

Kleinwachter P, Dahse HM, Luhmann U, Schlegel B, Dornberger K (2001) Epicorazine C, an antimicrobial metabolite from *Stereum hirsutum* HKI 0195. J Antibiot 54:521–525

Ko W, Sohn JH, Jang JH, Ahn JS, Kang DG, Lee HS, Kim JS, Kim YC, Oh H (2016) Inhibitory effects of alternaramide on inflammatory mediator expression through TLR4-MyD88-mediated inhibition of NF-KB and MAK pathway signaling in lipopolysaccharide-stimulated RAW264.7 and BV2 cells. Chem Biol Interact 244:16–26

Kobayashi M, Kawashima H, Takemori K, Ito H, Murai A, Masuda S, Yamada K, Uemura D, Horio F (2012) Ternatin, a cyclic peptide isolated from mushroom, and its derivative suppress hyperglycemia and hepatic fatty acid synthesis in spontaneously diabetic KK-Ay mice. Biochem Biophys Res Commun 427:299–304

Kobbe B, Cushman M, Wogan GN, Demain AL (1977) Production and antibacterial activity of malformin C, a toxic metabolite of *Aspergillus niger*. Appl Environ Microbiol 33:996–997

Kong F, Wang Y, Liu P, Dong T, Zhu W (2014) Thiodiketopiperazines from the marine-derived fungus *Phoma* sp. OUCMDZ-1847. J Nat Prod 77:132–137

Krasnoff SB, Keresztes I, Gillilan RE, Szebenyi DME, Donzelli BGG, Vhurchill ACL, Gibson DM (2007) Serinocyclins A and B, cyclic heptapeptides from *Metarhizium anisopliae*. J Nat Prod 70:1919–1924

Krasnoff SB, Englich U, Miller PG, Shuler ML, Glahn RP, Donzelli BGG, Gibson DM (2012) Metacridamides A and B, macrocycles from conidia of the entomopathogenic fungus *Metarhizium acridum*. J Nat Prod 75:175–180

Kürnsteiner H, Zinner M, Kück U (2002) Immunosuppressants. In: Osiewacz HD (ed) Industrial applications (Mycota, X). Springer, Berlin, pp 129–155

Laatsch H (2017) AntiBase 2017, a database for rapid dereplication of microbial natural products, and annual updates. Wiley-VCH, Germany

Leal SM, Roy S, Vareechon C, de Jesus Carrion S, Clark H, Lopez-Berges MS, di Pietro A, Schrettl M, Beckmann N, Redl B, Haas H, Pearlman E (2013) Targeting iron acquisition blocks infection with the fungal pathogens *Aspergillus fumigatus* and *Fusarium oxysporum*. PLoS Pathog:e1003436

Lee KK, Gloer JB, Scott JA, Malloch D (1995) Petriellin A: a novel antifungal depsipeptide from the coprophilous fungus *Petriella sordida*. J Org Chem 60:5384–5385

Leistner E, Steiner U (2017) The genus Periglandula and its symbiotum with morning glory plants (Convolvulaceae). In: Anke T, Schüffler A (eds) Physiology and genetics. Springer, Heidelberg

Lewis JR (2002) Amaryllidaceae, *Sceletium*, imidazole, oxazole, thiazole, peptide and miscellaneous alkaloids. Nat Prod Rep 19:223–258

Li C, Oberlies NH (2005) The most widely recognized mushroom: chemistry of the genus Amanita. Life Sci 78:532–538

Li Y, Li X, Kim S-K, Kang JS, Choi HD, Rho JR, Son BW (2004) Golmaenone, a new diketopiperazine alkaloid from the marine-derived fungus *Aspergillus* sp. Chem Pharm Bull 52:375–376

Li X, Kim S-K, Nam KW, Kang JS, Choi HD, Son BW (2006) A new antibacterial dioxopiperazine alkaloid related to gliotoxin from a marine isolate of the fungus *Pseudallescheria*. J Antibiot 59:248–250

Li YY, Hu ZY, Shen YM (2011) Two new cyclopeptides and one new nonenolide from *Xylaria* sp. 101. Nat Prod Commun 6:1843–1846

Li YY, Liu P, Tang Y, Li HM, Tang YL, Liang XH, Tang YJ (2013) Novel cerebrosides isolated from the fermentation mycelia of *Tuber indicum*. Helv Chim Acta 96:702–709

Li C, Wang J, Luo C, Ding W, Cox DG (2014) A new cyclopeptide with antifungal activity from the co-culture broth of two marine mangrove fungi. Nat Prod Res 28:616–621

Li G, Kusari S, Goz C, Strohmann C, Spiteller M (2016) Three cyclic pentapeptides and a cyclic lipopeptide produced by endophytic *Fusarium decemcellulare* LG53. RSC Adv 6:54092–54099

Liermann JC, Opatz T, Kolshorn H, Antelo L, Hof C, Anke H (2009) Omphalotins E-I, five oxidatively modified nematicidal cyclopeptides from *Omphalotus olearius*. Eur J Org Chem 8:1256–1262

Lira SP, Vita-Marques AM, Seleghim MHR, Bugni TS, LaBarbera DV, Sette LD, Sponchiado SRP, Ireland CM, Berlinck RGS (2006) New destruxins from the marine-derived fungus *Beauveria felina*. J Antibiot 59:553–563

Liu J-K (2005) N-containing compounds of macromycetes. Chem Rev 105:2723–2744

Liu S, Shen Y (2011) A new cyclic peptide from the marine fungal strain *Aspergillus* sp. AF119. Chem Nat Compd 47:786–788

Lorenz P, Jensen PR, Fenical W (1998) Mactanamide, a new fungistatic diketopiperazine produced by a marine *Aspergillus* sp. Nat Prod Lett 12:55–60

Luangsa-Ard JJ, Berkaew P, Ridkaew R, Hywel-Jones NL, Isaka M (2009) A beauvericin hot spot in the genus *Isaria*. Mycol Res 113:1389–1395

Ma YM, Liang XA, Zhang HC, Liu R (2016) Cytotoxic and antibiotic cyclic pentapeptide from an endophytic *Aspergillus tamarii* of *Ficus carica*. J Agric Food Chem 64:3789–3793

Maligie MA, Selitrennikoff CP (2005) *Cryptococcus neoformans* resistance to echinocandins: (1,3)β-glucan synthase activity is sensitive to echinocandins. Antimicrob Agents Chemother 49:2851–2856

Malmstrom J, Ryager A, Anthoni U, Nielsen PH (2002) Unguisin C, a GABA-containing cyclic peptide from the fungus *Emericella unguis*. Phytochemistry 60:869–887

Martins MB, Carvalho I (2007) Diketopiperazines: biological activity and synthesis. Tetrahedron 64:9923–9932

Matha V, Jegorov A, Weiser J, Pillai JS (1992) The mosquitocidal activity of conidia of *Tolypocladium tundrense* and *Tolypocladium terricola*. Cytobios 69:163–170

Matsuda D, Namatame I, Tomoda H, Kobayashi S, Zocher R, Kleinkauf H, Omura S (2004) New beauverolides produced by amino acid-supplemented fermentation of *Beauveria* sp. FO-6979. J Antibiot 57:1–9

Mayer A, Sterner O, Anke H (1997) Omphalotin, a new cyclic peptide with potent nematicidal activity from *Omphalotus olearius*. 1. Fermentation and biological activity. Nat Prod Lett 10:25–33

Mayer A, Kilian M, Hoster B, Sterner O, Anke H (1999) *In vitro* and *in vivo* nematicidal activities of the cyclic dodecapeptide omphalotin A. Pest Sci 55:27–30

Miyado S, Kawasaki H, Aoyagi K, Yaguchi T, Okada T, Sugiyama J (2000) Taxonomic position of the fungus producing the anthelmintic PF1022 based on the 18S rRNA gene base sequence. Nippon Kinzoku Gakkai Kaiho 41:183–188

Mochizuki K, Ohmori K, Tamura H, Shizuri Y, Nishiyama S, Miyoshi E, Yamamura S (1993) The structures of bioactive cyclodepsipeptides, beauveriolides I and II, metabolites of entomopathogenic fungi *Beauveria* sp. Bull Chem Soc Jpn 66:3041–3046

Molnar I, Gibson DM, Krasnoff SB (2010) Secondary metabolites from entomopathogenic hypocrealan fungi. Nat Prod Rep 27:1241–1275

Monma S, Sunazuka T, Nagai K, Arai T, Shiomi K, Matsui R, Mura S (2006) Verticilide: elucidation of absolute configuration and total synthesis. Org Lett 8:5601–5604

Mori H, Urano Y, Abe F, Furukawa S, Tsurumi Y, Sakamoto K, Hashimoto M, Takase S, Hino M, Fujii T (2003) FR235222, a fungal metabolite, is a novel immunosuppressant that inhibits mammalian histone deacetylase (HDAC) 1. Taxonomy, fermentation, isolation, and biological activities. J Antibiot 56:72–79

Morino T, Masuda A, Yamada M, Nishimoto Y, Nishikiori T, Saito S, Shimada (1994) Stevastelins, novel immunosuppressants produced by *Penicillium*. J Antibiot 47:1341–1343

Morris SA, Schwartz RE, Sesin DF, Masurekar P, Hallada TC, Schmatz DM, Bartizal K, Hensens OD, Zink DL (1994) Pneumocandin D_0, a new antifungal agent and potent inhibitor of *Pneumocystis carinii*. J Antibiot 47:755–764

Morrison VA (2006) Echinocandin antifungals: review and update. Expert Rev Anti-Infect Ther 4:325–342

Müller J, Feifel SC, Schmiederer T, Zocher R, Süssmuth RD (2009) In vitro synthesis of new cyclodepsipeptides of the PF1022-type: probing the a-D-hydrox acid tolerance of the PF1022 synthetase. Chembiochem 10:323–328

Nagano N, Umemura M, Izumikawa M, Kawano J, Ishii T, Kikuchi M, Tomii K, Kumagai T, Yoshimi A, Machida M, Abe K, Shin-ya K, Asai K (2016) Class of cyclic ribosomal peptide synthetic genes in filamentous fungi. Fungal Genet Biol 86:58–70

Namatame I, Zomoda H, Ishibashi S, Omura S (2004) Antiatherogenic activity of fungal beauverolides, inhibitors of lipid droplet accumulation in macrophages. Proc Natl Acad Sci U S A 101:737–742

Nilanonta C, Isaka M, Chanphen R, Thong-orn N, Tanticharoen M, Thebtaranonth Y (2003) Unusual enniatins produced by the insect pathogenic fungus *Verticillium hemipterigenum*: isolation and studies on precursor-directed biosynthesis. Tetrahedron 59:1015–1020

Odds FC, Brown AJ, Gow NA (2003) Antifungal agents: mechanisms of action. Trends Microbiol 11:272–279

Oh DC, Ca K, Jensen PR, Fenical W (2007) Induced production of emericellamides A and B from the marine-derived fungus *Emericella* sp. in competing co-culture. J Nat Prod 70:515–520

Ohshiro T, Rudel LL, Omura S, Tomoda H (2007) Selectivity of microbial acyl-CoA:cholesterol acyltransferase inhibitors towards isoenzymes. J Antibiot 60:43–51

Ohyama T, Kurihara Y, Ono Y, Ishikawa T, Miyakoshi S, Hamano K, Arai M, Suzuki T, Igari H, Suzuki Y, Inukai M (2000) Arborcandins A, B, C, D, E, and F, novel 1,3-beta-glucan synthase inhibitors: production and biological activities. J Antibiot 53:1108–1116

Oide S, Moeder W, Krasnoff S, Gibson D, Haas H, Yoshioka K, Turgeon BG (2006) *NPS6*, encoding a nonribosomal peptide synthetase involved in siderophore-mediated iron metabolism, is a conserved virulence determinant of plant pathogenic ascomycetes. Plant Cell 18:2836–2853

Oide S, Krasnoff SB, Gibson DM, Turgeon BG (2007) Intracellular siderophores are essential for ascomycete sexual development in heterothallic *Cochliobolus heterostrophus* and homothallic *Gibberella zeae*. Eukaryot Cell 6:1339–1353

Ortíz-López FJ, Monteiro MC, González-Menéndez V, Tormo JR, Genilloud O, Bills GF, Vicente F, Zhang C, Roemer T, Singh SB, Reyes F (2015) Cyclic

colisporifungin and linear cavinafungins, antifungal lipopeptides isolated from *Colispora cavincola*. J Nat Prod 78:468–475

Overy DP, Bayman P, Kerr RG, Bills GF (2014) An assessment of natural product discovery from marine (sensu strictu) and marine-derived fungi. Mycology 5:145–167

Panaccione DC, Cipoletti JR, Sedlock AB, Blemings KP, Schradl CL, Machado C, Seidel GE (2006) Effects of ergot alkaloids on food preference and satiety in rabbits, as assessed with gene-knockout endophytes in perennial ryegrass *(Lolium perenne*). J Agric Food Chem 54:4582–4587

Pasqualotto AC, Denning DW (2008) New and emerging treatments for fungal infections. J Antimicrob Chemother 61(Suppl 1):i19–i30

Patil BB, Wakharkar RD, Chincholkar SB (1995) Siderophores of *Cunninghamella blakesleeana* NCIM 687. World J Microbiol Biotechnol 15:265–268

Pedley KF, Walton JD (2001) Regulation of cyclic peptide biosynthesis in a plant pathogenic fungus by a novel transcription factor. Proc Natl Acad Sci U S A 98:14174–14179

Pedras MSC, Zaharia LI, Ward DE (2002) The destruxins: synthesis, biosynthesis, biotransformation, and biological activity. Phytochemistry 59:579–596

Pejin B, Jovanovic KK, Mojovic S, Aleksandar G (2013) New and highly potent antitumor natural products from marine-derived fungi: covering the period from 2003 to 2012. Curr Top Med Chem 13:2745–2766

Peng J, Gao H, Zhang X, Wang S, Wu C, Gu Q, Guo P, Tianjiao Zhu T, Dehai L (2014) Psychrophilins E−H and versicotide C, cyclic peptides from the marine-derived fungus *Aspergillus versicolor* ZLN-60. J Nat Prod 77:2218–2223

Pérez-Victoria I, Martín J, González-Menéndez V, de Pedro N, El Aouad N, Ortiz-López FJ, Tormo JR, Platas G, Vicente F, Bills GF, Genilloud O, Goetz MA, Reyes F (2012) Isolation and structural elucidation of cyclic tetrapeptides from *Onychocola sclerotica*. J Nat Prod 75:1210–1214

Pomilio AB, Battista ME, Vitale AA (2006) Naturally-occurring cyclopeptides: structures and bioactivity. Curr Org Chem 10:2075–2121

Prasad C (1995) Bioactive cyclic peptides. Peptides 16:151–164

Rees NH, Penfold DJ, Rowe ME, Chowdhry BZ, Cole SCJ, Samuels RI, Turner DL (1996) NMR studies of the conformation of destruxin A in water and in acetonitrile. Magn Reson Chem 34:237–241

Renshaw JC, Robson GD, Trinci APJ, Wiebe MG, Livens FR, Collison DC, Taylor RJ (2002) Fungal siderophores: structures, functions and applications. Mycol Res 106:1123–1142

Roth BD (1998) ACAT inhibitors: evolution from cholesterol-absorption inhibitors to antiatherosclerotic agents. Drug Disc Today 3:19–25

Rouxel T, Chupeau Y, Fritz R, Kollmann A, Bousquet J-F (1988) Biological effects of sirodesmin PL, a phytotoxin produced by *Leptosphaeria maculans*. Plant Sci 57:45–53

Rüegger A, Kuhn M, Lichti H, Loosli HR, Huguenin R, Quiquerez C, von Wartburg A (1975) Cyclosporin A, ein immunsuppressiv wirksamer Peptidmetabolit aus *Trichoderma polysporum* (Link ex Pers.) *Rifai*. Helv Chim Acta 59:1075–1092

Rukachaisirikul V, Chantaruk S, Tansakul C, Saithong S, Chaicharernwimonkoon L, Pakawatchai C, Isaka M, Intereya K (2006) A cyclopeptide from the insect pathogenic fungus *Cordyceps* sp. BCC 1788. J Nat Prod 69:305–307

Saeger B, Schmitt-Wrede HP, Dehnhardt M, Benten WP, Krucken J, Harder A, von Samson-Himmelstjerna G, Wiegand H, Wunderlich F (2001) Latrophilin-like receptor from the parasitic nematode *Haemonchus contortus* as target for the anthelmintic depsipeptide PF1022A. FASEB J 15:1332–1334

Saito T, Hirai H, Kim Y-J, Kojima Y, Matsunaga Y, Nishida H, Sakakibara T, Suga O, Sujaku T, Kojima N (2002) CJ 15208, a novel kappa opioid receptor antagonist from a fungus, *Ctenomyces serratus* ATCC15502. J Antibiot 55:847–854

Sarabia F, Chammaa S, Sánchez Ruiz A, Martín Ortiz L, López Herrera FJ (2004) Chemistry and biology of cyclic depsipeptides of medicinal and biological interest. Curr Med Chem 11:1309–1332

Sasaki T, Takagi M, Yaguchi T, Miyado S, Okada T, Koyama M (1992) A new anthelmintic cyclodepsipeptide, PF1022. J Antibiot 45:692–697

Sato T, Ishiyama D, Honda R, Senda H, Konno H, Tokumasu S, Kanazawa S (2000) Glomosporin, a novel cyclic depsipeptide from *Glomospora* sp. I. Production, isolation, physico-chemical properties, and biological activities. J Antibiot 53:597–602

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340

Schepper de S, Bruwiere H, Verhulst T, Steller U, Andries L, Wouters W, Janicot M, Arts J, Heusden van J (2003) Inhibition of histone deacylases by chlamydocin induces apoptosis and proteasome-mediated degradation of survivin. J Pharmacol Exp Ther 304:881–888

Scherkenbeck J, Jeschke P, Harder A (2002) PF1022A and related cyclodepsipeptide—a novel class of anthelmintics. Curr Top Med Chem (7):759–777

Scherlach K, Graupner K, Hertweck C (2013) Molecular bacteria-fungi interactions: effects on environment, food, and medicine. Annu Rev Microbiol 67:375–397

Schmidt FR (2002) Beta-lactam antibiotics: aspects of manufacture and therapy. In: Osiewacz HD (ed) Industrial applications (Mycota, X). Springer, Berlin, pp 69–91

Schrettl M, Haas H (2011) Iron homeostasis-Achilles' heel of *Aspergillus fumigatus*? Curr Opin Microbiol 14:400–405

Schrettl M, Bignell E, Kragl C, Sabiha Y, Loss O, Eisendle M, Wallner A, Arst HN Jr, Haynes K, Haas H (2007) Distinct roles for intra- and extracellular siderophores during *Aspergillus fumigatus* infection. PLoS Pathog 3:1195–1207

Scott PM, Polonsky J, Merrien MA (1979) Configuration of the 3,12 double bond of roquefortine. J Agric Food Chem 27:201–202

Seto Y, Takahasi K, Matsuura H, Kogami Y, Yada H, Yoshihara T, Nabeta K (2007) Novel cyclic peptide, epichlicin, from the endophytic fungus, *Epichloe typhina*. Biosci Biotechnol Biochem 71:1470–1475

Shimokawa K, Mashima I, Asai A, Yamada K, Kita M, Uemura D (2006) Ternatin, a highly N-methylated cyclic heptapeptide that inhibits fat accumulation: structure and synthesis. Tetrahedron Lett 47:445–4448

Shiono Y, Tschuchinari M, Shimanuki K, Miyajima T, Murayama T, Koseki T, Laatsch H, Funakoshi T, Takanami K, Suzuki K (2007) Fusaristatins A and B, two new cyclic lipopeptides from an endophytic *Fusarium* sp. J Antibiot 60:309–316

Singh SB, Zink DL, Liesch JM, Mosley RT, Dombrowski AW, Bills GF, Darkin-Rattray SJ, Schmatz DM, Goetz MA (2002) Structure and chemistry of apicidins, a class of novel cyclic tetrapeptides without a terminal α-keto epoxide as inhibitors of histone deacetylase with potent antiprotozoal activities. J Org Chem 67:815–825

Singh SB, Ondeyka J, Harris G, Herath K, Zink D, Vicente F, Bills G, Collado J, Platas G, González del Val A, Martin J, Fernando Reyes F, Hao Wang H, Nielsen Kahn J, Galuska S, Giacobbe R, Abruzzo G, Roemer T, Xu D (2013) Isolation, structure, and biological activity of phaeofungin, a cyclic lipodepsipeptide from a *Phaeosphaeria* sp. using the genome-wide *Candida albicans* fitness test. J Nat Prod 76:334–345

Skrobek A, Butt TM (2005) Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiol Lett 251:23–28

Sterner O, Etzel W, Mayer A, Anke H (1997) Omphalotin, a new cyclic peptide with potent nematicidal activity from *Omphalotus olearius*. II. Isolation and structure determination. Nat Prod Lett 10:33–38

Stoppacher N, Neumann NKN, Burgstaller L, Zeilinger S, Degenkolb T, Brückner H, Schuhmacher R (2013) The comprehensive peptaibiotics database. Chem Biodivers 10:734–743

Sugui JA, Pardo J, Chang YC, Zarember KA, Nardone G, Galvez EM, Müllbacher A, Gallin JI, Simon MM, Kwon-Chung KJ (2007) Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. Eukaryot Cell 6:1562–1569

Supothina S, Isaka M, Kirtikara K, Tanticharoen M, Thebtaranonth Y (2004) Enniatin production by the entomopathogenic fungus *Verticillium hemipterigenum* BCC 1449. J Antibiot 57:732–738

Takahashi C, Numata A, Matsumura E, Minoura K, Eto H, Shingu T, Ito T, Hasgawa T (1994) Leptosins I and J, cytotoxic substances produced by a *Leptosphaeria* sp. physico-chemical properties and structures. J Antibiot 47:1242–1249

Tan NH, Zhou J (2006) Plant cyclopeptides. Chem Rev 106:840–895

Tan LT, Cheng XC, Jensen PR, Fenical W (2003) Scytalidamides A and B, new cytotoxic cyclic heptapeptides from a marine fungus of the genus *Scytalidium*. J Org Chem 68:8767–8773

Tan RX, Jensen PR, Williams PG, Fenical W (2004) Isolation and structure assignments of Rostratins A-D, cytotoxic disulfides produced by the marine-derived fungus *Exserohilum rostratum*. J Nat Prod 67:1374–1382

Tan QW, Gao FL, Wang FR Chen QJ (2015) Anti-TMV activity of malformin A1, a cyclic pentapeptide produced by an endophytic fungus *Aspergillus tubingensis* FJBJ11. Int J Mol Sci 16:5750–5761

Tani H, Fujii Y, Nakajima H (2001) Chlamydocin analogues from the soil fungus *Peniophora* sp.: structures and plant growth-retardant activity. Phytochemistry 58:305–310

Taunton J, Hassig CA, Schreiber SL (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 272:408–411

Tobiasen C, Aahman J, Ravnholt KS, Bjerrum MJ, Grell MN, Giese H (2007) Nonribosomal peptide synthetase (NPS) genes in *Fusarium graminearum*, *F. culmorum* and *F. pseudograminearium* and identification of NPS2 as the producer of ferricrocin. Curr Genet 51:43–58

Traber R, Dreyfuss MM (1996) Occurrence of cyclosporins and cyclosporin-like peptolides in fungi. J Ind Microbiol 17:397–401

Tsukui T, Umemura M, Kumagai T, Terai G, Machida M, Nagano N, Asai K (2015) Ustiloxins, fungal cyclic peptides, are ribosomally synthesized in *Ustilaginoidea virens*. Bioinformatics 31:981–985

Turner WB, Aldridge DC (1983) Diketopiperazines and related compounds. In: Fungal metabolites II. Academic, London, pp 405–423

Ueno T, Nakashima T, Hayashi Y, Fukami H (1975) Structures of AM-toxin I and II, host-specific phytotoxic metabolites produced by *Alternaria mali*. Agric Biol Chem 39:1115–1122

Umeyama A, Takahashi K, Grudniewska A, Shimizu M, Hayashi S, Kato M, Okamoto Y, Suenaga M, Ban S, Kumada T, Ishiyama A, Iwatsuki M, Otoguro K, Omura S, Hashimoto T (2014) In

vitro antitrypanosomal activity of the cyclodepsipeptides, cardinalisamides A-C, from the insect pathogenic fungus *Cordyceps cardinalis* NBRC 103832. J Antibiot 67:163–166

Ványolós A, Dékány M, Kovács B, Krámos B, Bérdi P, Zupkó I, Hohmann J, Béni Z (2016) Gymnopeptides A and B, cyclic octadecapeptides from the mushroom *Gymnopus fusipes*. Org Lett 18:2688–2691

Verekar SA, Mishra PD, Sreekumar ES, Deshmukh SK, Fiebig HH, Kelter G, Maier A (2014) Anticancer activity of new depsipeptide compound isolated from an endophytic fungus. J Antibiot 67:697–701

Vey A, Matha V, Dumas C (2002) Effects of the peptide mycotoxin destruxin E on insect haemocytes and on dynamics and efficiency of the multicellular immune reaction. J Invertebr Pathol 80:177–187

Vijayakumar EKS, Roy K, Chatterjee S, Deshmukh SK, Ganguli BN, Fehlhaber HW, Kogler H (1996) Arthrichitin. A new cell wall active metabolite from *Arthrinium phaeospermum*. J Org Chem 61:6591–6593

von Bargen KW, Niehaus EM, Bergander K, Brun R, Tudzynski B, Humpf H-U (2013) Structure elucidation and antimalarial activity of apicidin F: an apicidin-like compound produced by *Fusarium fujikuroi*. J Nat Prod 76:136–2140

von Samson-Himmelstjerna G, Harder A, Sangster NC, Coles GC (2005) Efficacy of two cyclooctadepsipeptides, PF022A and emodepside, against anthelmintic-resistant nematodes in sheep and cattle. Parasitology 130:343–347

Vongvanich N, Kittakoop P, Isaka M, Trakulnaleamsai S, Vimuttipong S, Tanticharoen M, Thebtaranonth Y (2002) Hirsutellide A, a new antimycobacterial cyclohexadepsipeptide from the entomopathogenic fungus *Hirsutella kobayasii*. J Nat Prod 65:1346–1348

Walton JD, Hallen-Adams HE, Luo H (2010) Ribosomal biosynthesis of the cyclic peptide toxins of *Amanita* mushrooms. Biopolymers 94:659–664

Walton JD, Luo H, Hallen-Adams H (2012) Ribosomally encoded cyclic toxins from mushrooms. Methods Enzymol 516:63–77

Wang S-Y, Xu Z-L, Wang H, Li C-R, Fu L-W, Pang J-Y, Li J, She Z-G, Lin Y-C (2012) Total synthesis, absolute configuration, and biological activity of xyloallenoide A. Helv Chim Acta 95:973–982

Wang JP, Lin WH, Wray V, Lai DW, Proksch P (2013) Induced production of despsipeptides by co-culturing *Fusarium tricinctum* and *Fusarium begoniae*. Tetrahedron Lett 54:2492–2496

Wang WX, Kusari S, Sezgin S, Lamshoeft M, Kusari P, Kayser O, Spiteller M (2015) Hexacyclopeptides secreted by an endophytic fungus *Fusarium solani* N06 act as crosstalk molecules in *Narcissus tazetta*. Appl Microbiol Biotechnol 99:7651–7662

Waring P, Beaver J (1996) Gliotoxin and related epipolythiodioxopiperazines. Gen Pharmacol 27:1311–1316

Waring P, Eichner RD, Müllbacher A (1988) The chemistry and biology of the immunomodulating agent gliotoxin and related epipolythiodioxopiperazines. Med Res Rev 8:499–524

Weber D, Erosa G, Sterner O, Anke T (2006) Cyclindrocyclin A, a new cytotoxic cyclopeptide from *Cylindrocarpon* sp. J Antibiot 59:495–499

Wei W, Jiang N, Mei YN, Chu YL, Ge HM, Song YC, Ng SW, Tan RX (2014) An antibacterial metabolite from *Lasiodiplodia pseudotheobromae* F2. Phytochemistry 100:103–109

Welker M, von Döhren H (2006) Cyanobacterial peptides – nature's own combinatorial biosynthesis. FEMS Microbiol Rev 30:530–563

Welzel K, Eisfeld K, Antelo L, Anke T, Anke H (2005) Characterization of the ferrichrome A biosynthetic gene cluster in the homobasidiomycete *Omphalotus olearius*. FEMS Microbiol Lett 249:157–163

Winkelmann W, Drechsel H (1997) Microbial siderophores. In: Kleinkauf H, von Döhren H (eds) Products of secondary metabolism. (Biotechnology, 7). VCH, Weinheim, pp 199–246

Winterberg B, Uhlmann S, Linne U, Lessing F, Marahiel M, Eichhorn H, Kahmann R, Schirawski J (2010) Elucidation of the complete ferrichrome A biosynthetic pathway in *Ustilago maydis*. Mol Microbiol 75:1260–1271

Wolstenholme WA, Vining LC (1966) Determination of amino acid sequences in oligopeptides by mass spectrometry VIII. The structure of isariin. Tetrahedron Lett 7:2785–2791

Wu W, Dai H, Bao L, Ren B, Lu J, Lu Y, Guo L, Zhang L, Liu H (2011) Isolation and structural elucidation of proline-containing cyclopentapeptides from an endolichenic *Xylaria* sp. J Nat Prod 74:1303–1308

Xu Y, Wijeratne EMK, Espinosa-Artiles P, Gunatilaka AAL, Monar I (2009) Combinatorial mutasynthesis of scrambled beauvericins, cyclooligomer depsipeptide cell migration inhibitors from *Beauveria bassiana*. Chembiochem 10:345–354

Xu J, Zhao S, Yang X (2014) A new cyclopeptide metabolite of marine gut fungus from *Ligia oceanica*. Nat Prod Res 28:994–997

Yano T, Aoyagi A, Kozuma S, Kawamura Y, Tanaka I, Suzuki Y, Takamatsu Y, Takatsu T, Inukai M (2007) Pleofungins, novel inositol phosphorylceramide synthase inhibitors, from *Phoma* sp. SANK 13899. J Antibiot 60:136–142

Ye P, Shen L, Jiang W, Ye Y, Chen C-TA, Wu X, Wang K, Wu B (2014) Zn-Driven discovery of a hydrothermal vent fungal metabolite clavatustide C, and an experimental study of the anti-cancer mechanism of clavatustide B. Mar Drugs 12:3203–3217

Yin WQ, Zou JM, She ZG, Vrijmoed LLP, Jones EBG, Lin YC (2005) Two cyclic peptides produced by the

endophytic fungus 2221 from *Castaniopsis fissa* on the South China sea cost. Chin Chem Lett 16:219–222

Yoshioka H, Nakatsu K, Sato M, Tatsuno T (1973) The molecular structure of cyclochlorotine, a toxic chlorine-containing peptapeptide. Chem Lett (12):1319–1322

Zhang P, Chen Z, Hu J, Wei B, Zhang Z, Hu W (2005) Production and characterization of amanitin toxins from a pure culture of *Amanita exitialis*. FEMS Microbiol Lett 252:223–228

Zhao M, Lin H-C, Tang Y (2016) Biosynthesis of the alpha-nitro-containing cyclic tripeptide psychrophilin. J Antibiot 69:571–573

Zheng CJ, Oark SH, Koshino H, Kim YH, Kim WG (2007) Verticillin G, a new antibacterial compound from *Bionectria byssicola*. J Antibiot 60:61–64

Zheng J, Zhu H, Hong K, Wang X, Peng X, Zhu W (2009) Novel cyclic hexapeptides from marine-derived fungus *Aspergillus sclerotiorum* PT06-1. Org Lett 11:5262–5265

Zheng J, Xu Z, Wang Y, Hong K, Liu P, Zhu W (2010) Cyclic tripeptides from the halotolerant fungus *Aspergillus sclerotiorum* PT06-1. J Nat Prod 73:1133–1137

Zhou ZF, Yang XH, Liu HL, Gu YC, Ye BP, Guo YW (2014) A new cyclic peptide and a new steroid from the fungus *Penicillium* sp. GD6 isolated from the mangrove *Bruguiera gymnorrhiza*. Helv Chim Acta 97:1564–1570

Zhuang Y, Teng X, Wang Y, Liu P, Wang H, Li J, Li G, Zhu W (2011) Cyclopeptides and polyketides from coral-associated fungus, *Aspergillus versicolor* LCJ-5-4. Tetrahedron 67:7085–7089

Zimmermann G (2007a) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocont. Sci Technol 17:553–596

Zimmermann G (2007b) Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci Technol 17:879–920

Zou X, Niu S, Ren J, Li E, Liu X, Che Y (2011) Verrucamides A-D, antibacterial cyclopeptides from *Myrothecium verrucaria*. J Nat Prod 74:1111–1116

Polyketide Synthase–Nonribosomal Peptide Synthetase Hybrid Enzymes of Fungi

Shinji Kishimoto[1], Yuichiro Hirayama[1], Kenji Watanabe[1]

CONTENTS

I. Introduction

People have been long attracted to the bioactivity of natural products that are found in various organisms in nature. Usefulness of such compounds often surpasses that of the chemicals that can be artificially synthesized de novo in the laboratory. Thus, it is important for us to continue discovering new bioactive natural products in the present age for our academic, clinical, and industrial interests. However, discovery of new compounds, especially those with new bioactivities, has become progressively difficult, because natural products that can be obtained easily from the huge number of organisms examined to date have been isolated exhaustively. Recently, development of cheaper and faster sequencing technologies has accelerated dramatically the process of uncovering genomic information of many microbes. Results from genome sequencing studies revealed that there were many more biosynthetic gene clusters that are potentially capable of producing natural products in the genome of a single microorganism than the number of compounds that can be isolated from that microorganism (Brakhage and Schroeckh 2011). This tendency is particularly pronounced in streptomycetes and fungi, two families of microorganisms that are known to produce a wide array of natural products. This finding suggested that many of the biosynthetic genes encoded in the genome of a microbe are not activated to produce natural products under the conventional culture conditions used in the laboratory. More recent studies have indicated that epigenetic control of gene expression in those microorganisms is partly responsible for the lack of activation of silent natural product biosynthetic gene clusters (Bok et al. 2009). What this finding implicates is that our past effort of screening microorganisms for useful compounds may have missed identifying microbes that are capable of producing compounds that exhibit unique or potent bioactivities. However, this also poses an opportunity to discover natural products having unprecedented structural framework or high bioactivities from already known microorganisms, if we could control the expression of natural product biosynthetic genes in those organisms. More-

[1] Department of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan; e-mail: kenji55@u-shizuoka-ken.ac.jp

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

over, once biosynthetic gene clusters are identified in various organisms, such gene clusters can be transferred from the original microbes to other more conventional microorganisms that are amenable to genetic modifications and readily grow under a laboratory setup. Once the genes can be transferred to a convenient heterologous host, it becomes feasible to examine and engineer the biosynthetic pathways for the production of those novel natural products and their analogs. Such heterologous biosynthetic systems would also allow production of those compounds at a large scale for commercial distribution. Here, we review the construction of model fungi *Chaetomium globosum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans*, and *Aspergillus oryzae* that can serve as platform organisms having high versatility to allow production of new natural products and their analogs. Those organisms can also serve as a very powerful tool for identifying biosynthetic intermediates or examining the activity of specific enzymes for elucidating the detailed mechanisms of how complex natural products are biosynthesized.

II. Engineering of Fungi for Making Them Amenable to Molecular Genetics Manipulations

A. *Chaetomium globosum*

To date, many biosynthetic gene clusters have been found in various different organisms through recent genome and metagenome sequencing efforts, and this is especially true for fungi (Primm and Franzblau 2007). Those clusters carry genes encoding enzymes responsible for the production of the backbone structure of secondary metabolites, such as polyketides (PKs), nonribosomal peptides (NRPs), and mixed PK–NRPs. Those clusters also contain genes for multiple auxiliary enzymes that are responsible for the modification of the backbone structure. However, many fungi are often difficult to culture at a large scale (Ezaki et al. 2008). Furthermore, despite the existence of an upward of 40 biosynthetic gene clusters in a fungus, oftentimes only a few compounds can be isolated from a fungal culture that is grown under typical growth conditions (Brakhage and Schroeckh 2011). This is exactly the case for *C. globosum*, where the genome sequencing shows the presence of approximately 33 polyketide biosynthetic genes despite the fact that only 11 polyketide products can be isolated from the culture grown under typical growth conditions (Tsunematsu et al. 2012). One way of forcing the fungus to turn on a silent biosynthetic gene cluster is to artificially activate the expression of a transcription factor gene present within the target gene cluster. For example, analysis of the *C. globosum* genome sequence identified a gene cluster named *cgs* containing seven genes, including a PK synthase (PKS) gene *cgsA* and a GAL4-like transcription factor gene *cgsG*. Upon introduction of a copy of *cgsG* under the control of a constitutively active actin promoter, the *cgs* gene cluster was activated. This resulted in the identification that the cluster was responsible for the biosynthesis of a small aromatic compound called shanorellin (Tsunematsu et al. 2012).

While the kind of "brute force" method of activating a silent gene cluster described above can be successful, a more direct modification of the genome, such as gene knockout or substitution, would allow more efficient analyses of the functions of unknown biosynthetic gene clusters and the genes found within those clusters. Such genome modifications can be effectively performed by homologous recombination between the exogenous DNA and a specific locus within the genome of the target organism. However, many fungi, including *C. globosum*, carry out high level of nonhomologous random recombination with foreign DNA molecules, making site-specific genome modifications practically impossible (Ishibashi et al. 2006). Therefore, to engineer the wild-type *C. globosum* into a homologous recombination-compatible strain, the *CgligD* gene, a homolog of a Lig4-type DNA ligase responsible for the nonhomologous random recombination found in *C. globosum*, was deleted from the genome (Nakazawa et al. 2013). This *ΔCgligD* strain, which was designated as CGKW10 (Fig. 1), was shown to be able to perform targeted

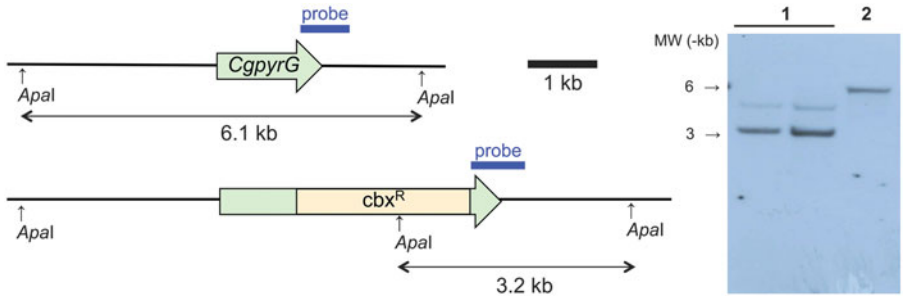


Fig. 1 Strategy for deleting the orotidine-5′-phosphate decarboxylase gene *CgpyrG* by inserting a carboxin resistance gene (*cbx*^{R}) in the *ΔCgligD C. globosum* strain CGKW10 for developing a high-efficiency gene-targeting system. The carboxin resistance gene was integrated into the *CgpyrG* locus in CGKW10 to generate the *CgpyrG*-mutated strain of *Chaetomium globosum*, CGKW12. Southern blotting analyses were performed on the *Apa*I-digested genomic DNA from the desired transformant CGKW12 and the parent strain CGKW10 using probes designed to anneal to regions indicated by blue bars. Lane 1, *CgpyrG*::cbxR/*CgligD*::*hph* (CGKW12); Lane 2, *CgligD*::*hph* (CGKW10) as a control

homologous recombination efficiently. Furthermore, a convenient positive and negative selection system, equivalent of the *URA3* gene of *Saccharomyces cerevisiae* (Boeke et al. 1984), that is based on nutritional (uridine) requirement and metabolite toxicity (5-fluoroorotic acid), respectively, was also established by deleting *CgpyrG* (Weidner et al. 1998), an orotidine-5′-phosphate decarboxylase gene, from the genome of CGKW10. The resulting *ΔCgligD*/*ΔCgpyrG* strain named CGKW12 (Fig. 1) made it possible to prepare various gene knockout strains, which accelerated dramatically the investigation into natural product biosynthesis by *C. globosum*. Later, the hygromycin resistance gene *hph* used to disrupt *CgligD* in CGKW10 and CGKW12 was eliminated to yield another *ΔCgligD*/*ΔCgpyrG* strain, CGKW14. Using CGKW14, knockout of several transcriptional regulator genes associated with epigenetic silencing of secondary metabolite biosynthetic pathways was performed successfully. Those knockout mutants were shown to produce a total of 11 compounds from *C. globosum* grown on a simple oatmeal agar medium, including 2 compounds mollipilin A and B that had not been isolated from *C. globosum* previously (Nakazawa et al. 2013). Those results confirmed the usefulness of CGKW14 for studying natural product biosynthesis in *C. globosum*. CGKW14 was also used extensively for deciphering the mechanism of biosynthesis of natural products, including PK–NRP hybrid compounds chaetoglobosin A and Sch 210972. Details of those studies are discussed in detail below.

B. *Aspergillus fumigatus*

It is well known that *A. fumigatus* is capable of biosynthesizing PKs, NRPs, and PK–NRP hybrid compounds, such as fumagillin (Lin et al. 2013, 2014), spirotryprostatin B (Tsunematsu et al. 2013), and pseurotin A (Tsunematsu et al. 2014; Wiemann et al. 2013; Zou et al. 2014) (Fig. 2). However, it is frequently difficult to resolve the biosynthetic pathway that employs multiple enzymes to generate various intermediates for the formation of a family of complex final products. For deciphering a complex biosynthetic pathway, preparation of knockout strains lacking each of the genes in the target biosynthetic gene cluster is indispensable. By identifying missing intermediates in the knockout mutants and performing biochemical assays with the enzyme coded by the deleted gene, details of the biosynthetic pathway can be elucidated.

As discussed earlier, the ability to perform targeted homologous recombination is crucial for performing gene knockout, where a specific gene can be inactivated in the genome of the target producer organism. However, as is the

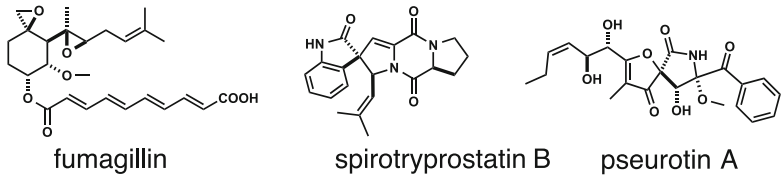


Fig. 2 Chemical structures of natural products isolated from *A. fumigatus*

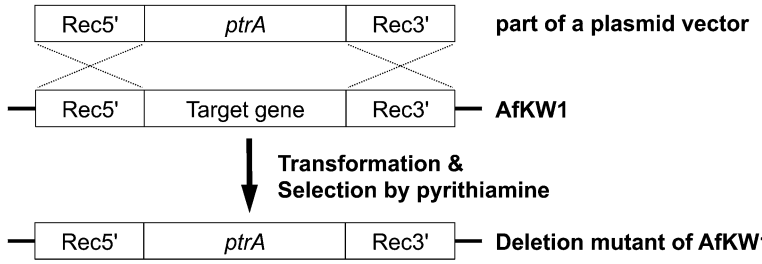


Fig. 3 A schematic diagram showing the construction of a disruption cassette-containing plasmid using the yeast-based homologous recombination method (Kubodera et al. 2002) for preparation of a mutant of AfKW1 in which the target gene is deleted

case with *C. globosum*, *A. fumigatus* also possesses a native random DNA recombination activity. To suppress non-specific DNA integration and improve the efficiency of targeted gene disruption significantly in *A. fumigatus*, it was necessary to eliminate *ku70* gene, which codes for a protein involved in the nonhomologous end-joining pathway (Ninomiya et al. 2004). Therefore, the *ku70*-deficient *A. fumigatus* strain A1159 (Krappmann et al. 2006) was chosen as a parent strain for detailed investigations of secondary metabolite biosynthesis in this fungus. However, two additional techniques needed to be applied to make this strain more useful for the study. Firstly, like in *C. globosum*, the orotidine-5′-phosphate decarboxylase gene *pyrG* was deleted in *A. fumigatus* A1159 to generate a *ΔpyrG/Δku70* strain, which was named AfKW1 (Weidner et al. 1998; Tsunematsu et al. 2014). This strain allowed the same negative/positive selection described earlier for *C. globosum* to be applied to *A. fumigatus*. In addition, to facilitate the selection of desired deletion mutants, a pyrithiamine resistance gene *ptrA* (Kubodera et al. 2002) was also used as a selection marker in the gene disruption cassette (Fig. 3). The use of AfKW1 and *ptrA* selection system facilitated our identification of biosynthetic genes and investigation of the detailed mechanisms of the biosynthesis of the pseurotin-type complex natural products (Tsunematsu et al. 2014).

C. *Aspergillus niger*

A. niger, like other fungi discussed here, is known to produce a number of natural products. One such class of compounds is pyranonigrins, which is a group of antioxidative natural products that has a characteristic fused γ-pyrone core (Hiort et al. 2004; Schlingmann et al. 2007). The backbone structure of pyranonigrins is biosynthesized by PynA, a megaenzyme that is a hybrid of a PKS and an NRP synthetase (NRPS). Previously, Abe et al. attempted activation of the pyranonigrin biosynthetic gene cluster by expressing the pathway-specific transcriptional regulator *pynR* under the control of an *aga* (arginase) promoter from a plasmid in *A. niger* ATCC 1015 (Awakawa et al. 2013). This study is another example of a study, where activation of a silent or a poorly expressed biosynthetic gene cluster is accomplished by inducing the expression of a pathway-specific transcription factor. However,

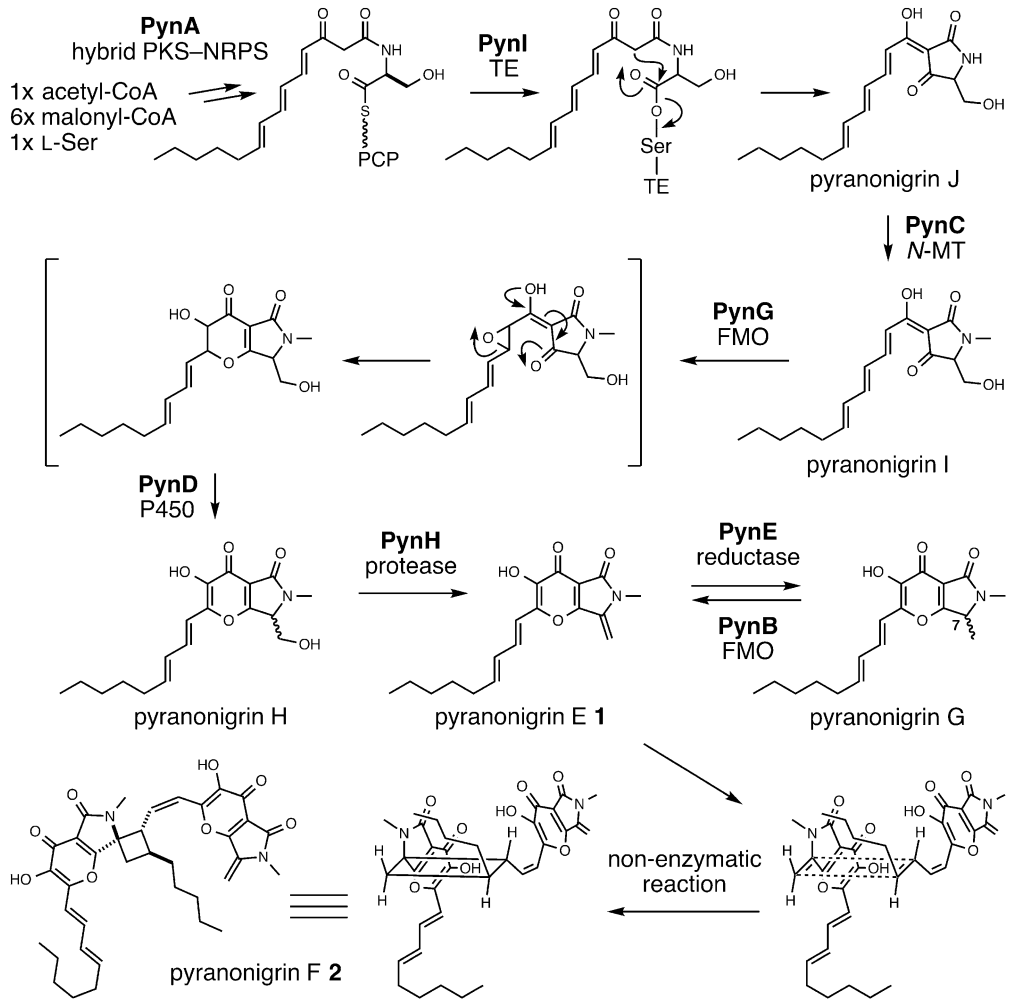


Fig. 4 A proposed pathway for the biosynthesis of pyranonigrin F **2**. *TE* thioesterase, *N-MT N*-methyltransferase, *FMO* flavin-containing monooxygenase

it resulted in only a modest induction of the production of pyranonigrin E **1** (Fig. 4). To effect a greater activation of the *pyn* gene cluster, Watanabe et al. took advantage of the *kusA*- and *pyrG*-deficient *A. niger* A1179 strain that allowed efficient targeted chromosome modification (Meyer et al. 2007). Just like CGKW14 and AfKW1 described earlier, *A. niger* was modified to be suppressed in the random integration of DNA into its chromosome and efficient in performing targeted homologous recombination by knocking out *kusA*, an ortholog of the *ku70* described above. In addition, transformant selection was made more reliable by the use of the *pyrG*-based selection system. This *kusA*- and *pyrG*-deficient *A. niger* strain was used in deciphering the mechanism of biosynthesis of spirotryprostatins (Tsunematsu et al. 2013). Furthermore, the original promoter for *pynR* was replaced with a strong *glaA* promoter (Ganzlin and Rinas 2008) in *A. niger* A1179 to generate AnKW2 (Yamamoto et al. 2015). AnKW2 was able to produce **1** at a yield of 1 g/L. In addition, AnKW2 was able to produce a new compound, which was named pyranonigrin F **2**. This compound had an unusual spiral cyclobutane core structure, which was predicted to be formed through dimerization of **1** (Fig. 4). This high-level activation of the gene cluster, combined with specific gene dele-

tion via site-specific homologous recombination, provided an opportunity to conduct a detailed study of the biosynthesis of pyranonigrins. Through the study, the complete mechanism of how pyranonigrins are produced was revealed successfully. For instance, the γ-pyrone core formation, which was previously proposed to take place spontaneously, was shown to be catalyzed by the flavin-containing monooxygenase PynG and the cytochrome P450 PynD, highlighting the importance of the ability to induce sufficient transcription of necessary biosynthetic genes and to perform deletion of specific genes in the fungus being studied when attempting to unravel the mechanism of the biosynthesis of complex natural products in detail (Yamamoto et al. 2015).

D. *Aspergillus nidulans* and *Aspergillus oryzae*

As illustrated earlier, the ability to generate knockout strains is vital to conducting studies on natural product biosynthesis in fungi. Another way to exploit the potential of fungi and their genomic information for drug discovery and development is to establish a heterologous production system using a convenient host organism capable of expressing the exogenous biosynthetic genes and producing the corresponding compounds. Typical host organisms used in this type of efforts are *Escherichia coli* and *Saccharomyces cerevisiae*. However, reconstitution of a fungal secondary metabolite biosynthetic pathway in those hosts, especially in *E. coli*, often encounters difficulty in producing fungal proteins in their active forms, leading to lack of formation of desired products. Naturally, expression of fungal genes is handled more reliably by fungi. In particular, fungi are more competent in producing massive enzymes like PKSs, NRPSs, and their hybrid enzymes, as well as redox enzymes like cytochrome P450s and flavin-containing monooxygenases. These properties make fungal model hosts particularly suitable for heterologous reconstitution of natural product biosynthetic pathways of interest. Heterologous reconstitution of a biosynthetic pathway is a very effective way of deciphering how a natural product is formed. Reconstitution allows extraction of the target pathway from the background of complex metabolic pathways and easy modification of the pathway, making it easier to identify and characterize each step of the biosynthesis of the target compound. For such studies, engineered *A. nidulans* (Chiang et al. 2013) and *A. oryzae* (Pahirulzaman et al. 2012) are frequently used. Among other examples, those heterologous host systems are successfully used for studying the biosynthesis of Sch 210972 (Sato et al. 2015) and cytochalasin (Fujii et al. 2013; Song et al. 2015) as discussed below in depth.

III. Chemical Structures of PK–NRP Hybrid Natural Products

A. Natural Product Biosynthesis Involving Enzymes Catalyzing Diels–Alder Reactions

Despite the fact that Diels–Alder (DA) reaction is one of the most important transformations employed in chemical synthesis (Corey 2002), biogenic and biocatalytic DA reactions are still recognized poorly. However, since proteins (Xu et al. 2004; Preiswerk et al. 2014) and nucleic acids (Jäschke and Seelig 2000) have been engineered to catalyze DA reactions, biomolecules are expected to be competent catalysts for this type of cycloaddition reaction. While DA reaction has been implicated as a key transformation during the biosynthesis of a growing number of natural products (Oikawa 2010), the identification of enzymes, Diels–Alderases (DAases), for the cycloaddition has proven challenging. Thus far, only a handful of enzymes has been identified as DAases. Many of the DAases are found in fungi, including lovastatin nonaketide synthase LovB (Auclair et al. 2000) and solanapyrone synthase (Kasahara et al. 2010). However, only the bacterial DAases, SpnF involved in the biosynthesis of spinosyn A (Kim et al. 2011) and VstJ responsible for the formation of the spirotetronate framework of versipelostatin (Hashimoto et al. 2015), have the specific rate for accelerating the

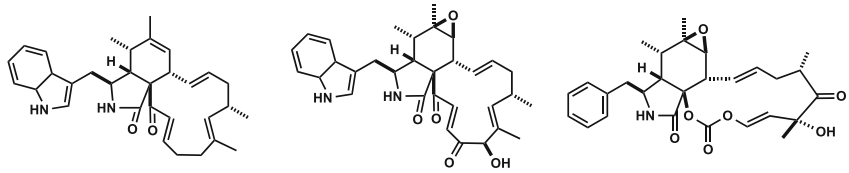


Fig. 5 Chemical structures of the representative members of the cytochalasan family of natural products

[4 + 2] cycloaddition reaction determined experimentally. Nevertheless, the limited understanding of enzymatic DA reactions and the potential synthetic utility of DAases warrant further studies for establishing the existence and catalytic modes of natural DAases. Below, we will discuss fungal PK–NRP hybrid compounds whose biosynthetic process is considered to employ a DA reaction.

B. Chaetoglobosins

Fungal natural products often exhibit biological activities of medicinal importance. Among them is chaetoglobosin A **3** (Fig. 5), which has a unique inhibitory activity against actin polymerization in mammalian cells (Löw et al. 1979; Scherlach et al. 2010). The first discovery of the gene cluster responsible for the biosynthesis of **3** in *Penicillium expansum* was accomplished using an siRNA technology (Schümann and Hertweck 2007). Based on this study, the core structure of **3** was predicted to be formed by a PKS–NRPS hybrid enzyme [CheA in *P. expansum* and CHGG_01239 in *C. globosum* (Ishiuchi et al. 2013)] and a stand-alone enoyl reductase (ER) (CheB in *P. expansum* and CHGG_01240 in *C. globosum*) found within the gene cluster. This stand-alone ER is proposed to work in trans with the PKS–NRPS for reduction of olefins in the polyketide backbone. It is commonly found in gene clusters responsible for the biosynthesis of similar fungal metabolites and has been shown to be essential for the formation of the final products (Boettger and Hertweck 2013; Ma et al. 2009; Qiao et al. 2011). How the straight PK–NRP chain off-loaded from the PKS–NRPS remains poorly established for the biosynthesis of **3**. However, for a related compound cytochalasin E **4** from *A. clavatus*, the C-terminal reductase (R) domain of the PKS–NRPS was considered to perform a reductive release of the PK–NRP intermediate as an aldehyde. Then, the aldehyde is thought to undergo a cyclization to form a 2-pyrrolidinone moiety via a Knoevenagel condensation (Qiao et al. 2011; Fujii et al. 2013). The same mechanism is thought to apply for the biosynthesis of **3**. Once the 2-pyrrolidinone moiety is formed, it is thought to act as a dienophile for the DA reaction that achieves cyclization of the released intermediate to form prochaetoglobosin I 5 (Ishiuchi et al. 2013). However, the detailed reaction mechanism involving the 2-pyrrolidinone formation and the DA reaction is yet to be uncovered. Further investigation is currently ongoing in our laboratory to reveal the mechanism of chaetoglobosin biosynthesis by using the genetically modified *C. globosum* strain CGKW14 described above.

C. Sch 210972

Sch 210972 **6** is another PK–NRP compound produced by *C. globosum* whose biosynthetic process is thought to involve a DA reaction (Fig. 6). This compound is characterized by a tetramic acid moiety, which is also found in a number of similar natural products, such as equisetin **7** (Fig. 6). In those compounds, the tetramic acid moiety is attached to a decalin core. This bicyclic core structure is also found in other fungal secondary metabolites, such as lovastatin **8**, and it is proposed to be formed via a DA reaction (Ma et al. 2009; Kakule et al. 2013; Sato et al. 2015). To be able to examine the biosynthesis of **6** in detail, the corresponding biosynthetic genes were pursued. A BLASTP

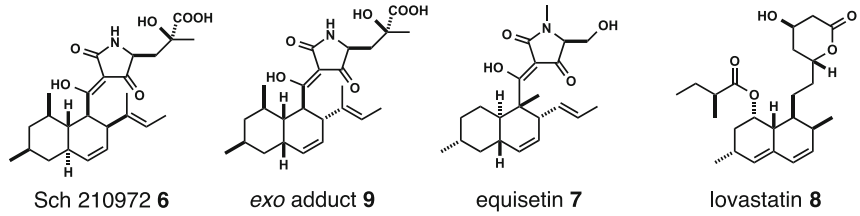


Fig. 6 Natural products with a decalin core whose biosynthesis is proposed to proceed via a Diels–Alder reaction

(Johnson et al. 2008) search of the genome of *C. globosum* identified three recognizable hybrid PKS–NRPS genes: CHGG_01239, CHGG_05286, and CHGG_02374–CHGG_02378. Inspection of the sequence of CHGG_02374 through CHGG_02378 suggested that the five annotated open reading frames (ORFs) were actually a single ORF coding for a hybrid PKS–NRPS. As discussed above, CHGG_01239 codes for the PKS–NRPS responsible for the formation of **3** (Ishiuchi et al. 2013). In addition, deletion of CHGG_05286 did not affect the production of 6. Therefore, the reassigned ORF CHGG_02374–CHGG_02378, which was named *cghG*, was predicted and later confirmed by homologous recombination-mediated targeted gene disruption to code for the PKS–NRPS responsible for the biosynthesis of **6**. Functionally very similar PKS–NRPSs, CheA (Schümann and Hertweck 2007)/CHGG_01239 (Ishiuchi et al. 2013), CcsA (Qiao et al. 2011), and EqxS (Kakule et al. 2013, 2015), are also found in the biosynthetic gene clusters responsible for the production of **3**, **4**, and **7**, respectively (Fig. 7). As mentioned earlier, this class of PKS–NRPSs is often accompanied by a stand-alone ER, and they are CghC (CHGG_02368), CheB/CHGG_01240, CcsC, and EqxC for the biosynthesis of **6**, **3**, **4**, and **7**, respectively. Disruption of this ER abolishes the assembly of the PK–NRP intermediate, such as **5**, by the PKS–NRPS (Sato et al. 2015). For **3**, **4**, and **7**, the extender unit for the NRPS portion of the hybrid enzyme is a natural amino acid; it is L-tryptophan, L-phenylalanine, and L-serine for **3**, **4**, and **7**, respectively. For **6**, however, it was predicted to be an unusual amino acid, (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid. Knockout strains prepared with the use of the engineered strain CGKW14 allowed determination of the function of CghB, a predicted aldolase, as the enzyme responsible for the production of (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid through dimerization of two molecules of pyruvic acid (Fig. 8). As in the case of chaetoglobosin biosynthesis, the amino acid portion of the PK–NRP intermediate for **6** undergoes a cyclization upon release from the PKS–NRPS, although a tetramic acid moiety, not a 2-pyrrolidinone moiety, is formed. Unlike for the biosynthesis of **3**, the heterocyclic moiety is not predicted to be involved in the intramolecular DA reaction for the formation of the decalin core of **6** (Fig. 9).

During the examination of the involvement of DA reaction in the biosynthesis of the natural products discussed here, it became apparent that the biosynthetic gene cluster for **3**, **4**, **6**, and **7** all contained a gene that coded for a small protein of unknown function. One such gene CHGG_01241 (CheC in *P. expansum*) found in the gene cluster responsible for the biosynthesis of **3** was knocked out in CGKW14 to investigate its role in the biosynthesis of **3**. However, deletion of CHGG_01241 completely abolished the formation of **3** despite the high titer of 100 mg or more of **3** per liter of culture attained with the wild-type strain. The straight chain form of **5**, i.e., the intermediate before undergoing an intramolecular DA reaction, could not be observed, either (Ishiuchi et al. 2013). Thus, insight into the role of DA reaction during the biosynthesis of **3** could not be obtained from this study. Likewise, the role of the proteins coded in other clusters, CcsF for **4**, which was suggested to play a role in the formation of the isoindolone core via a DA reaction (Qiao et al. 2011), and Eqx3 for the formation of the decalin core of **7** (Kakule et al. 2013, 2015) have not been investigated in

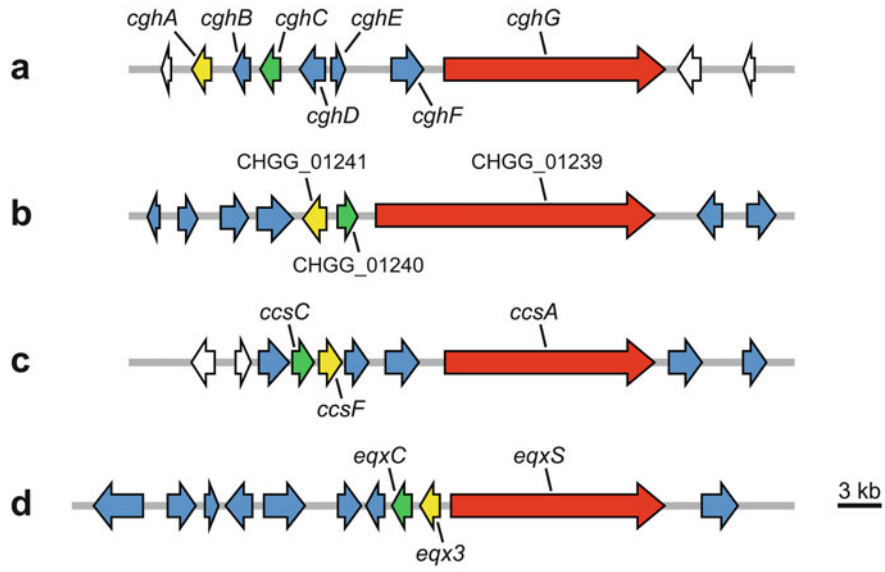


Fig. 7 The gene clusters proposed to contain a gene coding for a Diels–Alderase. The overall organization of (**a**) the *cgh* gene cluster for the biosynthesis of Sch 210972 **6** from *Chaetomium globosum*; (**b**) the CHGG gene cluster for the biosynthesis of chaetoglobosin A **3** from *C. globosum*; (**c**) the *ccs* gene cluster for the biosynthesis of cytochalasin E **4** from *Aspergillus clavatus*; and (**d**) the *eqx* gene cluster for the biosynthesis of equisetin **7** from *Fusarium heterosporum*. Predicted Diels–Alderase gene is colored in yellow, while the PKS–NRPS and its associated stand-alone ER genes are shown in red and green, respectively

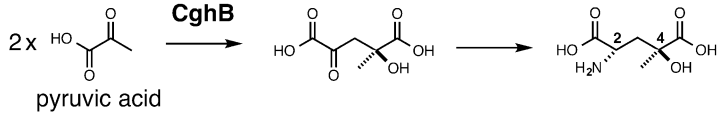


Fig. 8 Formation of an unusual amino acid, (2*S*,4*S*)-4-hydroxy-4-methylglutamic acid, via dimerization of two molecules of pyruvic acid catalyzed by an aldolase CghB

details. However, a very interesting result was obtained during the examination of the biosynthetic gene cluster for **6**. When *cghA*, the proposed DAase-coding gene, was knocked out in CGKW14 (Fig. 9), the deletion strain produced the original *endo* adduct **6** and its diastereomeric exo adduct **9**, both being expected products of the proposed DA reaction, albeit at a much lower overall yield (2 mg/L of **6** and 1 mg/L of **9**) than in the wild-type strain (60 mg/L of **6** only). The stereochemistry of the products was determined by NMR spectroscopy for both compounds and X-ray crystallography for **9**. In addition, reconstitution of the biosynthetic pathway for **6** in an engineered *A. nidulans* strain confirmed that four genes, *cghG* (PKS–NRPS), *cghC* (ER), *cghA* (speculative DAases), and *cghB* ((2*S*,4*S*)-4-hydroxy-4-methylglutamic acid-forming aldolase), were sufficient for the formation of **6**, with *cghA* being required for the strict stereocontrol over the product formation (Sato et al. 2015). Without CghA in the reaction, the decalin-forming [4 + 2] cycloaddition reaction proceeds in a non-stereoselective manner, suggesting that CghA is a DAase responsible for controlling the stereoselectivity of the intramolecular DA cycloaddition of the PK–NRP straight chain intermediate. Furthermore, processing of the straight chain intermediate by CghG appears to be diminished significantly in the absence of CghA, presumably because CghA-catalyzed decalin formation in the inter-

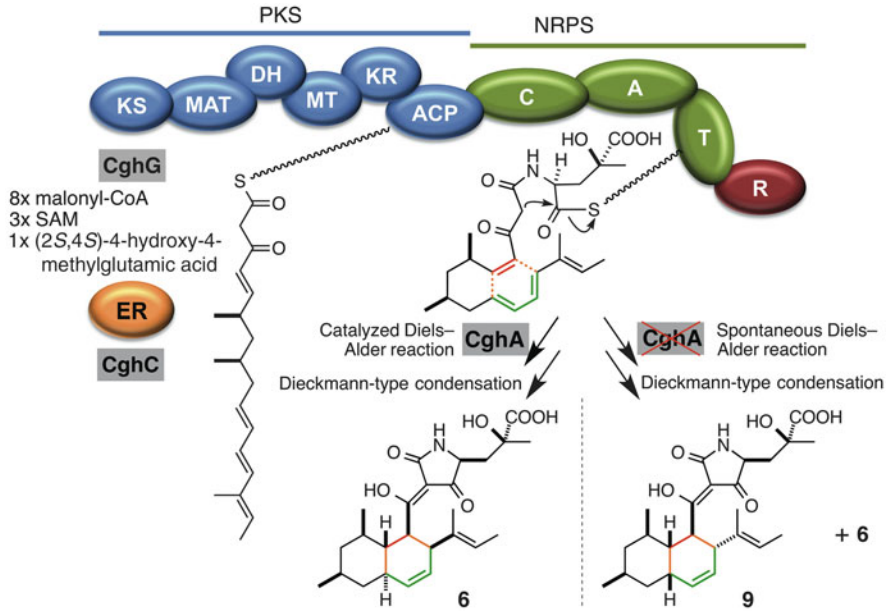
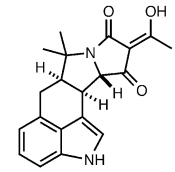


Fig. 9 Proposed biosynthetic pathway of **6**. CghG-catalyzed synthesis of a linear intermediate and subsequent cyclization for the formation of a tetramic acid moiety are shown. Two diastereomeric products Sch 210972 **6** and **9** can be formed via a Diels–Alder reaction in this pathway, depending on the presence of the proposed Diels–Alderase, CghA. *KS* ketosynthase, *MAT* malonyl-CoA acyltransferase, *DH* dehydratase, *MT* methyltransferase, *KR* ketoreductase, *ACP* acyl carrier protein, *C* condensation, *A* adenylation, *T* thiolation, *R* reductase, *ER* enoyl reductase, *SAM* *S*-adenosyl-L-methionine

mediate that is covalently bound to the thiolation (T) domain of CghG is required for subsequent processing for an efficient release from the PKS–NRPS. The release of the intermediate from the PKS–NRPS is thought to be catalyzed by the C-terminal R domain of the PKS–NRPS through a Dieckmann-type condensation that forms the tetramic acid moiety in the final product. This aspect of the biosynthesis of **6** and related compounds is discussed in greater depth below. Again, the engineered *C. globosum* strain CGKW14 is being used effectively for a further investigation of the biosynthetic mechanism of **6**.

D. Equisetin

Equisetin **7**, which is already mentioned in the previous section, is a PK–NRP compound produced by various *Fusarium* fungi, including *Fusarium equiseti* (Burmeister et al. 1974),



cyclopiazonic acid **10**

Fig. 10 Chemical structure of cyclopiazonic acid **10**

Fusarium heterosporum (Sims et al. 2005), and *F.* sp. FN080326 (Kato et al. 2015), that is highly similar to **6** in its structure (Fig. 6). Another compound, cyclopiazonic acid **10** (Fig. 10), isolated from various *Aspergillus* and *Penicillium* fungi (Liu and Walsh 2009), is also related to **6** and **7** in that it has a tetramic acid moiety in its structure. However, **10** is comprised of a tryptophan-containing PK–NRP core with a prenyl side chain that undergoes multiple cyclization reactions to form a fused pentacyclic framework. All of the PKS–

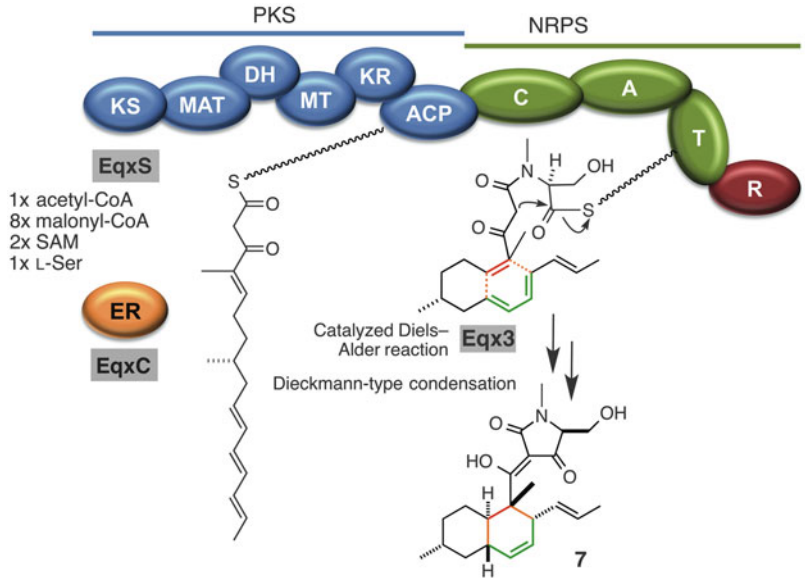


Fig. 11 Proposed biosynthetic pathway of **7**

NRPS responsible for the biosynthesis of **3**, **4**, **6**, and **7** contain a C-terminal R domain. As described for the biosynthesis of **3** and **4**, the R domain can perform a reductive cleavage of the PK–NRP intermediate from the T domain to release the product as an aldehyde. However, the R domain found in EqxS responsible for the biosynthesis of **7** was shown not to bind NAD(P)H or perform a reduction to form an aldehyde but to form a tetramic acid via a Dieckmann-type condensation (Fig. 11) (Sims and Schmidt 2008; Scherlach et al. 2010). The same transformation was proposed to be involved in the biosynthesis of a fungal PK–NRP product tenellin (Halo et al. 2008) and **6** (Sato et al. 2015). A possible explanation of why certain R domains perform a reductive cleavage of a thioester linkage while other R domains perform a Dieckmann-type condensation for concomitant tetramic acid formation and product release is discussed below. As to the involvement of a DA reaction in the biosynthesis of **7**, an investigation into the function of the uncharacterized protein Fsa2 [Eqx3 in *F. heterosporum* (Kakule et al. 2015)], a homolog of CghA, through deletion of *fsa2* in *Fusarium* sp. FN080326 identified it to be a likely DAase (Kato et al. 2015).

E. Pyrrolocins

Pyrrolocin C **13** and its diastereomer pyrrolocin B **12**, along with an *N*-methylated analog of **13** pyrrolocin A **11** (Jadulco et al. 2014), comprise a group of antituberculosis agents that is structurally related to **6** and **7** (Fig. 12). Pyrrolocins are produced by a strain of endophytic fungus designated as NRRL 50135 that was identified as a phylogenetically new strain. This strain mainly produced **11**, while only a negligible amount of the desmethylated compounds **12** and **13** were isolated. During the isolation and characterization of this series of compounds, NRRL 50135 stopped producing **11** under the standard culture conditions examined. To salvage the production of **11** for examining its chemical structure and bioactivity, the biosynthetic locus for the formation of **11** was isolated from NRRL 50135 and transferred to a filamentous fungus *F. heterosporum* ATCC 74349, which was known to produce **7** at a very high titer (Kakule et al. 2015), for heterologous production of **11**. For the production of **11**, an engineered *F. heterosporum* ATCC 74349 strain was used. In this modified strain, the equisetin biosynthetic gene cluster was knocked out, and the expression of the equisetin biosynthetic

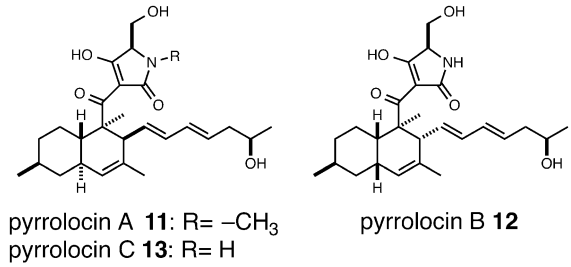


Fig. 12 Chemical structures of pyrrolocin A **11**, B **12**, and C **13**

gene regulator gene *eqxR* was placed under the control of an inducible *A. nidulans alcA* promoter. Furthermore, like CGKW14 and AfKW1 described earlier, the *pyrG* gene was also knocked out for auxotrophic selection (Kakule et al. 2013). Two biosynthetic genes, *prlS* (PKS–NRPS) and *prlC* (ER), were successfully cloned from the genome of NRRL 50135. However, the third gene coding for a methyltransferase (MT) presumably necessary for the *N*-methylation to form **11** could not be located in the vicinity of the *prl* gene cluster. Thus, *prlS* and *prlC* were cloned into a plasmid and placed under the control of *PeqxS*, the promoter for the equisetin PKS–NRPS gene *eqxS*. In place of the missing *prl* MT gene, the gene coding for the equisetin MT *eqxD* was provided in the engineered *F. heterosporum* ATCC 74349 strain. This heterologous system only produced less than 10 mg of **11** per kilogram of culture medium. However, it produced roughly 800 mg/kg of **12** and **13** in an approximately 2-to-1 ratio (Kakule et al. 2013, 2015). Loss of production of **11**, the only *N*-methylated member of pyrrolocins, is likely due to lack of correct MT in the system. The compound **12** contains a cis-decalin, which is a more favored endo adduct of a DA cycloaddition, whereas **13** has a trans-decalin formed through a less favored exo transition state. Thus the higher yield of **12** over **13** is consistent with the notion that the decalin formation during the pyrrolocin biosynthesis occurs through an intramolecular DA reaction. When only the PKS–NRPS and the stand-alone ER but not the presumed DAase like CghA for the biosynthesis of Sch 210972 are provided to the heterologous host, the system produces more of the chemically more favored endo product, **12**. In contrast, **12** is not produced in the original producer where the presumed DAase would be present. The fact that products are formed efficiently by the pyrrolocin-forming enzymes in the absence of a DAase suggests that the biosynthetic mechanism, especially the mechanism involving the decalin formation, might be somewhat different between pyrrolocins and other related compounds like **6**. For the biosynthesis of **6**, knockout of the presumed DAase-coding gene *cghA* abolishes the production of any products (Sato et al. 2015). It is possible that the C-terminal R domain of the PKS–NRPS, which is responsible for the chain release and tetramic acid formation, requires the decalin to be present in the substrate. Whether products are formed efficiently by a DAase gene-knockout strain may depend on the relative efficiency of the spontaneous decalin core formation.

Oftentimes, identification of different intermediates formed during the biosynthesis of a target natural product provides valuable insight for deciphering how the final product is biosynthesized. Unfortunately, the straight chain PK–NRP intermediate has not been isolated from the biosynthetic systems responsible for the production of **6**, **7**, or **11** to date. Interestingly, however, isolation of a possible intermediate was reported for the cytochalasin biosynthetic system by Oikawa et al. (1995) (Fig. 13a). For cytochalasin biosynthesis, the straight chain intermediate is thought to be off-loaded from the PKS–NRPS by the C-terminal R domain as an aldehyde. The isolated compound was not an aldehyde **14** but a primary alcohol **15** (Fig. 13b) (Fujii et al. 2013). For this study, the auxotrophic *A. oryzae* strain

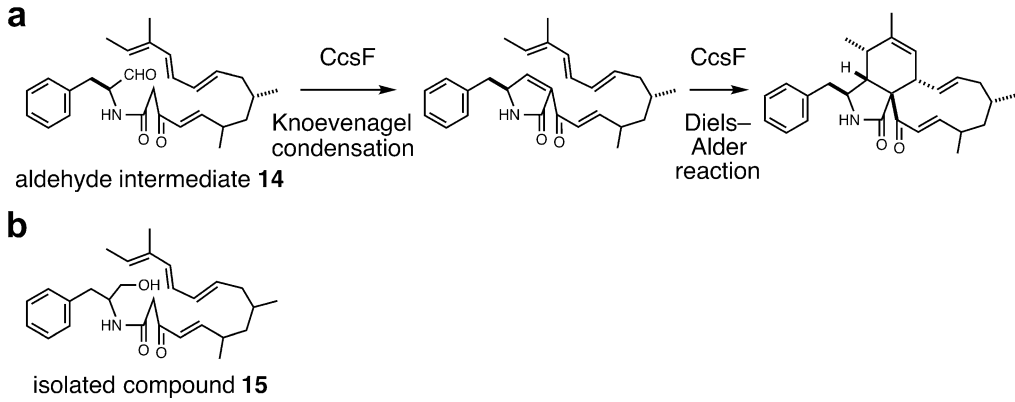


Fig. 13 Proposed biosynthetic pathway of cytochalasins. (**a**) Speculative functions of CcsF, catalyzing a Knoevenagel condensation on a straight-chain aldehyde intermediate **14** to form a 2-pyrrolidinone moiety and a Diels–Alder reaction to form a decalin core. (**b**) Isolated alcohol **15** as a shunt product produced by an unexpected reduction in *A. niger*

NSAR1 (*niaD*$^{-}$, *sC*$^{-}$, *ΔargB*, *adeA*$^{-}$) (Jin et al. 2004) was used as the heterologous host for the plasmid-based expression of the PKS–NRPS gene *ccsA* and the stand-alone ER gene *ccsC* without including the gene for the presumed DAase *ccsF*. Isolation of **15** from this heterologous system is a good indication that the C-terminal R domain of CcsA actually catalyzes off-loading of the PK–NRP intermediate via a reduction of the thioester linkage between CcsA and the bound intermediate. It is not clear whether the R domain is actually catalyzing only a two-electron reduction only to form an aldehyde or a four-electron reduction (Li et al. 2008; Song et al. 2015) to form the primary alcohol found in **15**. Nonetheless, this observation suggests that the pyrrolinone moiety of **4** is formed through a Knoevenagel condensation, not through a Dieckmann-type cyclization as proposed for **6**, **7**, and **11**. This observation is consistent with the amino acid sequence of the CcsA R domain having an intact NAD(P)H-binding motif GXSXXG and the Ser–Tyr–Lys catalytic triad conserved among the short-chain dehydrogenase/reductase family of proteins (Liu and Walsh 2009; Qiao et al. 2011). The reason the R domain of the PKS–NRPS for the biosynthesis of **4** performs a reductive cleavage of the thioester linkage while the R domain for the biosynthesis of **6**, **7**, and **11** catalyzes a Dieckmann-type condensation for concomitant tetramic acid formation may have to do with the subsequent transformation required for the biosynthesis of the final product. For the formation of **4**, 3-pyrrolin-2-one is thought to act as a dienophile for the DA reaction that forms the six-membered ring in the core structure. A Dieckmann-type condensation would result in the formation of a pyrrolidine-2,4-dione, which would have to undergo additional reduction and dehydration of the 4-carbonyl to be converted into 3-pyrrolin-2-one. However, a Knoevenagel condensation of the aldehyde intermediate would lead to direct formation of 3-pyrrolin-2-one. Thus, it makes sense for the R domain of the chaetoglobosin- and the cytochalasin-forming PKS–NRPSs to possess a reductase activity. On the other hand, the tetramic moiety of **6**, **7**, and **11** does not participate in a DA reaction. Thus, the R domains of the PKS–NRPSs that form those compounds could gain a Dieckmann-type condensation activity. Based on this idea, the R domain of CHGG_01239 for the biosynthesis of **3** is predicted to catalyze a reduction, not a Dieckmann-type cyclization. The engineered *C. globosum* strain CGKW14 combined with in vitro biochemical assays can be used to characterize the activity of the CHGG_01239 R domain.

IV. Conclusions

In this review, we mainly summarized the findings from recent studies that focused on engineering of important fungi, primarily *C. globosum*, *A. fumigatus*, *A. niger*, *A. nidulans*, and *A. oryzae*. Creation of those engineered fungal strains has enabled many of the studies described in this review. The engineering typically focuses on eliminating the fungi's native ability to randomly recombine exogenous DNA into their genome. Abolishing the random recombination activity in those fungi leaves the site-specific homologous recombination activity intact, allowing targeted modifications of the genome. Another modification focuses on establishing selection schemes that improve the efficiency of isolating correctly manipulated strains. Those engineered strains make knockout and other genome editing experiments, such as promoter exchange studies, reliable. Those strains also make heterologous reconstitution of biosynthetic pathways of interest straightforward and efficient. Those experiments allow isolation and characterization of biosynthetic pathway intermediates that provide critical insight into what genes are involved in the formation of certain natural products and how such complex natural products are biosynthesized.

Among the interesting chemical transformations found in fungal secondary metabolite biosynthesis, we focused on the biosynthetic steps that are considered to involve enzyme-catalyzed DA reactions. Among them, we paid a particular attention to those proposed for the biosynthesis of chaetoglobosin, Sch 210972, equisetin, and pyrrolocin. There is a shared aspect in the biosynthesis of these compounds, namely, the scaffolding formed by a PKS–NRPS hybrid enzyme with a stand-alone ER, and the cyclization of the linear intermediate by a DA reaction. The DA reaction is thought to be catalyzed by a small protein whose function is unknown due to lack of amino acid sequence homology to other known proteins. Use of engineered fungal strains discussed in the first part of this chapter made it possible to perform targeted gene deletion and heterologous reconstitution of the biosynthetic pathways. Investigation into the biosynthesis of chaetoglobosin, Sch 210972, equisetin, and pyrrolocin with those engineered fungal strains provided strong indication of the involvement of enzyme-catalyzed DA reaction and identification of the uncharacterized proteins as a bona fide DAase. Detailed biochemical analyses and X-ray crystallographic characterizations of the DAases are currently ongoing to understand how those proteins catalyze DA reactions. Those studies are combined with theoretical calculations that can model the reaction and compute transition states of the DA reaction leading to the formation of each of the four compounds discussed in this chapter. Experimentally determined structural information of the DAases will be combined with the results from the computational studies using quantum-mechanical calculations to gain thorough insight into the mechanism of enzyme-catalyzed DA reactions. Those efforts will expand our understanding of how nature biosynthesizes complex chemical structures found in various natural products.

Acknowledgments We wish to thank the financial support from the Japan Society for the Promotion of Science (JSPS) Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers (No. G2604) (K.W.). This work was also supported in part by the Japan Society for the Promotion of Science (JSPS) (K.W., 15KT0068, 26560450), the Takeda Science Foundation (K.W.), the Institution of Fermentation at Osaka (K.W.), the Japan Antibiotics Research Association (K.W.), the Uehara Memorial Foundation (K.W.), and the Tokyo Biochemical Research Foundation (K.W.).

References

Auclair K, Sutherland A, Kennedy J, Witter DJ, Van den Heever JP, Hutchinson CR, Vederas JC (2000) Lovastatin nonaketide synthase catalyzes an intramolecular Diels–Alder reaction of a substrate analogue. J Am Chem Soc 122:11519–11520

Awakawa T, Yang XL, Wakimoto T, Abe I (2013) Pyranonigrin E: a PKS-NRPS hybrid metabolite from *Aspergillus niger* identified by genome mining. Chembiochem 14:2095–2099

Boeke JD, LaCroute F, Fink GR (1984) A positive selection for mutants lacking orotidine-5′-phosphate

decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol Gen Genet 197:345–346

Boettger D, Hertweck C (2013) Molecular diversity sculpted by fungal PKS–NRPS hybrids. Chembiochem 14:28–42

Bok JW, Chiang YM, Szewczyk E, Reyes-Dominguez Y, Davidson AD, Sanchez JF, Lo HC, Watanabe K, Strauss J, Oakley BR, Wang CC, Keller NP (2009) Chromatin-level regulation of biosynthetic gene clusters. Nat Chem Biol 5:462–424

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites—strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22

Burmeister HR, Bennett GA, Vesonder RF, Hesseltine CW (1974) Antibiotic produced by *Fusarium equiseti* NRRL 5537. Antimicrob Agents Chemother 5:634–639

Chiang YM, Oakley CE, Ahuja M, Entwistle R, Schultz A, Chang SL, Sung CT, Wang CC, Oakley BR (2013) An efficient system for heterologous expression of secondary metabolite genes in *Aspergillus nidulans*. J Am Chem Soc 135:7720–7731

Corey EJ (2002) Catalytic enantioselective Diels–Alder reactions: methods, mechanistic fundamentals, pathways, and applications. Angew Chem Int Ed Engl 41:1650–1667

Ezaki M, Muramatsu H, Takase S, Hashimoto M, Nagai K (2008) Naphthalecin, a novel antibiotic produced by the anaerobic bacterium, *Sporotalea colonica* sp. nov. J Antibiot (Tokyo) 61:207–212

Fujii R, Minami A, Gomi K, Oikawa H (2013) Biosynthetic assembly of cytochalasin backbone. Tetrahedron Lett 54:2999–3002

Ganzlin M, Rinas U (2008) In-depth analysis of the *Aspergillus niger* glucoamylase (*glaA*) promoter performance using high-throughput screening and controlled bioreactor cultivation techniques. J Biotechnol 135:266–271

Halo LM, Marshall JW, Yakasai AA, Song Z, Butts CP, Crump MP, Heneghan M, Bailey AM, Simpson TJ, Lazarus CM, Cox RJ (2008) Authentic heterologous expression of the tenellin iterative polyketide synthase nonribosomal peptide synthetase requires coexpression with an enoyl reductase. Chembiochem 9:585–594

Hashimoto T, Hashimoto J, Teruya K, Hirano T, Shinya K, Ikeda H, Liu HW, Nishiyama M, Kuzuyama T (2015) Biosynthesis of versipelostatin: identification of an enzyme-catalyzed [4+2]-cycloaddition required for macrocyclization of spirotetronate-containing polyketides. J Am Chem Soc 137:572–575

Hiort J, Maksimenka K, Reichert M, Perović-Ottstadt S, Lin WH, Wray V, Steube K, Schaumann K, Weber H, Proksch P, Ebel R, Müller WE, Bringmann G (2004) New natural products from the sponge-derivedfungus *Aspergillus niger*. J Nat Prod 67:1532–1543

Ishibashi K, Suzuki K, Ando Y, Takakura C, Inoue H (2006) Nonhomologous chromosomal integration of foreign DNA is completely dependent on MUS-53 (human Lig4 homolog) in *Neurospora*. Proc Natl Acad Sci U S A 103:14871–14876

Ishiuchi K, Nakazawa T, Yagishita F, Mino T, Noguchi H, Hotta K, Watanabe K (2013) Combinatorial generation of complexity by redox enzymes in the chaetoglobosin A biosynthesis. J Am Chem Soc 135:7371–7377

Jadulco RC, Koch M, Kakule TB, Schmidt EW, Orendt A, He H, Janso JE, Carter GT, Larson EC, Pond C, Matainaho TK, Barrows LR (2014) Isolation of pyrrolocins A-C: cis- and trans-decalin tetramic acid antibiotics from an endophytic fungal-derived pathway. J Nat Prod 77:2537–2544

Jäschke A, Seelig B (2000) Evolution of DNA and RNA as catalysts for chemical reactions. Curr Opin Chem Biol 4:257–262

Jin FH, Maruyama J, Juvvadi PR, Akioka M, Kitamoto K (2004) Development of a novel quadruple auxotrophic host transformation system by *argB* gene disruption using *adeA* gene and exploiting adenine auxotrophy in *Aspergillus oryzae*. FEMS Microbiol Lett 239:79–85

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36:W5–W9

Kakule TB, Sardar D, Lin Z, Schmidt EW (2013) Two related pyrrolidinedione synthetase loci in *Fusarium heterosporum* ATCC 74349 produce divergent metabolites. ACS Chem Biol 8:1549–1557

Kakule TB, Jadulco RC, Koch M, Janso JE, Barrows LR, Schmidt EW (2015) Native promoter strategy for high-yielding synthesis and engineering of fungal secondary metabolites. ACS Synth Biol 4:625–633

Kasahara K, Miyamoto T, Fujimoto T, Oguri H, Tokiwano T, Oikawa H, Ebizuka Y, Fujii I (2010) Solanapyrone synthase, a possible Diels-Alderase and iterative type I polyketide synthase encoded in a biosynthetic gene cluster from *Alternaria solani*. Chembiochem 11:1245–1252

Kato N, Nogawa T, Hirota H, Jang JH, Takahashi S, Ahn JS, Osada H (2015) A new enzyme involved in the control of the stereochemistry in the decalin formation during equisetin biosynthesis. Biochem Biophys Res Commun 460:210–215

Kim HJ, Ruszczycky MW, Choi SH, Liu YN, Liu HW (2011) Enzyme-catalysed [4+2] cycloaddition is a key step in the biosynthesis of spinosyn A. Nature 473:109–112

Krappmann S, Sasse C, Braus GH (2006) Gene targeting in *Aspergillus fumigatus* by homologous recombination is facilitated in a nonhomologous end- joining-deficient genetic background. Eukaryot Cell 5:212–215

Kubodera T, Yamashita N, Nishimura A (2002) Transformation of *Aspergillus* sp. and *Trichoderma reesei* using the pyrithiamine resistance gene (*ptrA*)

of *Aspergillus oryzae*. Biosci Biotechnol Biochem 66:404–406

Li Y, Weissman KJ, Müller R (2008) Myxochelin biosynthesis: direct evidence for two- and four-electron reduction of a carrier protein-bound thioester. J Am Chem Soc 130:7554–7555

Lin HC, Chooi YH, Dhingra S, Xu W, Calvo AM, Tang Y (2013) The fumagillin biosynthetic gene cluster in *Aspergillus fumigatus* encodes a cryptic terpene cyclase involved in the formation of β-trans-bergamotene. J Am Chem Soc 135:4616–4619

Lin H, Tsunematsu Y, Dhingra S, Xu W, Fukutomi M, Chooi YH, Cane DE, Calvo AM, Watanabe K, Tang Y (2014) Generation of complexity in fungal terpene biosynthesis: discovery of a multifunctional cytochrome P450 in the fumagillin pathway. J Am Chem Soc 136:4426–4436

Liu X, Walsh CT (2009) Cyclopiazonic acid biosynthesis in *Aspergillus* sp.: characterization of a reductase-like R* domain in cyclopiazonate synthetase that forms and releases cyclo-acetoacetyl-L-tryptophan. Biochemistry 48:8746–8757

Löw I, Jahn W, Wieland T, Sekita S, Yoshihira K, Natori S (1979) Interaction between rabbit muscle actin and several chaetoglobosins or cytochalasins. Anal Biochem 95:14–18

Ma SM, Li JWH, Choi JW, Lee KKM, Moorthie VA, Xie X, Kealey JT, Da Silva NA, Vederas JC, Tang Y (2009) Complete reconstitution of a highly reducing iterative polyketide synthase. Science 326:589–592

Meyer V, Arentshorst M, El-Ghezal A, Drews AC, Kooistra R, van den Hondel CA, Ram AF (2007) Highly efficient gene targeting in the *Aspergillus niger kusA* mutant. J Biotechnol 128:770–775

Nakazawa T, Ishiuchi K, Sato M, Tsunematsu Y, Sugimoto S, Gotanda Y, Noguchi H, Hotta K, Watanabe K (2013) Targeted disruption of transcriptional regulators in *Chaetomium globosum* activates biosynthetic pathways and reveals transcriptional regulator-like behavior of aureonitol. J Am Chem Soc 135:13446–13455

Ninomiya Y, Suzuki K, Ishii C, Inoue H (2004) Highly efficient gene replacements in *Neurospora* strains deficient for nonhomologous end-joining. Proc Natl Acad Sci U S A 101:12248–12253

Oikawa H (2010) Diels-Alderases. In: Liu H-W, Mander L (eds) Comprehensive natural products II, vol 8. Elsevier, Oxford, pp 277–314

Oikawa H, Katayama K, Suzuki Y, Ichihara A (1995) Enzymatic activity catalyzingexo-selective Diels–Alder reaction in solanapyrone biosynthesis. J Chem Soc Chem Commun 13:1321–1322.

Pahirulzaman KA, Williams K, Lazarus CM (2012) A toolkit for heterologous expression of metabolic pathways in *Aspergillus oryzae*. Methods Enzymol 517:241–260

Preiswerk N, Beck T, Schulz JD, Milovník P, Mayer C, Siegel JB, Baker D, Hilvert D (2014) Impact of scaffold rigidity on the design and evolution of an artificial Diels-Alderase. Proc Natl Acad Sci U S A 111:8013–8018

Primm TP, Franzblau SG (2007) Recent advances in methodologies for the discovery of antimycobacterial drugs. Curr Bioact Compd 3:201–208

Qiao K, Chooi YH, Tang Y (2011) Identification and engineering of the cytochalasin gene cluster from *Aspergillus clavatus* NRRL 1. Metab Eng 13:723–732

Sato M, Yagishita F, Mino T, Uchiyama N, Patel A, Chooi YH, Goda Y, Xu W, Noguchi H, Yamamoto T, Hotta K, Houk KN, Tang Y, Watanabe K (2015) Involvement of lipocalin-like CghA in decalin-forming stereoselective intramolecular [4+2] cycloaddition. Chembiochem 16:2294–2298

Scherlach K, Boettger D, Remme N, Hertweck C (2010) The chemistry and biology of cytochalasans. Nat Prod Rep 27:869–886

Schlingmann G, Taniguchi T, He H, Bigelis R, Yang HY, Koehn FE, Carter GT, Berova N (2007) Reassessing the structure of pyranonigrin. J Nat Prod 70:1180–1187

Schümann J, Hertweck C (2007) Molecular basis of cytochalasan biosynthesis in fungi: gene cluster analysis and evidence for the involvement of a PKS-NRPS hybrid synthase by RNA silencing. J Am Chem Soc 129:9564–9565

Sims JW, Schmidt EW (2008) Thioesterase-like role for fungal PKS–NRPS hybrid reductive domains. J Am Chem Soc 130:11149–11155

Sims JW, Fillmore JP, Warner DD, Schmidt EW (2005) Equisetin biosynthesis in *Fusarium heterosporum*. Chem Commun 2:186–188

Song Z, Bakeer W, Marshall JW, Yakasai AA, Khalid RM, Collemare J, Skellam E, Tharreau D, Lebrun MH, Lazarus CM, Bailey AM, Simpson TJ, Cox RJ (2015) Heterologous expression of the avirulence gene *ACE1* from the fungal rice pathogen *Magnaporthe oryzae*. Chem Sci 6:4837–4845

Tsunematsu Y, Ichinoseki S, Nakazawa T, Ishikawa N, Noguchi H, Hotta K, Watanabe K (2012) Overexpressing transcriptional regulator in *Chaetomium globosum* activates a silent biosynthetic pathway: evaluation of shanorellin biosynthesis. J Antibiot (Tokyo) 65:377–380

Tsunematsu Y, Ishikawa N, Wakana D, Goda Y, Noguchi H, Moriya H, Hotta K, Watanabe K (2013) Distinct mechanisms for spiro-carbon formation reveal biosynthetic pathway crosstalk. Nat Chem Biol 9:818–825

Tsunematsu Y, Fukutomi M, Saruwatari T, Noguchi H, Hotta K, Tang Y, Watanabe K (2014) Elucidation of pseurotin biosynthetic pathway points to trans-acting *C*-methyltransferase: generation of chemical diversity. Angew Chem Int Ed 53:8475–8479

Weidner G, d'Enfert C, Koch A, Mol PC, Brakhage AA (1998) Development of a homologous transformation system for the human pathogenic fungus *Aspergillus fumigatus* based on the *pyrG* gene

encoding orotidine 5′-monophosphate decarboxylase. Curr Genet 33:378–385

Wiemann P, Guo CJ, Palmer JM, Sekonyela R, Wang CC, Keller NP (2013) Prototype of an intertwined secondary-metabolite supercluster. Proc Natl Acad Sci U S A 110:17065–17070

Xu Y, Yamamoto N, Janda KD (2004) Catalytic antibodies: hapten design strategies and screening methods. Bioorg Med Chem 12:5247–5268

Yamamoto T, Tsunematsu Y, Noguchi H, Hotta K, Watanabe K (2015) Elucidation of pyranonigrin biosynthetic pathway reveals a mode of tetramic acid, fused γ-pyrone and exo-methylene formation. Org Lett 17:4992–4995

Zou Y, Xu W, Tsunematsu Y, Tang MC, Watanabe K, Tang Y (2014) Methylation-dependent acyl transfer between polyketide synthase and nonribosomal peptide synthetase modules in fungal natural product biosynthesis. Org Lett 16:6390–6393

Biosynthesis of Fungal Polyketides

Russell J. Cox[1], Elizabeth Skellam[1], Katherine Williams[1]

CONTENTS

I. Introduction

Fungal polyketides are a large and diverse group of compounds united by a common biosynthetic origin, whereby the carbon backbone of a polyketide is biosynthesised from the condensation of simple acyl CoA thioesters. Diversity is introduced into the polyketide structure through differences in starter and extender units, chain length, level of reduction, methylation pattern and release mechanism and through modifications introduced by tailoring enzymes that can 'decorate' and rearrange the polyketide backbone. Examples of fungal polyketides include lovastatin **1** (Alberts et al. 1980), the progenitor for statin pharmaceuticals, citrinin **2** (Hetherington and Raistrick 1931), a mycotoxin and the tropolones, for example, puberulic acid **3** (Birkinshaw and Raistrick 1932) which possesses potent antiplasmodial activity (Fig. 1).

Despite the wide range of bioactivity shown by fungal natural products, the percentage of fungal compounds employed in healthcare is low (Bérdy 2005). This is due to a number of difficulties inherent in the commercialisation of fungal natural products, for example, the native producer can be difficult to culture or produces the compound in low titres; additionally the compounds themselves may have many chiral centres, complex ring structures and specific stereochemistries, which make them hard to produce synthetically (Feher and Schmidt 2003). Genetic, biochemical and chemical characterisation of fungal natural product pathways can further our understanding of these complex compounds and provide us with new routes towards their application.

The basis of our understanding of fungal polyketide biosynthesis is founded upon classical radioisotope experiments coupled with degradation studies and subsequently upon stable isotope feeding studies coupled with NMR (Birch et al. 1955; Simpson 1998). Since the 1990s, molecular methods such as cloning and PCR, and more recently, genome sequencing, have allowed a much deeper understanding of the biosynthesis of these complex molecules,

[1] Institute for Organic Chemistry and Biomolekulares Wirkstoffzentrum (BMWZ), Leibniz Universität Hannover, Hannover, Germany; e-mail: russell.cox@oci.uni-hannover.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

demonstrating that almost all fungal polyketides are synthesised by iterative, multidomain polyketide synthases (PKS). Despite these advances, much is still to be understood, particularly regarding the cryptic programming present within the PKS enzyme and how this programming can create such great diversity (Cox 2007).

II. Biological Activity

Fungal polyketides are as diverse in their bioactivities as they are in their structures. They range from the pharmaceutically relevant drugs lovastatin [Mevacor] **1**, an orally active cholesterol-lowering agent (Alberts et al. 1980), and griseofulvin **4**, an orally administered antifungal drug used in animals and humans (Goldman et al. 1960), to one of the most potent natural carcinogenic compounds known, aflatoxin B1 **5** (Squire 1981).

Despite fungal polyketides being infamous mycotoxins, they display distinct promise in clinical settings. For example, squalestatin S1 **6**, isolated from *Phoma* sp., is a potent and selective inhibitor of squalene synthase, a key enzyme in cholesterol biosynthesis (Baxter et al. 1992), with additional activity in protecting against prion neurotoxicity (Bate et al. 2004). In fact, entire classes of fungal polyketide compounds such as the strobilurins, sorbicillinoids, cytochalasans, and resorcylic acid lactones [RALs] have demonstrated interesting biological activities (vide infra) (Fig. 2).

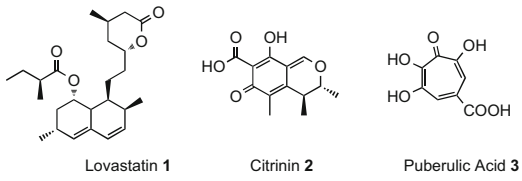


Fig. 1 Typical fungal polyketides

The strobilurins are a group of compounds used in agriculture as QoI fungicides which interfere with energy production in the fungal cell by preventing ATP formation. These natural fungicides were improved with chemical modifications to reduce their degradation by sunlight, e.g. strobilurin A **7**, isolated from *Strobilurus tenacellus* (Anke et al. 1977), which was the lead compound for the development of kresoxim-methyl **8** and azoxystrobin **9**, two of the most commercially successful agricultural fungicides (Fig. 3).

The unique structural features of the sorbicillinoids, as exemplified by trichodimerol **10**, are attractive candidates for developing new anticancer and antioxidant agents. Trichodimerol **10**, first isolated from *Trichoderma longibrachiatum* (Andrade et al. 1992), is also a potent inhibitor of tumour necrosis factor-α which has been implicated in a diverse range of inflammatory, infectious and malignant conditions (Warr et al. 1996; Bradley 2008) (Fig. 4).

The cytochalasans exert a wide range of biological effects, and as microfilament-targeting molecules, they interfere with several cellular processes such as cytokinesis, intracellular motility as well as exo- and endocytosis (Foissner and Wasteneys 2007; Berger et al. 1997; Hirose et al. 1990; Peterson and Mitchison 2002). For example, cytochalasin B **11** slows down the elongation of actin filaments, and since actin is a major component of the cells of amoebae, cytochalasin B is able to significantly inhibit the growth of *Entamoeba invadens*, a reptile parasite (Makioka et al. 2004). Other positive activities of cytochalasin B include its ability to interfere with the monosaccharide transport systems, its inhibition of thyroid secretion and release of growth hormones and its inhibition of the biosynthesis of

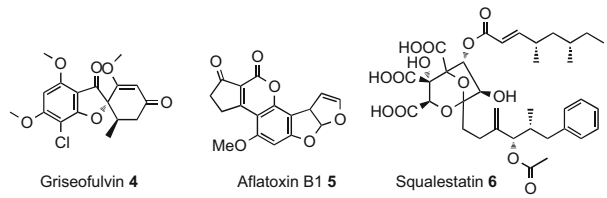


Fig. 2 Bioactive fungal polyketides

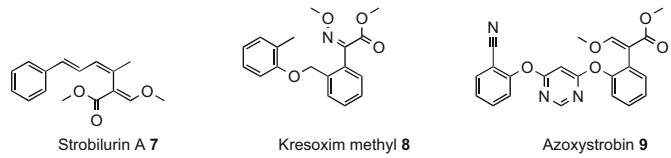


Fig. 3 Strobilurin A and its commercially developed derivatives

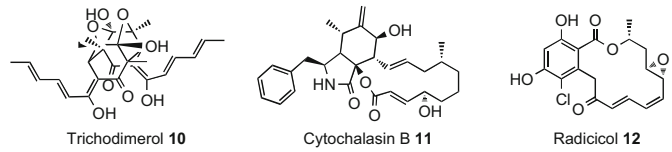


Fig. 4 Biologically active fungal polyketides

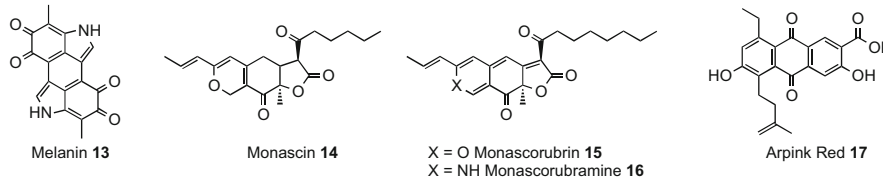


Fig. 5 Examples of some fungal pigments

phosphatidylcholine and phosphatidylethanolamine (Rampal et al. 1998; Schofield 1971; Williams and Wolff 1971; George et al. 1991).

The resorcylic acid lactone (RAL) family of polyketides have quite diverse biological activities considering the small changes in functional groups. Several RALs inhibit kinases and ATPase, which interferes with gene expression, cell growth and apoptosis (Winssinger and Barluenga 2007). Radicicol **12**, isolated from *Monosporium bonorden*, was originally reported to have moderate antibiotic activity as well as a mild sedative activity (McCapra et al. 1964); however, it is most well known as a potent and selective inhibitor of HSP90 (Schulte et al. 1998; Sharma et al. 1998), a molecular chaperone responsible for the maturation and stability of a number of oncogenic proteins (Fig. 4).

Fungal polyketides can also function as pigments, for example, melanin **13**, which is derived from the polyketide pathway. Studies of melanisation in *Cryptococcus neoformans* demonstrated that melanin promotes resistance to UV light, temperature extremes, enzymatic degradation and toxic heavy metals, indicating the role of melanin as a virulence factor in fungi (Gomez and Nosanchuk 2003). The azaphilone pigments, produced by *Monascus* species (Jung et al. 2003), have been commercially available as food colourants in Southeast Asia for hundreds of years (Dufosse 2006). These *Monascus* pigments include monascin **14** (yellow), monascorubrin **15** (orange) and monascorubramine **16** (purple-red); however, there is still controversy over their use in other parts of the world due to the co-production of the mycotoxin citrinin **2** (Blanc et al. 1995). Arpink Red **17** is a red anthraquinone pigment, first isolated from *Penicillium oxalicum* var. Armeniaca CCM 8242. In 2002, Arpink Red was approved for commercial use as a food dye, and it was also shown to display anticancer effects. Nowadays *P. oxalicum* var. Armeniaca CCM 8242 is used to produce an anticancer bioactive supplement under the brand name Penoxal (Fig. 5).

Mycotoxins are toxins produced by fungi with low molecular weights and which are toxic to vertebrates and other animal groups, in low concentrations (Bennett and Klich 2003). Mycotoxin-producing fungi can grow on a

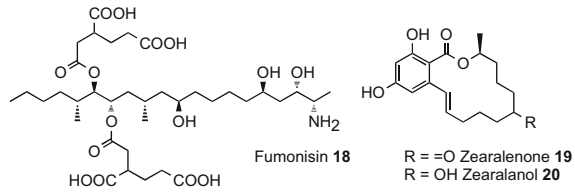


Fig. 6 Examples of some fungal polyketide mycotoxins

wide spectrum of food stuffs and can invade the food supply at any time during production, processing, transport or storage.

Aflatoxins, e.g. **5**, are produced mainly by *Aspergillus* sp., and human exposure to these toxins is typically through ingestion of contaminated food stuffs such as corn, peanuts and cotton seed (Williams et al. 2004). However, aflatoxins can also be detected in animal food products, e.g. meat, milk, cheese, etc. due to the animal's consumption of aflatoxin-contaminated feed (van Egmond 1989). Aflatoxins are most commonly known for causing acute or chronic liver disease but also display immunosuppressive, hepatotic, mutagenic, teratogenic and carcinogenic properties (Chao et al. 1991; Patten 1981).

Citrinin **2** is produced mainly by *Penicillium*, *Aspergillus* and *Monascus* species and can be found in contaminated wheat, oats, rye, corn, barley and rice (Abramson et al. 2001). While citrinin displays antibiotic and insecticidal activities, it also acts as a nephrotoxin (Dobias et al. 1980; Braunberg et al. 1992). The presence of citrinin in *Monascus* species cultures producing the azaphilone pigments is considered too great a risk to approve these *Monascus* pigments as food colourants in the European Union and the United States (Dufosse et al. 2014).

Fumonisins, e.g. **18**, are commonly found in corn, rice and maize and are produced by a number of *Fusarium* species. Fumonisins affect animals in different ways and are known to cause leukoencephalomalacia (hole in the head syndrome; Marasas et al. 1988) pulmonary oedema and hydrothorax (Harrison et al. 1990), as well as being hepatotoxic and carcinogenic (Gelderblom et al. 1988), while in humans they have a role in causing oesophageal cancer (Sydenham et al. 1991).

Fusarium species are also responsible for the production of zearalenone **19**, often considered a mycotoxin. However, although zearalenone **19** has potent bioactivity as a nonsteroidal oestrogen, it is not toxic. Adverse effects include disrupted conception, abortion and reproductive problems in farm animals (Kurtz and Mirocha 1978; El-Nezami et al. 2002). Zearalanol **20**, the reduced form of zearalenone **19**, has increased estrogenic activity and has been marketed successfully as Ralgro for use as an anabolic agent for cattle (Hodge et al. 1966) (Fig. 6).

III. Bioinformatics

With the advent of modern molecular techniques in the 1980s, the first PKS cluster [the *act* cluster] was cloned and shown to reproduce the biosynthesis of the polyketide, actinorhodin, in a heterologous host, *Streptomyces parvulus* (Malpartida and Hopwood 1984). Not long after, the first fungal PKS gene (6-MSAS) which synthesises 6-methyl salicylic acid (6-MSA) **21** was identified and sequenced from *Penicillium patulum* (Beck et al. 1990). Interestingly, comparison of 6-MSAS to known PKSs and FASs from bacteria showed limited sequence similarity, with much higher homology between 6-MSAS and vertebrate fatty acid synthase (FAS). In contrast to many bacterial Type I PKS, fungal Type I PKS are (to date) iterative (Beck et al. 1990). The intrinsic cryptic programming present in these enzymes has become the subject of much investigation (Fisch et al. 2011; Newman et al. 2014; Crawford et al. 2008; Liu et al. 2011, 2014; Huitt-Roehl et al. 2015; Xu et al. 2013a, b, 2014). The sequencing of further fungal PKS genes allowed them to be grouped into three different classes, the non-reducing (nrPKS), the partially reduc-

ing (prPKS) and the highly reducing (hrPKS) (Bingle et al. 1999; Nicholson et al. 2001). Later large-scale phylogenetic analyses have strongly supported this categorisation, although it is now thought that prPKSs are in fact a subclade of hrPKSs (Kroken et al. 2003; Brown and Proctor 2016). Also within the hrPKS category are the PKS-NRPS hybrids which synthesise a polyketide chain linked to an amino acid (Kroken et al. 2003).

Since the first fungal genome was sequenced in 2003 (Galagan et al. 2003), the number of fungal genomes available has increased exponentially. This is particularly promoted by sequencing initiatives such as '1000 Fungal Genome Project' at the JGI (Nordberg et al. 2014). Additionally, next-generation sequencing technology makes it feasible for individual laboratories to cost-effectively sequence fungal genomes for biosynthetic gene cluster (BGC) discovery (Fig. 7; van Dijk et al. 2014; Cacho et al. 2015). This is supported by the convenience of GUI (graphical user interface) software such as CLC Workbench that can allow non-experts to easily assemble raw sequence information into contigs or scaffolds.

The ready availability of fungal genome data can allow researchers to access BGCs encoding polyketide-based compounds without undertaking laborious library screening or chromosome walking experiments. However, the identification of a BGC of interest from whole genome data can be an intimidating task. Online software such as SMURF (Khaldi et al. 2010) or antiSMASH (Medema et al. 2011) can vastly speed up analysis of genome data by identifying putative BGCs (Fedorova et al. 2012). SMURF is specifically targeted at fungal genomes but requires gene co-ordinates as well as protein sequences as inputs. antiSMASH can analyse both bacterial and fungal genomes and can accept a nucleotide FASTA file as an input. Recent updates to antiSMASH (Weber et al. 2015) and to its sister data repository MIBiG (Medema et al. 2015; Li et al. 2016) have radically improved its ability to compare raw genome data to both publically available genomes and previously characterised BGCs. Therefore, antiSMASH can allow a researcher to identify the number of polyketide BGCs present within their genome of interest and to rapidly narrow down their target clusters through comparisons to known BGCs and/or comparisons to publically available genomes. antiSMASH also makes predictions about the boundaries of a BGC, can annotate the tailoring enzymes surrounding a PKS and can predict domains within a PKS. These results can be downloaded as a GenBank file for further analysis. For example, FGENESH (Solovyev et al. 2006) can be utilised for fungal-specific intron prediction within the identified target BGC, which can then allow more accurate function prediction using tools such as InterPro (Hunter et al. 2012).

It can be particularly useful to perform homology comparisons between clusters that are likely to contain similar sets of genes, i.e. for BGCs where a section of biosynthetic pathway is common (Williams et al. 2016). Artemis Comparison Tool [ACT] can visualise comparisons between two or more sequences and can be utilised up to whole genome scale, which makes it especially useful for BGC comparisons (Carver et al. 2005). Coupling the output from antiSMASH/MIBiG with multiple comparisons, using ACT may help to identify cluster boundaries and/or sets of genes responsible for specific biosynthetic steps (Fig. 7).

As mentioned above, antiSMASH can identify specific catalytic domains present within a PKS enzyme (Weber et al. 2015). There are several differences between the domain architecture of nrPKSs, prPKSs and hrPKSs which partially explains some level of programming within fungal PKSs (Cox 2007). Other methods exist for both identifying domains and domain boundaries, for example, by using InterPro (Hunter et al. 2012), by comparison to the mammalian FAS [where a crystal structure is known] (Maier et al. 2008) or the use of the Udwary-Merski algorithm (Udwary et al. 2002). It is essential to predict the correct domain boundaries for producing active chimaeric enzymes containing domain swaps and experiments which can produce novel 'unnatural' compounds and reveal insights into the programming of fungal PKSs (Xu et al. 2013a; Liu et al. 2011; Fisch et al. 2011).

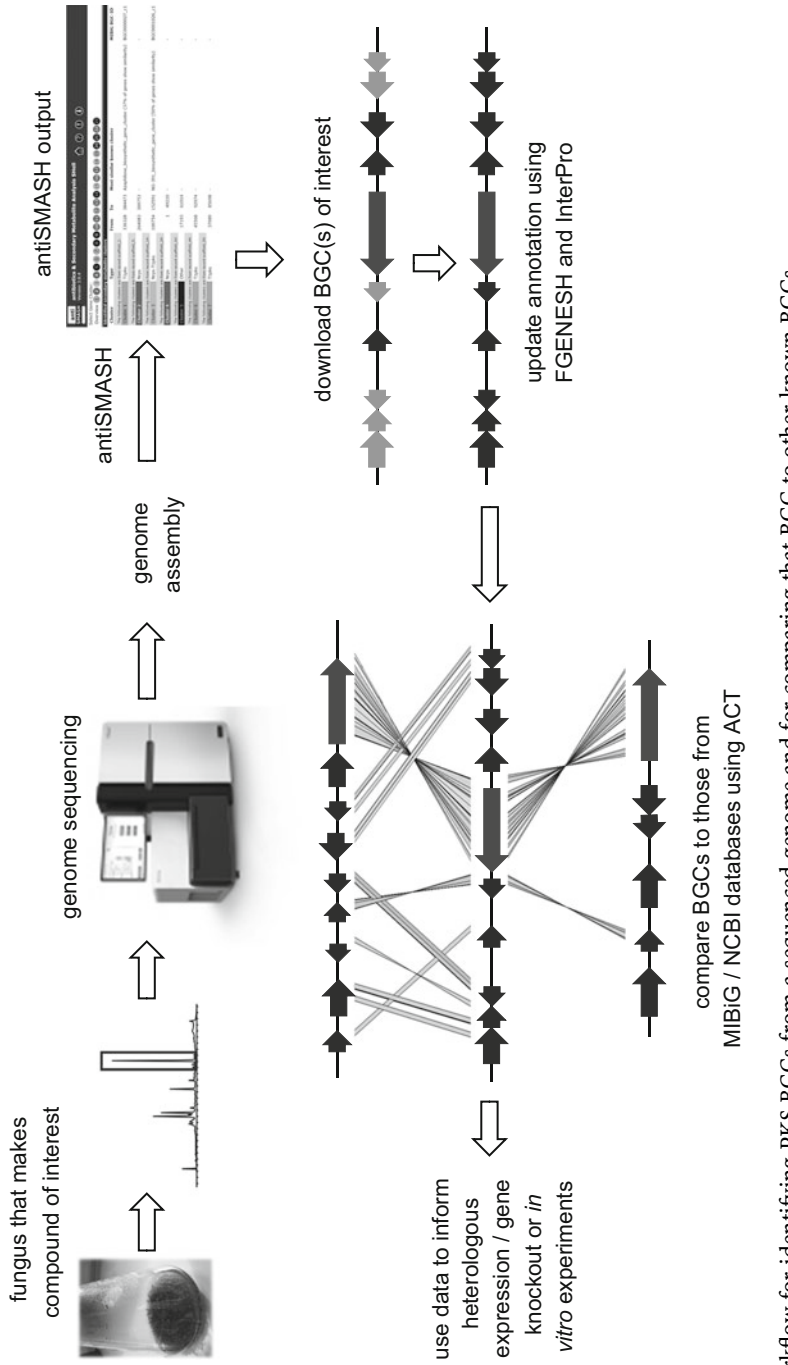


Fig. 7 Workflow for identifying PKS BGCs from a sequenced genome and for comparing that BGC to other known BGCs

Although the identification of the domains present within a fungal PKS, along with phylogenetic analysis, can categorise the type of PKS, for the most part, these analyses cannot provide concrete predictions about the structure of the compound that is biosynthesised. This is in contrast to bacterial modular Type I PKSs where one-to-one colinearity of PKS domains and biosynthetic steps can allow highly accurate prediction of the compound that is produced. Additionally, for bacterial acyl transferase (AT) domains, online software can predict substrate specificity (this functionality has been integrated into antiSMASH), but similar predictivity is not yet available for fungal systems (Weber et al. 2015; Yadav et al. 2003; Minowa et al. 2007).

Despite lagging behind analyses available for bacterial PKSs, tools are beginning to improve for fungal PKS compound prediction, for example, phylogenetic analysis and structural modelling of 661 product template (PT) domains (these domains mediate the regioselective cyclisation of the polyketide backbone) from both characterised and uncharacterised nrPKSs allowed the PT domains to be categorised into eight major groups. Each group is associated with a specific regioselective cyclisation mode and also with specific substrate sizes (Liu et al. 2015). This may allow the prediction of the structural type of aromatic polyketide produced by an uncharacterised nrPKS by sequence information alone. A similar analysis has been conducted for fungal ketosynthase (KS) domains, whereby structural modelling demonstrated that the number of iterations catalysed by the KS may be controlled by key residues within the active site pocket as well as the active site cavity volume (Yadav et al. 2009). However for hrPKS-NRPSs, at least, it has been demonstrated that the ketoreductase (KR) domain, not the KS domain, is likely to be responsible for the chain length of the polyketide portion of the molecule (Fisch et al. 2011).

Prediction of the amino acid that is incorporated into hybrid fungal polyketide non-ribosomal peptide compounds is also an important step towards in silico prediction of the compound produced. Several tools have been developed that can predict the specificity of the adenylation (A) domain from bacterial NRPSs (Stachelhaus et al. 1999; Challis et al. 2000); however, these are generally not reliable for fungal A domains in either PKS-NRPS or NRPS systems. Recent work has developed tools that have some applicability to fungal A-domains, but further research is required for highly accurate prediction (Roettig et al. 2011; Khayatt et al. 2013; Lee et al. 2015).

IV. Types of Fungal PKS

Observations of the chemical structures of fungal polyketides suggested that they fell into two broad chemical categories: aromatic (often multicyclic) and aliphatic (cyclic or acyclic) compounds. These ideas were refined and linked to the domain structures of the different types of PKS as non-reducing PKS (nrPKS) where there are no reductive steps during chain construction, partially reducing PKS (prPKS) where there are limited (in practice usually only one) reductions during chain extension and highly reducing PKS (hrPKS) where the level of reduction is varied and clearly subject to a high level of programming control (Fig. 8; Nicholson et al. 2001). Almost all fungal PKS currently known are Type I systems, but they differ from their Type I bacterial counterparts in being iterative. They also differ in the mode of methylation—while bacterial systems can incorporate methylmalonate as an extender unit, fungal systems known to date exclusively methylate growing chains at the β-dicarbonyl stage, using a *C*-methyl transferase (*C*-MeT) catalytic domain which requires *S*-adenosyl methionine (SAM) as its cofactor. Fungi also possess Type III PKS (Seshime et al. 2005).

A. nrPKS

These enzymes usually consist of an N-terminal starter unit acyl transferase (SAT) domain followed by a ketoacyltransferase (KS), acyl transferase (AT), product template (PT) and acyl carrier protein (ACP) domains. These are the minimal domains required for chain

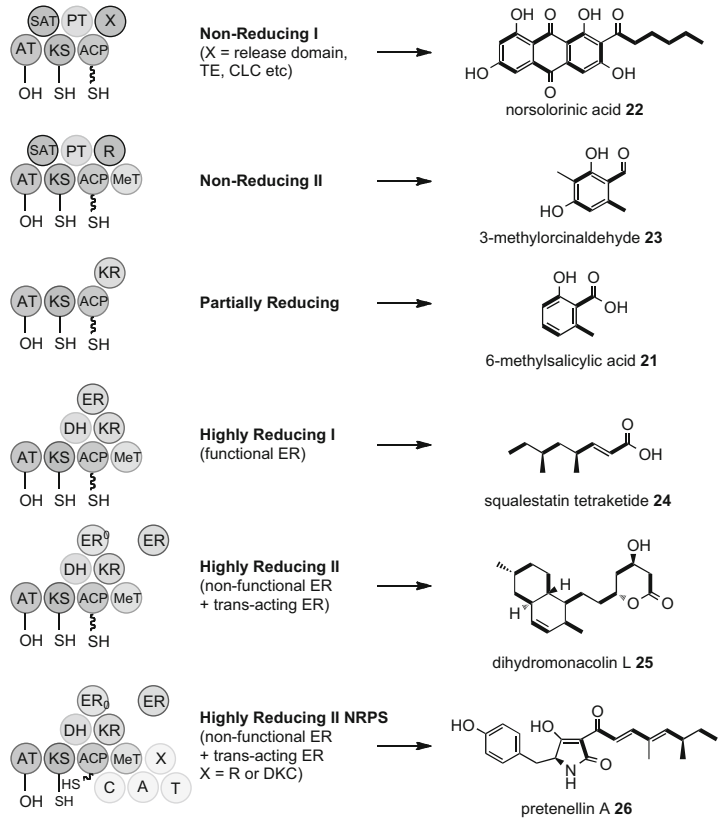


Fig. 8 Types of fungal PKS domain structures: *SAT* starter unit acyl transferase, *KS* β-ketoacyl synthase, *AT* acyl transferase, *ACP* acyl carrier protein, *PT* product template, TE thioesterase, *CLC* Claisen cyclase, *R* reductive release domain, *MeT* *C*-methyl transferase, *KR* keto reductase, *DH* dehydratase, *ER* enoyl reductase, ER^0 non-functional ER, *C* condensation, *A* adenylation, *T* thiolation (peptidyl carrier protein), *DKC* Dieckmann cyclase. Bold lines indicate intact acetate units

construction. The SAT selects and loads the starter unit which can be acetate, a fatty acyl chain or another polyketide (usually provided by another PKS, vide infra); the AT loads the malonate extender units, and the KS catalyses the chain extension of the ACP-bound acyl chain (Cox 2007). The PT domain is responsible for the cyclisation—it probably also forms a protective pocket for the growing poly-β-keto chain which would otherwise be highly unstable and cyclise chaotically. In the simplest nrPKS systems (nrPKS I), there is then a final release domain which is usually hydrolytic or in a common variation, such as that involved in norsolorinic acid biosynthesis, catalyses a Claisen cyclisation (CLC). A typical example of this group is norsolorinic acid **22** synthase (NSAS) involved in the first steps of aflatoxin biosynthesis in *Aspergillus flavus*.

Other nrPKS can have more than one C-terminal domain (nrPKS II). Reductive release is common, and this is known to form aromatic aldehydes. Many non-reduced polyketides are methylated, and in these cases a *C*-MeT domain is located between the ACP and the release domain—evidence suggests that methylation is processive, i.e. occurs as the chain extends. A typical example is that of methylorcinaldehyde **23** synthase (MOS; Bailey et al. 2007, 2010) from *Acremonium strictum* (an nrPKS II).

B. prPKS

Compared to the nrPKS and hrPKS types, prPKSs are rare. Almost all known examples are involved in the synthesis of the tetraketide 6-MSA **21**, although one example involved in biosynthesis of the pentaketide mellein (Sun et al. 2012) is also known. During 6-MSA **21** biosynthesis, a single reduction occurs at the triketide stage catalysed by the KR domain. Although prPKSs are more closely related to hrPKS and vertebrate FAS than they are to nrPKS, they are thought to exist as tetrameric species (Moriguchi et al. 2008).

C. hrPKS

Highly reducing PKSs are also very common in fungi. They are very similar in terms of sequence and domain organisation to the vertebrate FAS (and, in fact, quite different to the fungal FAS; Jenni et al. 2007) Interestingly when the structure of vertebrate FAS was solved (Maier et al. 2008), it became clear that it possessed a vestigial *C*-MeT domain in the same region as the often functional *C*-MeT domains of the hrPKS.

The hrPKS fall into two broad categories, those with functional enoyl reductase (ER) domains (such as squalestatin tetraketide **24** synthase (SQTKS) (Cox et al. 2004) and lovastatin diketide synthase (LDKS) (Hendrickson et al. 1999), hrPKS I) and those with missing or non-functional ER domains such as lovastatin nonaketide synthase (LNKS, which makes **25**; Hendrickson et al. 1999) and the tenellin **26** pentaketide synthase (TENS; Eley et al. 2007)—these are hrPKS II systems. In systems lacking a functional ER, there is often (but not always, e.g. fusarin C; Song et al. 2004) a *trans*-acting ER encoded within the BGC. The domain structure of the PKS usually consists of KS, AT, DH, *C*-MeT and then ΨKR, a structural component of the KR. The ER, when present and functional, is located between the ΨKR and KR sequences, in the gene sequence, and the final domain is usually the ACP. Unlike the nrPKS there is not usually an overt off-loading domain. However in the case of the hrPKS-NRPS hybrids, one can consider the NRPS to be an off-loading unit. The NRPS usually consists of condensation (C), adenylation (A) and thiolation (T, also known as peptidyl carrier protein (PCP)) domains. Final release is often catalysed by a reductive (R) domain, but in some cases this has undergone subtle changes (Liu and Walsh 2009) to become a Dieckmann release domain (DKC; Halo et al. 2008a) which gives acyl tetramic acids such as pretenellin A **26**. The first reported case of a fungal PKS-NRPS was that of fusarin (Song et al. 2004), but other hrPKS-NRPSs make the cytochalasans and the precursors for fungal 2-pyridones (vide infra).

V. Fungal PKS Gene Clusters

As discussed above, freely available software can quickly identify PKS genes in fungal genomes. Typically, most filamentous fungi can contain between 10 and 50 PKS genes, although more is not uncommon. These almost always occur with genes encoding tailoring functions, and clustered genes encoding transport functions and transcription factors are also not unusual, although by no means ubiquitous. While the broad function of the PKS genes can be predicted (i.e. non-reducing, partially reducing, highly-reducing), the function of tailoring genes is much harder to predict. Commercial software packages are also prone to the erroneous determination of gene boundaries from raw genomic data where introns are hard to determine. More accurate BGC determinations can be made where there is good-quality transcriptome or RT-PCR data and ORF positions, and thus automated annotation is more accurate. Similar problems are encountered in determining the boundaries of the entire cluster—since many secondary and primary metabolism proteins are related, it can be hard to determine where a secondary metabolism gene cluster ends. Again good transcriptome data can help to show a cotranscribed region, but this may not correspond precisely to the genes encoding the proteins actually functional during biosynthesis. A good example of this is the aspyridone

cluster in *A. nidulans* (Bergmann et al. 2007). Here Southern blotting identified a set of cotranscribed genes, but when these genes were transferred to the heterologous host *A. oryzae*, it was shown that the *A. nidulans* cluster contains both too many genes (i.e. some genes present were redundant) and too few genes (i.e. some functions were encoded outwith the cluster) (Wasil et al. 2013). Thus, cluster prediction in fungi remains challenging.

One solution to these problems is to examine two or more BGCs which encode the biosynthesis of identical or very similar metabolites in different fungi. The software tool Artemis Comparison Tool makes this approach fairly simple. The assumption that biosynthetic steps for related pathways are conserved in terms of chemistry and order of deployment is almost always valid, and this means that common genes encode common chemical steps. This allows the identification of genes which are common to both clusters to be involved in synthesis of the core metabolite, while genes which do not occur in all of the BGCs under analysis are likely to encode later-stage tailoring steps responsible for molecular differences. These types of analyses allow higher-order classifications of fungal PKS compounds and their associated clusters and also allow inferences about gene transfer and evolution of BGCs to be made, as well as relationships between different compound classes to be observed. The results are especially useful when at least one of the BGCs has already been properly understood by gene knockout (KO) or heterologous expression studies. Although this type of analysis is still mostly done 'by hand', the development of higher-order software tools and repositories such as MBiG will make this analysis easier and more common in the future. As the number of properly investigated clusters increases, these relationships will allow much better prediction of the possible products of fungal polyketide clusters. However, even with the limited number of examples available presently, it is already possible to make a number of interesting and useful observations. The following sections illustrate three examples from the authors' own research, but other structural types such as the xanthones and the resorcylic acid lactones can also be grouped using similar arguments.

A. The Aromatic Aldehyde Family: Oxidised Monoaromatic Polyketides

This family is best exemplified by citrinin **2**, a common product of *Penicillium* and *Monascus* species. The biosynthesis of citrinin has recently been correctly elucidated for the first time through heterologous expression of the whole pathway (He and Cox 2016; Fig. 9). The citrinin PKS is encoded by a typical nrPKS II which consists of SAT, KS, AT, PT, ACP, *C*-MeT and R-domains. The PKS is assisted in an as yet cryptic way by a hydrolase to produce a trimethylated pentaketide which is cyclised and released as an aldehyde by the C-terminal R-domain. The 12-methyl is then systematically oxidised, first to an alcohol by a non-haem iron oxygenase (CitB), then to an aldehyde (oxidoreductase, CitC) and then to a carboxylic acid (aldehyde dehydrogenase, CitD). Finally the 3-ketone is reduced, and the alcohol (CitE) thus formed closes on the aldehyde, and elimination of water forms the quinomethide skeleton of citrinin. Heterologous expression experiments in *A. oryzae* showed that when restricted gene sets are expressed (e.g. lacking the 3-ketone reductase CitE), many shunt compounds are formed—these include azaphilones and their amidated homologs.

In a parallel pathway, another nrPKS (MOS) with a reductive release domain makes the methylated tetraketide aldehyde methylorcinaldehyde **23** (Davison et al. 2012). This electron-rich aromatic is then oxidatively dearomatised by an FAD-dependent oxygenase (TropB), which forms the precursor for known compounds related to leptosphaerone A **27**. Action of a non-haem iron oxygenase analogous to CitB from the citrinin pathway then oxidises the methyl group, but because the ring is now dearomatised, there is an additional oxidative rearrangement (Cox 2014) to give a tropolone. This forms the tropolone series of compounds leading to stipitatic acid **28** via stipitatonic acid **29** (al Fahad et al. 2014a).

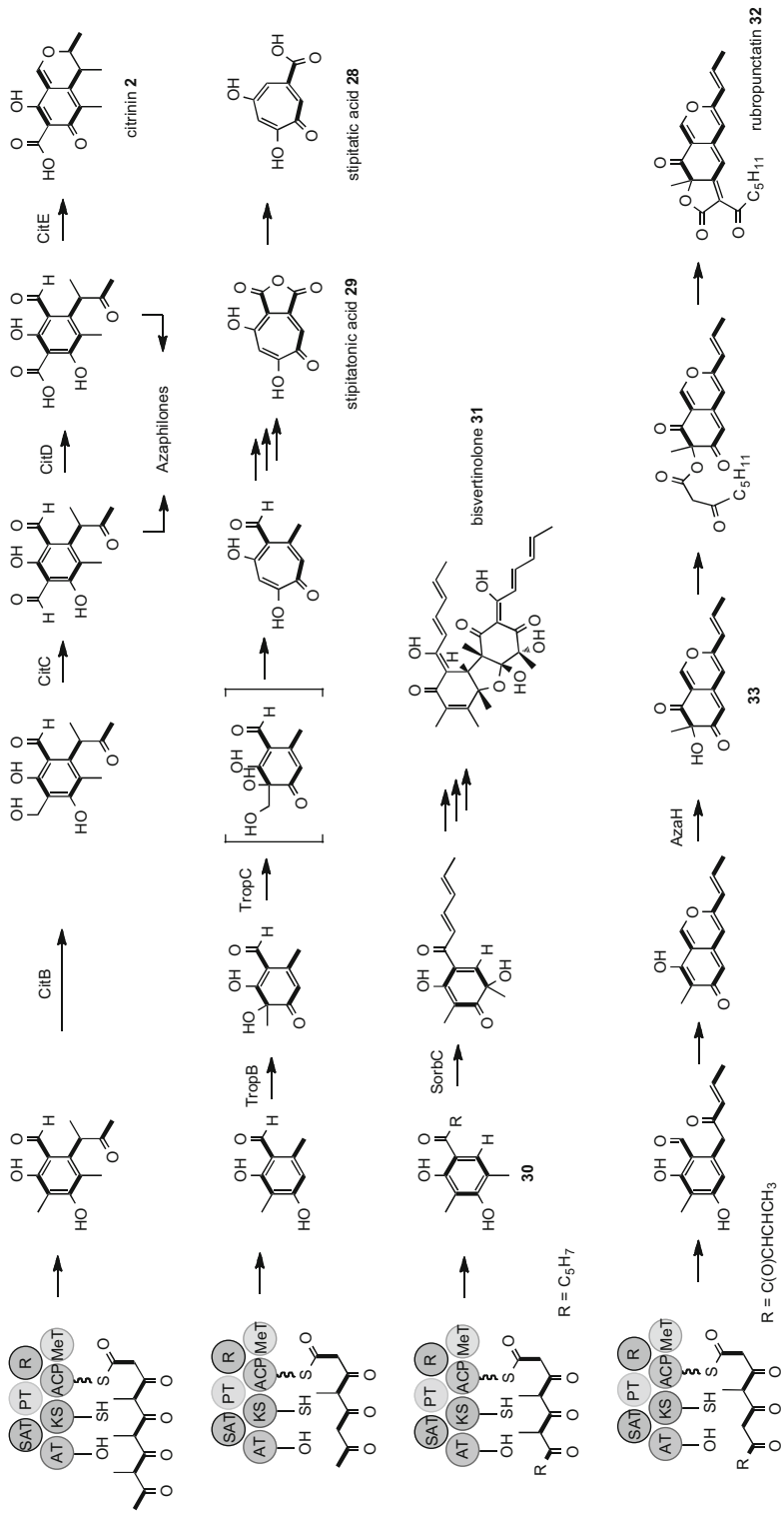


Fig. 9 The aromatic aldehyde family

Another parallel pathway starts with a different non-reduced polyketide. In the case of the sorbicillins (al Fahad et al. 2014b), an hrPKS makes the starter unit for an nrPKS triketide, which is again methylated and released reductively. A different mode of cyclisation leads to another electron-rich aromatic system **30**, and oxidation by the FAD-dependent SorbC, which is a homolog of TropB, then leads to the sorbicillinoid class of compounds exemplified by bisvertinolone **31**.

Finally a very similar pattern is observed during the synthesis of the azaphilones such as rubropunctatin **32**. An nrPKS again makes a methylated aromatic aldehyde—this time as a hexaketide. Once again ring oxidation (by AzaH, homologous to TropB and SorbC) leads to the key intermediate **33**, and reaction with a fatty acid then forms the structurally distinct γ-lactone ring of **32**.

Thus while sequence-based tools are not yet able to determine chain-length, cyclisation or methylation pattern in nrPKS systems, analysis of the clustered genes can give an indication of the class of compound specified by the BGC. The presence of a PKS with a reductive release underpins all of the above classes, but the presence or absence of key oxidase-encoding genes defines the final structural class. Ring oxidation can lead to azaphilones, while methyl oxidation can lead to citrinin-related compounds. The presence of both ring and methyl oxidases then specifies the likely synthesis of tropolones.

B. The Citrate Synthase Family, Maleidrides and Squalestatins

Maleidrides are a family of seven-, eight- and nine-membered carbocyclic compounds characterised by the presence of one or (usually) two maleic anhydride moieties (Szwalbe et al. 2015). Nonadrides and heptadrides (the nine- and seven-membered examples, respectively) are known to be produced by an hrPKS with functional ER domains (Williams et al. 2016). In the case of the nonadride byssochlamic acid **34**, the PKS probably produces an unsaturated triketide. The biosynthesis requires a separate hydrolase activity, and this may be involved in chain release from the PKS. A similar release strategy is used by the brefeldin PKS where a separate hydrolase releases the polyketide at a defined chain length (Zabala et al. 2014). The key reaction in the citrate family of compounds is then catalysed by a citrate synthase-like enzyme to condense the polyketide with oxaloacetate. In the case of maleidride biosynthesis, the reaction is followed by the activity of a dedicated dehydratase with similarity to the primary metabolism enzyme 2-methylcitrate dehydratase, but in other cases, such as that of viridiofungin A **35**, the biosynthesis stops here (Mandala et al. 1997). The precursor of viridiofungin **36** itself is probably the product of a typical fungal PKS-NRPS pathway (Section "C. The PKS-NRPS Family").

Expression of the PKS-, hydrolase-, citrate synthase- and dehydratase-encoding genes leads to the biosynthesis of monomeric precursors such as **37** (Williams et al. 2016). These compounds are well-known metabolites of fungi and are probably the precursors for compounds such as cordyanhydride **38** and related compounds (Isaka et al. 2000). In the case of the maleidrides, enzymes with homology to phosphatidylethanolamine binding proteins (PEBP) and ketosteroid isomerases (KI) catalyse dimerisation to form nonadrides and heptadrides (Williams et al. 2016). Gene clusters containing these genes are well represented in the available fungal genomes, and this reflects the common occurrence of maleidrides.

A third version of this pathway is represented by the squalestatins. These compounds, which are potent inhibitors of squalene synthase, possess a unique highly functionalised 4,8-dioxa-bicyclo[3.2.1]octane core, although many different substituted analogues are known (Bergstrom et al. 1995). Here it appears that a PKS and releasing hydrolase produce the polyketide **39** required by a citrate synthase. No citrate dehydratase is present in the cluster, and so the chemistry takes a different route—successive oxidations lead to the creation of the bicyclo[3.2.1]octane moiety (Bonsch et al. 2016) (Fig. 10).

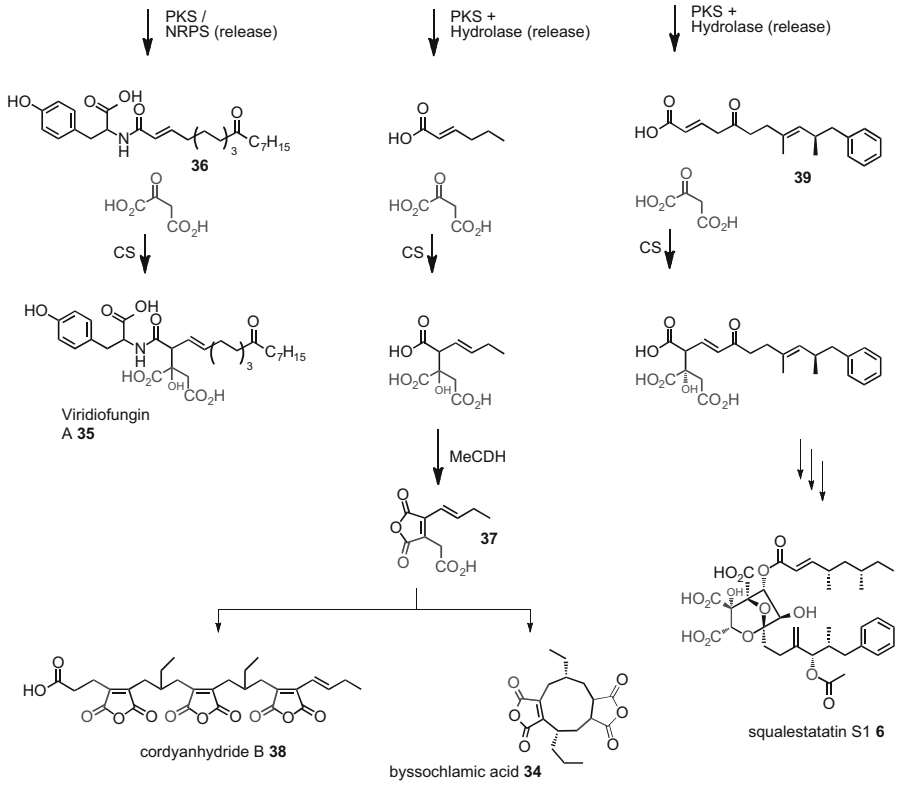


Fig. 10 The citrate family

C. The PKS-NRPS Family

PKS-NRPS hybrids are common in fungi. In the examples known to date, the PKS usually has a non-functional ER (designated ER^0). In cases where a *trans*-acting ER is not present, such as in the fusarin BGC, for example (Song et al. 2004), then a polyunsaturated compound is formed. Two types of release mechanism are known—reduction probably releases an aldehyde, but Dieckmann cyclisation is also common and results in the formation of the more stable acyl tetramic acid class of compounds. As in the previous examples, several variations on this theme lead to the creation of different well-known compound classes.

The first-discovered example was that of fusarin C **40** (Song et al. 2004). Here a PKS-NRPS hybrid, lacking a *trans*-acting ER, makes a polyunsaturated heptaketide fused to homoserine and releases its product as an aldehyde **41** (Song et al. 2004; Rees et al. 2007). A Knoevenagel ring closure creates the pyrrolone core, and this is followed by an as yet undefined series of oxidative reactions to form **40** (Niehaus et al. 2013). The polyunsaturated polyketide is rather unstable, and many *E/Z* isomers are known. This pathway can also lead to the large class of fungal acyl tetramic acids and pyridones, represented by pretenellin A **42** and tenellin **43** (Eley et al. 2007; Halo et al. 2008b, a). The tetramic acid precursors such as **42** are created when the release involves a Dieckmann cyclase (DKC). Oxidations of the tetramic acid can lead to oxidative rearrangement (Cox 2014) to form pyrones, and *N*-hydroxylation and subsequent *O*-methylation are also common pathways.

When the polyketide is longer (octa- and nonaketides) and polyunsaturated, then Diels-Alder (DA) reactions can occur. In the case of equisetin **45,** the PKS-NRPS creates an

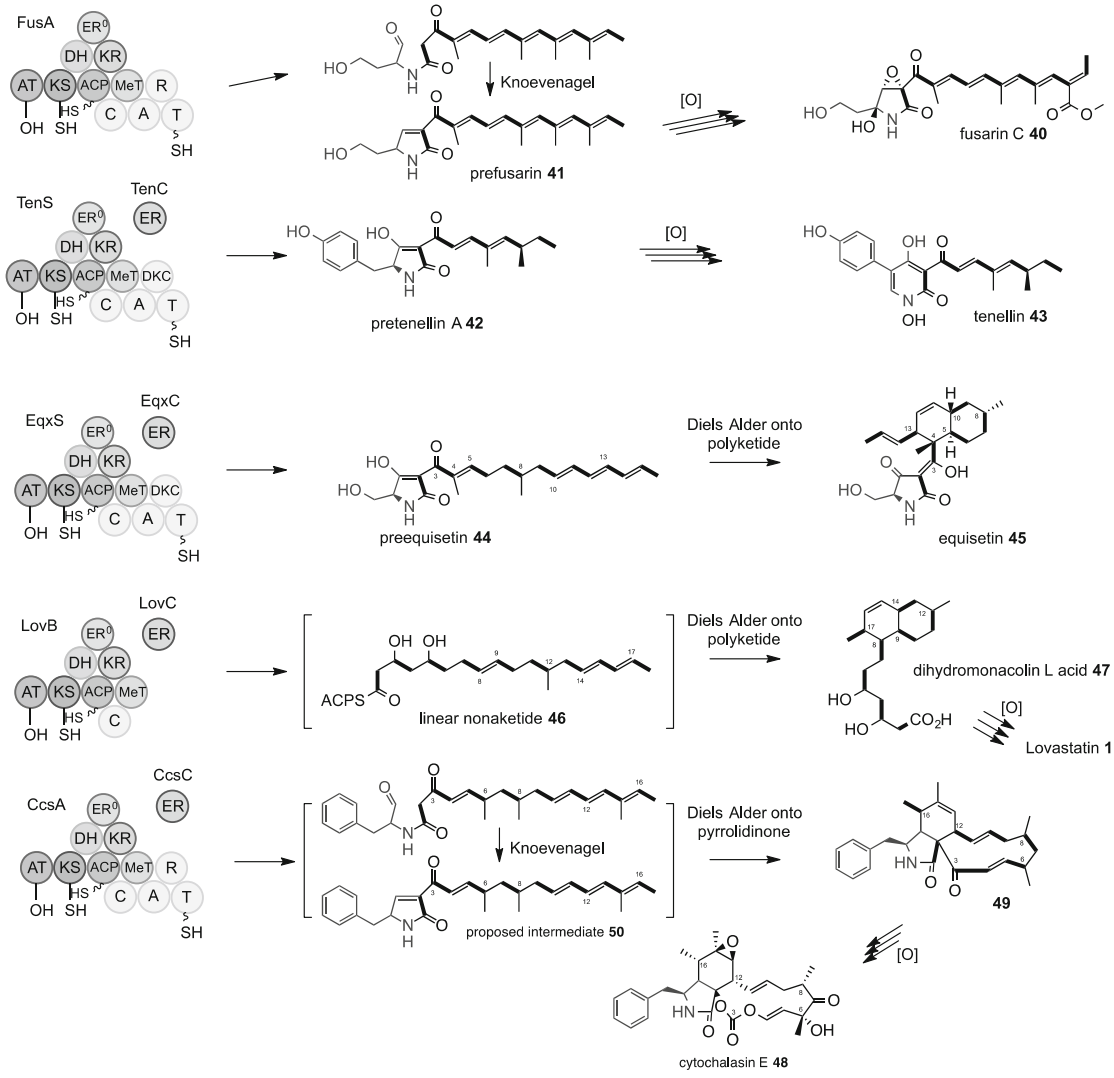


Fig. 11 The PKS-NRPS family. Bold bonds indicate intact acetate

octaketide fused to serine **44**, and the polyketide can undergo a DA reaction to form the trans-decalin of **45**. A further variation of this pathway involves PKS-NRPS systems in which much of the NRPS has been deleted so that no amino acid is added, forming putative intermediates such as **46**. An intra-polyketide Diels-Alder reaction gives the observed **47** which is a precursor of **1**. A good example of this is the lovastatin **1** pathway where a nonaketide is formed by LovB which undergoes a DA reaction (probably *during* its construction) to form the core decalin system of the statins (Hendrickson et al. 1999).

Finally, PKS-NRPS systems produce the precursors for the cytochalasan class of metabolites (Fujii et al. 2013). In these cases the PKS-NRPS probably releases an aldehyde such as **50** which could form the pyrrolone core via a Knoevenagel reaction (Fujii et al. 2013). A diene at the terminus of the polyketide reacts with the pyrrolone in a DA reaction to produce the familiar cytochalasan skeleton **49**, and further oxidations (Hu et al. 2014) would give cytochalasin E **48**. Variations in chain length, methylation pattern and amino acid selection by the NRPS lead to the very wide variety of compounds known in this class (Fig. 11).

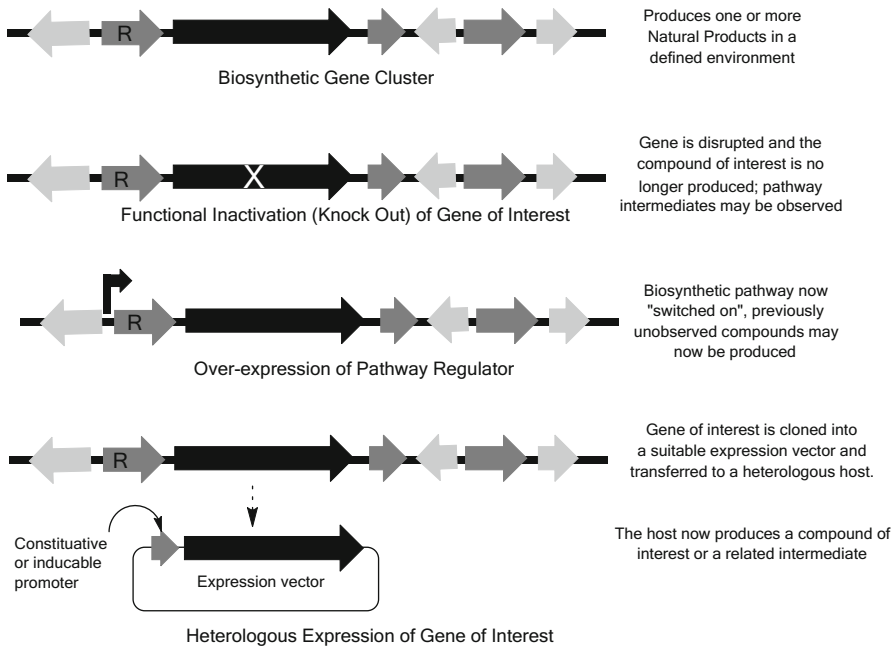


Fig. 12 Techniques for functional characterisation of biosynthetic gene clusters

VI. Engineering Fungal Polyketide Biosynthesis

Although fungal polyketides display a diverse range of bioactivities, only a handful are employed commercially. This in part is due to undesirable biological activities, low titres, complicated culturing procedures, toxicity and solubility issues. The strategy for producing non-native metabolites or 'unnatural' natural products by directly engineering polyketide pathways [combinatorial biosynthesis] is becoming an achievable goal. However, before a pathway can be properly engineered, its function must first be established (Fig. 12).

A. Inactivation of PKS Genes

A common method for determining the function of a gene is by disrupting or replacing the gene, i.e. gene knockout (KO). Unfortunately, in fungi, this procedure can be highly inefficient due to high levels of ectopic integration of the introduced DNA, not to mention limitations in suitable selection markers and difficulties associated with successfully introducing the knockout plasmid or construct (Weld et al. 2006). One of the first fungal PKS genes to be functionally characterised by gene KO was *wA* from *A. nidulans* (Tilburn et al. 1990; Mayorga and Timberlake 1990, 1992). *wA* is involved in the synthesis of a green pigment present in the conidia, and disruption of the gene led to colourless conidia. Since then, KO of fungal PKS genes has become a somewhat routine procedure in elucidating gene function. For example, all 15 PKS genes in *Gibberella zeae* (anamorph *Fusarium graminearum*) were disrupted using a single-crossover integration approach, and the functions of five genes were assigned as being responsible for the biosynthesis of zearalenone **19**, fusarin C **40**, aurofusarin **51** and a black perithecial pigment (Gaffoor et al. 2005).

While gene KO experiments give definitive data on the role of a gene in a pathway, the process is rather laborious and time-consuming and is not always feasible on a broader scale. An alternative is RNA-mediated gene silencing (knockdown), a post-transcriptional process in which double-stranded RNA (dsRNA) triggers the degradation of cognate mRNA in a sequence-specific manner. One of the first fungal PKS genes to be

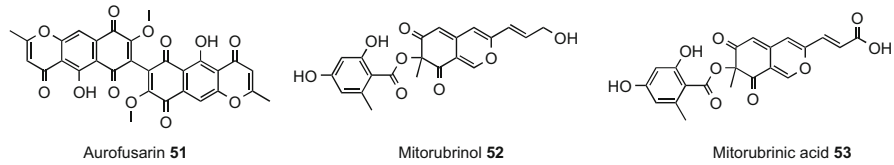


Fig. 13 Examples of some compounds identified through gene inactivation experiments

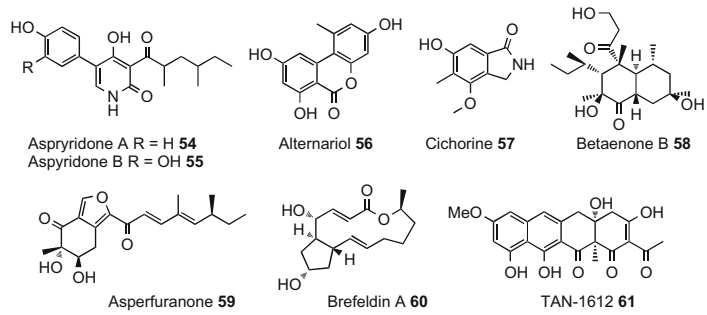


Fig. 14 Examples of some compounds identified by changing pathway regulation and heterologous expression

targeted for knockdown was a gene from *Magnaporthe oryzae*. The targeted gene had homology to melanin biosynthesis genes, and gene knockdown resulted in 34 of 48 transformants displaying a recognisable change in colony colour (Nakayashiki et al. 2005). In *Penicillium marneffei*, knockdown of all 23 PKS genes and 2 PKS-NRPS genes led to the discovery of the PKS genes involved in the biosynthesis of mitorubrinol **52** and mitorubrinic acid **53**, responsible for yellow pigment formation (Woo et al. 2012). While gene silencing has the advantage of being able to reduce gene expression levels relatively quickly and easily, a potential disadvantage is that suppression of gene expression may be partial, and varying degrees of silencing can make experimental results difficult to interpret, and it is not yet clear whether silencing is universally applicable in fungi (Fig. 13).

B. Engineering PKS Pathway Regulation

As the recent advances in genome sequencing have shown, microorganisms possess a far greater wealth of secondary metabolite biosynthesis potential than has been so far discovered using laboratory culturing conditions. Methods have now been developed to 'switch on' these 'silent' clusters to deduce their function. A silent PKS-NRPS cluster in *Aspergillus nidulans* could not be activated by varying the culture conditions alone, so one of the genes in the cluster, *apdR*, believed to be an activator gene, was overexpressed in *A. nidulans*, under the control of the inducible alcohol dehydrogenase promoter *alcAp*, leading to the production and isolation of aspyridones A **54** and B **55** (Bergmann et al. 2007). Other strategies for 'switching on' these silent clusters include using gene targeting to directly replace the promoters of fungal PKS genes. For example, in *A. nidulans*, the eight nrPKS genes without an assigned function had their native promoter replaced with the *alcA* promoter and were grown in inducing conditions. This led to the isolation and characterisation of seven novel compounds and identification of the nrPKS genes required for the biosynthesis of alternariol **56** and cichorine **57** (Ahuja et al. 2012) (Fig. 14).

C. Heterologous Expression

Heterologous expression is another method for deducing the function of an individual gene. This method has additional advantages in that

the heterologous host usually has 'clean' background (devoid of unwanted metabolites); optimal growth conditions for the host are already known; and genes are often expressed at relatively high levels due to careful selection of the promoter driving expression of the fungal gene (s). For example, the 6-MSAS gene has been heterologously expressed in *Aspergillus nidulans* and *Saccharomyces cerevisiae* (Fujii et al. 1996; Yalpani et al. 2001). Heterologous expression of fungal genes in fungal hosts usually overcomes problems associated with intron-splicing and post-translational modifications. *Aspergillus oryzae* has been used to express a number of fungal PKS genes and has been further developed so that it is able to express entire PKS gene clusters, e.g. tenellin **43** (Halo et al. 2008a), aspyridone **54** (Wasil et al. 2013) and betaenone **58** (Ugai et al. 2015). In addition, *A. nidulans* has been developed as a heterologous host for PKS expression; all nrPKS from *A. terreus* have been transferred to *A. nidulans*, along with additional genes for release of the products, allowing identification of six of these genes (Chiang et al. 2013). Furthermore all genes belonging to a cryptic azaphilone cluster were expressed to produce asperfuranone **59**, demonstrating that entire secondary metabolite pathways can be expressed using this system.

Saccharomyces cerevisiae (baker's yeast) is another common choice as a heterologous host as many tools are available for strain engineering, homologous recombination is highly efficient, and secondary metabolism is insignificant and so offers a clean background for metabolite analysis. Challenges of working with yeast as a heterologous host include the inability to splice most fungal introns and low production of necessary polyketide precursors. However, yeast as a heterologous expression host has been used to co-express the hrPKS gene and partnering thiohydrolase gene involved in brefeldin A **60** biosynthesis (Zabala et al. 2014) and characterise a cryptic nrPKS gene from *A. niger* involved in TAN-1612 **61** biosynthesis (Li et al. 2011). Significantly, yeast has been used to efficiently express the *A. terreus* LovB megasynthase to examine the influence of cofactors and its LovC partner on product formation, something not yet achieved in filamentous fungi (Ma et al. 2009).

D. Understanding the Programming of PKS Genes

The generation of novel 'unnatural' natural products via combinatorial biosynthesis has started to gain momentum in the fungal PKS field and has also begun to shed light on the intricate programming concealed within iterative PKS. Two main strategies are commonly used: in vivo domain swapping to generate novel PKS chimaeras and a deconstruction approach where the PKS is dissected into mono-, di- and tridomains which are studied in vitro.

E. Domain Swapping Experiments

The programming of the benzenediol lactone (BDL) PKS SAT domains was investigated by pairwise shuffling of four pairs of BDL hrPKS and nrPKS genes. In these experiments the PKS systems responsible for monocillin **62**, *trans*-resorcylide **63**, desmethyl-lasiodiplodin **64** and 10,11-dehydrocurularin **65** were used. In these systems an hrPKS synthesises a polyketide which acts as the starter unit for an nrPKS. The highly reduced PKS (either a tetraketide **63**, **65** or a pentaketide **62**, **64**) is passed to the SAT domain of the nrPKS which then extends the chain. The nrPKS extends the chain by either three (**64**) or four acetates (**62**, **63**, **65**) and catalyses different ring closures, either 2,7 (**62–64**) or 3,8 (**65**). The nrPKS for **63** and **64** also has the ability to stutter, that is, to produce aberrant longer pentaketide chains. Where an nrPKS was unable to accept the product of the new hrPKS partner, the SAT domain was modified to accept the product of its new partner. From 16 possible combinations including natural occurring pairs, 14 showed the formation of products, some of which (i.e. **66–68**) were novel (Xu et al. 2014; Boecker et al. 2016; Fig. 15).

Domain swaps to investigate the communication between fungal PKS-NRPS modules were achieved by swapping the PKS modules from EqxS, FsdS, Cpa, PsoA and LovB involved in the biosynthesis of equisetin **45**, fusaridione **69**, cyclopiazonic acid **70**, pseurotin **71** and lovastatin **1** nonaketide biosynthesis, respectively (Kakule et al. 2014; Fig. 16). The PKS-NRPS

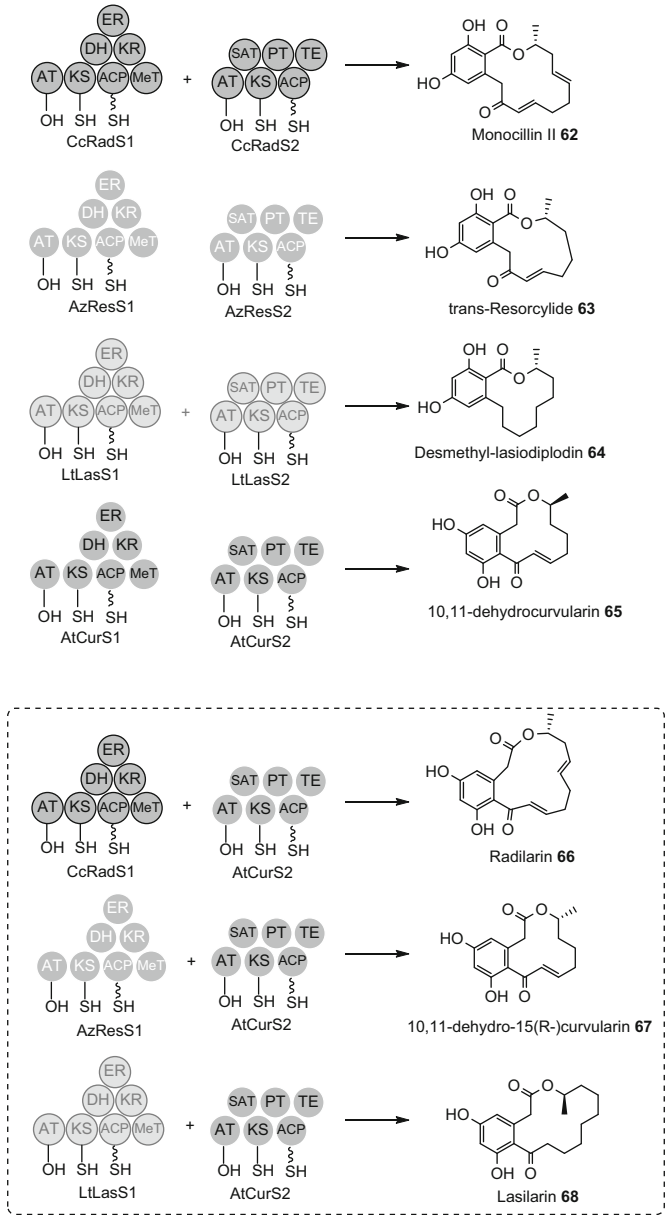


Fig. 15 BDL hrPKS nrPKS swaps. The naturally occurring pairings of the BDLs are shown at the top. Combinatorial biosynthesis of the BDLs, using AtCurS2 as an example of the nrPKS acceptor, (shown underneath), demonstrates how different BDL products were produced

hybrids resulted in the production of six novel polyketide compounds and highlighted the complex interplay between PKS and NRPS domains, as well as the roles of the ACP, C and KR domains for substrate recognition and processing.

Methodology has been developed in *A. nidulans* which allows exchange of the SAT domain from AfoE in the asperfuranone **59** pathway with the SAT domains from ten other nrPKS from *A. nidulans* in vivo, resulting in the generation of a novel aromatic polyketide

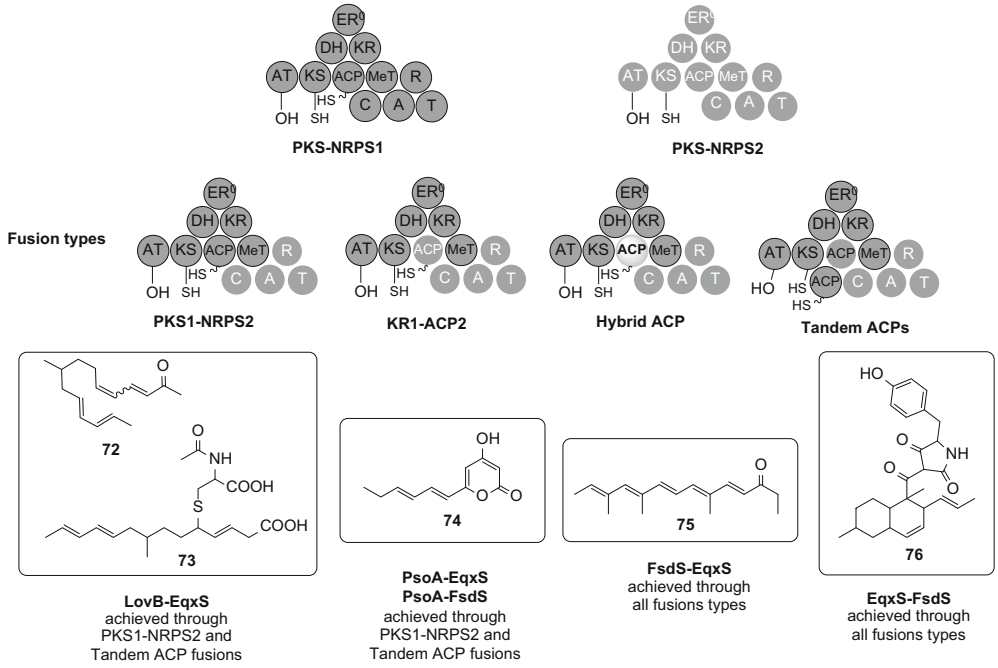


Fig. 16 Domain swap strategy for investigating the programming between fungal PKS-NRPS modules. The new compounds **72–76** generated from the various fusion strategies are shown

product **77** (Liu et al. 2014; Fig. 17a). The PT domains, involved in specific cyclisation cascades, have been investigated by swapping the PT domains between CcRADS2 and AtCURS2, involved in the biosynthesis of monocillin II **78** and 10, 11-dehydrocurvularin **79**, respectively, resulting in the production of two isocoumarins (**80** and **81**) and a unique eight-membered lactone **82** (Xu et al. 2013a; Fig. 17b). Swapping of the terminal release domains has also been successful for the DtbA protein from *A. niger* which naturally produces the two polyketides 2,4-dihydroxy-3,5,6-trimethylbenzaldehyde **85** and 6-ethyl-2, 4-dihydroxy-3, 5-dimethylbenzaldehyde **84**. Swapping the naturally occurring R domain for a TE release domain led to the production of the two corresponding carboxylic acids **86** and **87** (Yeh et al. 2013; Fig. 17c).

Rational domain swaps between TENS and DMBS, responsible for the biosynthesis of pretenellin A **42** and predesmethylbassianin A **87**, respectively, led to the production of reprogrammed compounds **89** and **90** in which the changes to methylation and chain length could be mapped to the domain swaps and revealed important information about the overall PKS programming (Fisch et al. 2011). The domain swaps combined with co-expression of two tailoring P450 genes *tenA* and *tenB* led to the resurrection of the extinct metabolite bassianin **91** (Fig. 18).

F. PKS Deconstruction

In parallel to these in vivo domain swaps, the deconstruction of PKS proteins into smaller mono- to multidomain fragments has been a crucial tool for the mechanistic understanding of these highly programmed enzymes. The deconstruction method enables the rapid and selective in vitro recombination of PKS activity, allowing the individual domain functions to be

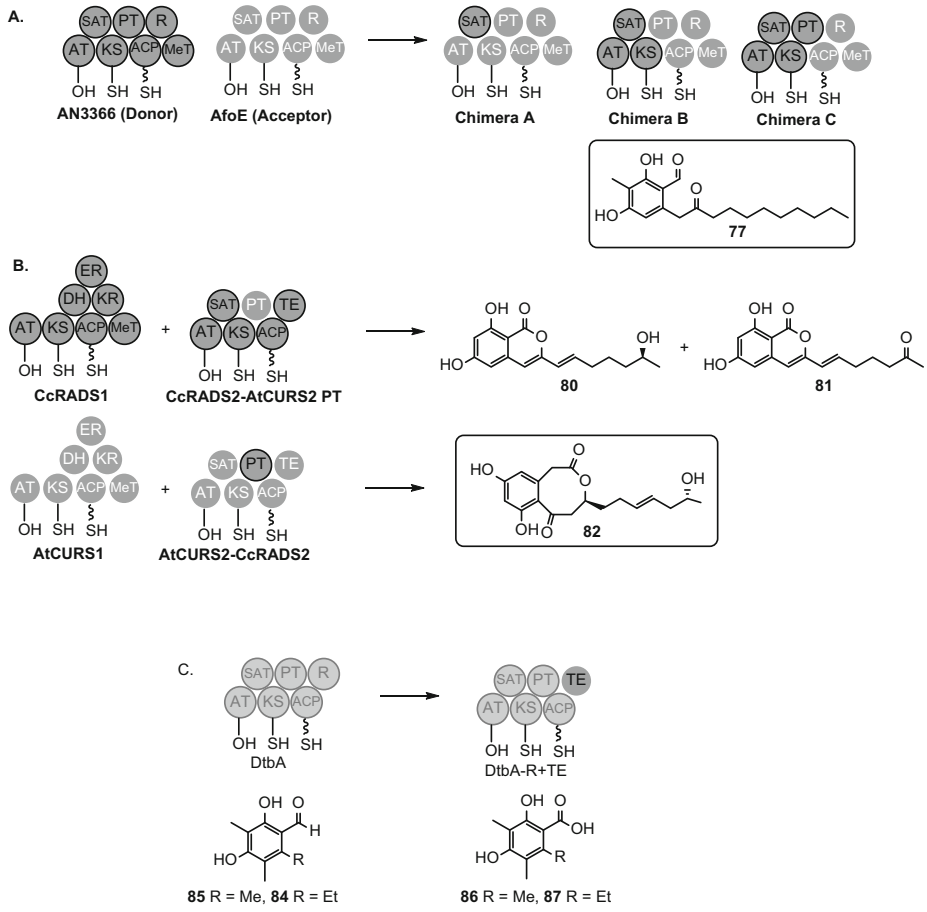


Fig. 17 Examples of domain swap experiments in fungal PKS. (**a**) Domain swaps in *A. nidulans* using AfoE as the acceptor led to the production of the novel polyketide **77**. (**b**) PT domain swaps between CcRADS2 and AtCURS2 BDL pairs led to unnatural compounds. (The natural products of these BDL pairs are shown in Fig. 15.) (**c**) The R domain of DtbA was swapped with a TE domain, converting the aldehyde products to carboxylic acids

deduced and unnatural PKS enzymes to be constructed. One of the first examples of the deconstruction approach in fungal PKS was in part to deduce the function of the newly identified SAT domain from PksA, involved in norsolorinic acid **92** biosynthesis (Fig. 19). The SAT and ACP domains were cloned and expressed in *E. coli*, and it was shown that the SAT domain could transfer a hexanoyl starter unit to the ACP domain, while there was only minimal transfer of malonyl-CoA (Crawford et al. 2006). Complementary experiments that involved cloning and expressing the AT and ACP domains of PksA, also in *E. coli*, demonstrated that the AT domain could only transfer malonyl-CoA to the ACP domain, while hexanoyl-CoA was not transferred.

The deconstruction approach was expanded to dissect the domains of PksA into various mono-, di- and tridomains and reassemble them, to reveal how the domains are able to control polyketide length, cyclisation and product release, also leading to the discovery of the PT domain (Crawford et al. 2008; Fig. 19). Deconstruction of CTB1, the PKS involved in cercosporin **93** biosynthesis, demonstrated that the TE domain catalysed pyrone formation, a previously unknown function of fungal nrPKS TE domains (Newman et al. 2012; Fig. 20).

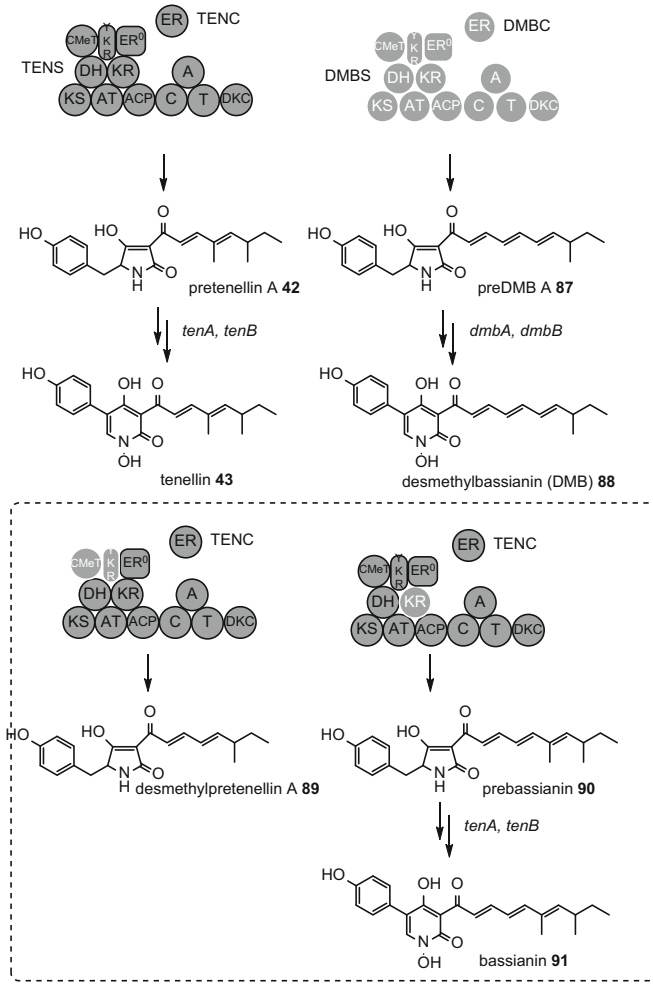


Fig. 18 Examples of domain swaps between TENS and DMBS illustrating that even swapping single domains between highly similar PKSs can have a significant effect on the overall programming

Based on the new understanding of how individual domains function, experiments involving swapping the N-terminal SAT-KS-MAT tridomain fragments from six nrPKS with the C-terminal monodomains from six non-cognate nrPKS resulted in 72 individual combinations and enabled the study of PKS starter unit selection, chain-length control, cyclisation control and product release mechanisms (Newman et al. 2014; Fig. 21). These experiments provided evidence that at the very least, a matched SAT and KS pair is crucial in the development of a non-cognate nrPKS; the PT domain also appears to assert control over chain length; and the TE domain has a crucial role in generating non-native products.

VII. Conclusion

Fungal polyketides are common components of almost all known fungi, found in both ascomycetes and basidiomycetes. Their biosynthesis is increasingly understood at chemical, biochemical and genetic levels, and their biosynthetic gene clusters are easier than ever to find and investigate. Cluster investigation tools such as Artemis and the MBiG database make it easier to begin to determine the general classes of compounds which may be produced, although precise structure prediction is still out of reach because it is not yet possible to predict the programming of the iterative Type I PKS themselves or the precise selectivity and activity of

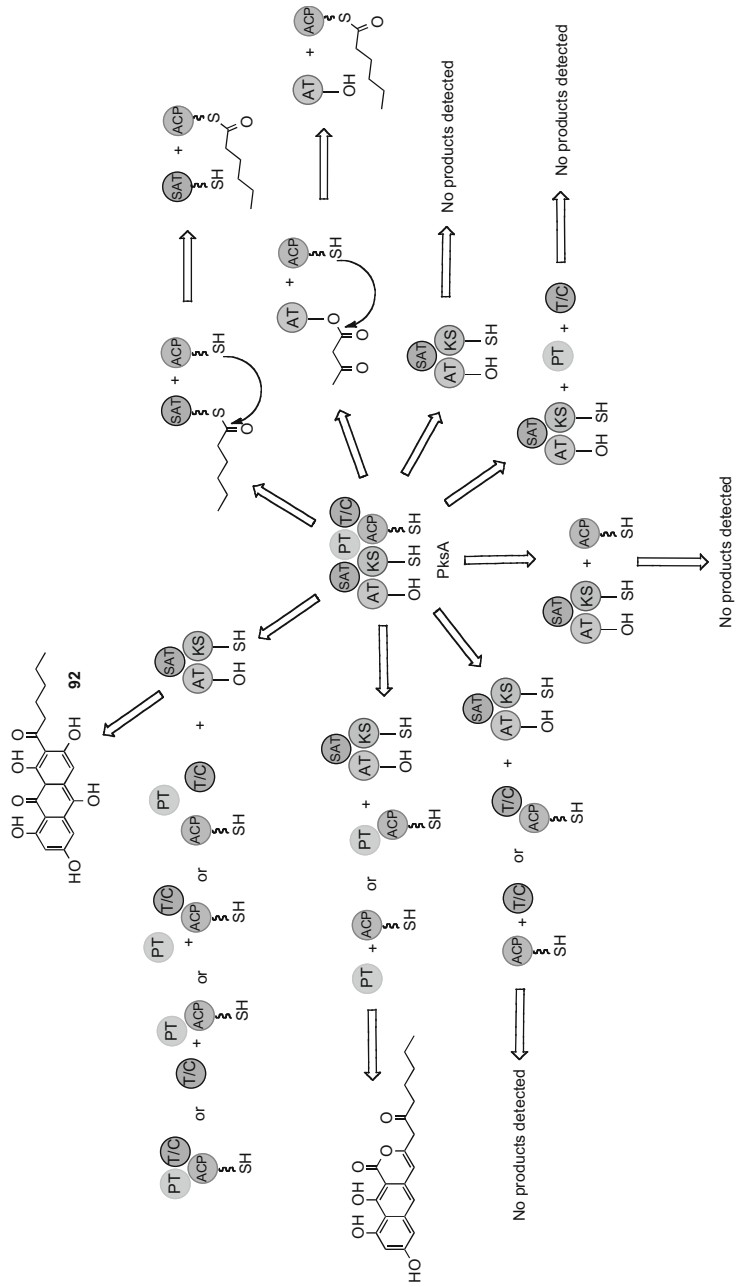


Fig. 19 The various deconstruction experiments in PksA involving generating different mono-, di- and tridomains. These deconstruction experiments formally identified and characterised the SAT and PT domains and revealed important information about programming rules

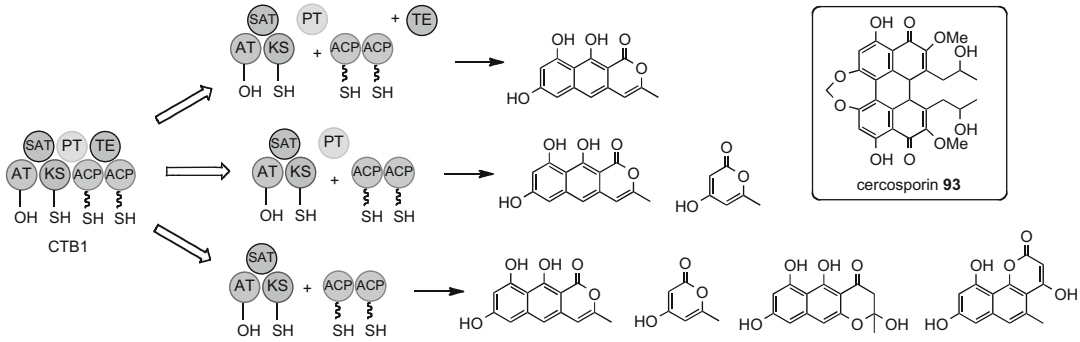


Fig. 20 Deconstruction of CTB1 led to the discovery that the TE domain was able to catalyse pyrone formation

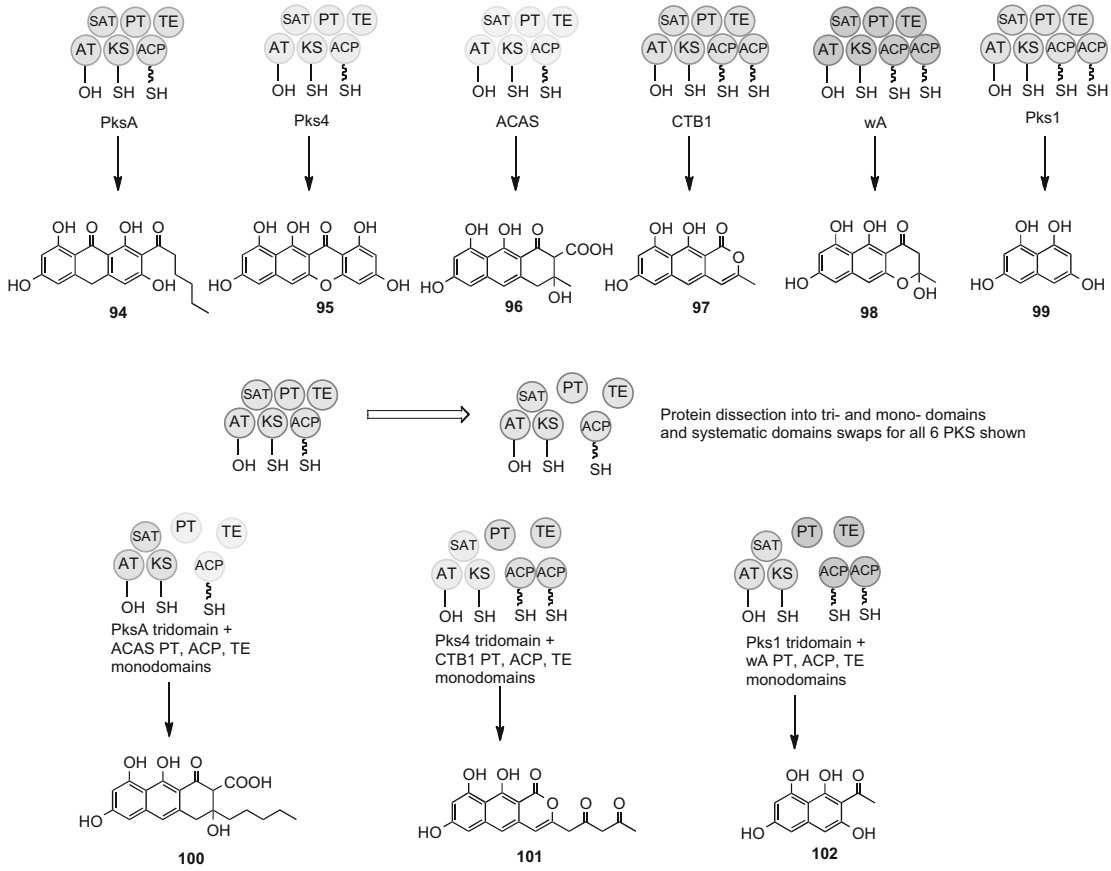


Fig. 21 Systematic domain swaps between the N-terminal SAT-KS-MAT tridomain fragments and C-terminal monodomains from other non-cognate nrPKS led to increased understanding of PKS programming

many of the tailoring enzymes. However, the ability to engineer fungal PKS is beginning to be a reality. Since very many fungal PKSs are biologically active, the prospects appear good for the development of new fungal polyketides with new biological properties.

References

Abramson D, Usleber E, Märtlbauer E (2001) Immunochemical method for citrinin. In: Mycotoxin protocols, vol 157. Humana, Totowa, NJ, pp 195–204

Ahuja M, Chiang Y-M, Chang S-L et al (2012) Illuminating the diversity of aromatic polyketide synthases in *Aspergillus nidulans*. J Am Chem Soc 134(19):8212–8221

al Fahad A, Abood A, Simpson TJ, Cox RJ (2014a) The biosynthesis and catabolism of the maleic anhydride moiety of stipitatonic acid. Angew Chem Int Ed Engl 53(29):7519–7523

al Fahad A, Abood A, Fisch KM et al (2014b) Oxidative dearomatisation: the key step of sorbicillinoid biosynthesis. Chem Sci 5(2):523–527

Alberts AW, Chen J, Kuron G et al (1980) Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc Natl Acad Sci USA 77(7):3957–3961

Andrade R, Ayer WA, Mebe Paul P (1992) The metabolites of *Trichoderma longibrachiatum*. Part 1. Isolation of the metabolites and the structure of trichodimerol. Can J Chem 70(10):2526–2535

Anke T, Oberwinkler F, Steglich W, Schramm G (1977) Strobilurins - new antifungal antibiotics from Basidiomycete *Strobilurus tenacellus* (Pers Ex Fr) Sing. J Antibiot 30(10):806–810

Bailey AM, Cox RJ, Harley K et al (2007) Characterisation of 3-methylorcinaldehyde synthase (MOS) in *Acremonium strictum*: first observation of a reductive release mechanism during polyketide biosynthesis. Chem Commun (39):4053–4055

Bailey AM, Fisch KM, Skellam E et al (2010) Catalytic role of the C-terminal domains of a fungal nonreducing polyketide synthase. Chem Commun 46 (29):5331–5333

Bate C, Salmona M, Diomede L, Williams A (2004) Squalestatin cures prion-infected neurons and protects against prion neurotoxicity. J Biol Chem 279(15):14983–14990

Baxter A, Fitzgerald BJ, Hutson JL et al (1992) Squalestatin 1, a potent inhibitor of squalene synthase, which lowers serum cholesterol *in vivo*. J Biol Chem 267(17):11705–11708

Beck J, Ripka S, Siegner A et al (1990) The multifunctional 6-methylsalicylic acid synthase gene of *Penicillium patulum* - its gene structure relative to that of other polyketide synthases. Eur J Biochem 192:487–498

Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16(3):497–516

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58(1):1–26

Berger W, Micksche M, Elbling L (1997) Effects of multidrug resistance-related ATP-binding-cassette transporter proteins on the cytoskeletal activity of cytochalasins. Exp Cell Res 237(2):307–317

Bergmann S, Schümann J, Scherlach K et al (2007) Genomics-driven discovery of PKS-NRPS hybrid metabolites from *Aspergillus nidulans*. Nat Chem Biol 3(4):213–217

Bergstrom JD, Dufresne C et al (1995) Discovery, biosynthesis, and mechanism of action of the zaragozic acids: potent inhibitors of squalene synthase. Annu Rev Microbiol 49(1):607–639

Bingle LEH, Simpson TJ, Lazarus CM (1999) Ketosynthase domain probes identify two subclasses of fungal polyketide synthase genes. Fungal Genet Biol 26:209–223

Birch AJ, Massywestropp RA, Moye CJ (1955) Studies in relation to biosynthesis 7. 2-Hydroxy-6-methylbenzoic acid in *Penicillium griseofulvum* Dierckx. Aust J Chem 8(4):539–544

Birkinshaw JH, Raistrick H (1932) Studies in the biochemistry of micro-organisms: puberulic acid C(8) H(6)O(6) and an acid C(8)H(4)O(6), new products of the metabolism of glucose by *Penicillium puberulum* Bainier and *Penicillium aurantiovirens* Biourge. with an appendix on certain dihydroxybenzenedicarboxylic acids. Biochem J 26(2):441–453

Blanc PJ, Laussac JP, Lebars J et al (1995) Characterization of monascidin A from *Monascus* as citrinin. Int J Food Microbiol 27(2-3):201–213

Boecker S, Zobel S, Meyer V, Süssmuth RD (2016) Rational biosynthetic approaches for the production of new-to-nature compounds in fungi. Fungal Genet Biol 89:89–101

Bonsch B, Belt V, Bartel C et al (2016) Identification of genes encoding squalestatin S1 biosynthesis and *in vitro* production of new squalestatin analogues. Chem Commun 52:6777–6780

Bradley JR (2008) TNF-mediated inflammatory disease. J Pathol 214(2):149–160

Braunberg RC, Gantt O, Barton C, Friedman L (1992) In vitro effects of the nephrotoxins ochratoxin A and citrinin upon biochemical function of porcine kidney. Arch Environ Contam Tocicol 22:464–470

Brown DW, Proctor RH (2016) Insights into natural products biosynthesis from analysis of 490 polyketide synthases from *Fusarium*. Fungal Genet Biol 89:37–51

Cacho RA, Tang Y, Chooi Y-H (2015) Next-generation sequencing approach for connecting secondary metabolites to biosynthetic gene clusters in fungi. Front Microbiol 5(e98212)

Carver TJ, Rutherford KM, Berriman M et al (2005) ACT: the Artemis comparison tool. Bioinformatics 21(16):3422–3423

Challis GL, Ravel J, Townsend CA (2000) Predictive, structure-based model of amino acid recognition by nonribosomal peptide synthetase adenylation domains. Chem Biol 7:211–224

Chao T-C, Maxwell SM, Wong S-Y (1991) An outbreak of aflatoxicosis and boric acid poisoning in Malaysia: a clinicopathological study. J Pathol 164 (3):225–233

Chiang Y-M, Oakley CE, Ahuja M et al (2013) An efficient system for heterologous expression of secondary metabolite genes in *Aspergillus nidulans*. J Am Chem Soc 135(20):7720–7731

Cox RJ (2007) Polyketides, proteins and genes in fungi: programmed nano-machines begin to reveal their secrets. Org Biomol Chem 5(13):2010–2026

Cox RJ (2014) Oxidative rearrangements during fungal biosynthesis. Nat Prod Rep 31(10):1405–1424

Cox RJ, Glod F, Hurley D et al (2004) Rapid cloning and expression of a fungal polyketide synthase gene involved in squalestatin biosynthesis. Chem Commun (Camb) (20):2260–2261

Crawford JM, Dancy BCR, Hill EA et al (2006) Identification of a starter unit acyl-carrier protein transacylase domain in an iterative type I polyketide synthase. Proc Natl Acad Sci USA 103(45):16728–16733

Crawford JM, Thomas PM et al (2008) Deconstruction of Iterative multidomain polyketide synthase function. Science 320(5873):243–246

Davison J, al Fahad A, Cai M et al (2012) Genetic, molecular, and biochemical basis of fungal tropolone biosynthesis. Proc Natl Acad Sci USA 109 (20):7642–7647

Dobias J, Betina V, Nemec P (1980) Insecticidal activity of ramihyfin-A, citrinin and rugulosin. Biologia 35 (6):431–434

Dufosse L (2006) Microbial production of food grade pigments. Food Technol Biotechnol 44(3):313–321

Dufosse L, Fouillaud M, Caro Y et al (2014) Filamentous fungi are large-scale producers of pigments and colorants for the food industry. Curr Opin Biotechnol 26:56–61

Eley KL, Halo LM, Song Z et al (2007) Biosynthesis of the 2-pyridone tenellin in the insect pathogenic fungus *Beauveria bassiana*. ChemBioChem 8 (3):289–297

El-Nezami H, Polychronaki N, Salminen S, Mykkanen H (2002) Binding rather than metabolism may explain the interaction of two food-grade *Lactobacillus* strains with zearalenone and its derivative alpha-zearalenol. Appl Environ Microbiol 68 (7):3545–3549

Fedorova ND, Moktali V, Medema MH (2012) Bioinformatics approaches and software for detection of secondary metabolic gene clusters. Methods Mol Biol 944:23–45

Feher M, Schmidt JM (2003) Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. J Chem Inf Comp Sci 43(1):218–227

Fisch KM, Bailey AM, Bakeer W et al (2011) Rational domain swaps decipher programming in fungal highly reducing polyketide synthases and resurrect an extinct metabolite. J Am Chem Soc 133 (41):16635–16641

Foissner I, Wasteneys GO (2007) Wide-ranging effects of eight cytochalasins and latrunculin A and B on intracellular motility and actin filament reorganization in characean internodal cells. Plant Cell Physiol 48(4):585–597

Fujii I, Ono Y, Tada H et al (1996) Cloning of the polyketide synthase gene *atX* from *Aspergillus terreus* and its identification as the 6-methylsalicylic acid synthase gene by heterologous expression. Mol Gen Genet 253:1–10

Fujii R, Minami A, Gomi K, Oikawa H (2013) Biosynthetic assembly of cytochalasin backbone. Tetrahedron Lett 54(23):2999–3002

Gaffoor I, Brown DW, Plattner R et al (2005) Functional analysis of the polyketide synthase genes in the filamentous fungus *Gibberella zeae* (Anamorph *Fusarium graminearum*). Eukaryot Cell 4 (11):1926–1933

Galagan JE, Calvo SE et al (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Gelderblom WC, Jaskiewicz AK et al (1988) Fumonisins–novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol 54:1806–1811

George TP, Cook HW et al (1991) Inhibition of phosphatidylcholine and phosphatidylethanolamine biosynthesis by cytochalasin B in cultured glioma cells: potential regulation of biosynthesis by Ca^{2+}-dependent mechanisms. Biochim Biophys Acta Lipids Lipid Metab 1084:185–193

Goldman L, Schwarz J et al (1960) Current status of griseofulvin: report on one hundred seventy-five cases. J Am Med Assoc 172:532–538

Gomez BL, Nosanchuk JD (2003) Melanin and fungi. Curr Opin Infect Dis 16:91–96

Halo LM, Heneghan MN et al (2008a) Late stage oxidations during the biosynthesis of the 2-pyridone tenellin in the entomopathogenic fungus *Beauveria bassiana*. J Am Chem Soc 130:17988–17996

Halo LM, Marshall JW et al (2008b) Authentic heterologous expression of the tenellin iterative polyketide synthase nonribosomal peptide synthetase requires coexpression with an enoyl reductase. ChemBioChem 9:585–594

Harrison LR, Colvin BM et al (1990) Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. J Vet Diagn Invest 2:217–221

He Y, Cox RJ (2016) The molecular steps of citrinin biosynthesis in fungi. Chem Sci 7:2119–2127

Hendrickson L, Davis C et al (1999) Lovastatin biosynthesis in *Aspergillus terreus*: characterization of blocked mutants, enzyme activities and a multifunctional polyketide synthase gene. Chem Biol 6:429–439

Hetherington C, Raistrick H (1931) On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by *Penicillium citrinum Thom*. Philos Trans R Soc B 220:269–295

Hirose T, Izawa Y et al (1990) The effects of new cytochalasins from *Phomopsis sp.* and the derivatives on cellular structure and actin polymerization. Chem Pharm Bull 38:971–974

Hodge EG, Hidy PH, and Wehrmeister HJ (1966) Estrogenic compounds and animal growth promoters. US Patent 3239345

Hu Y, Dietrich D et al (2014) A carbonate-forming Baeyer-Villiger monooxygenase. Nat Chem Biol 10:552–554

Huitt-Roehl CR, Hill EA et al (2015) Starter unit flexibility for engineered product synthesis by the nonreducing polyketide synthase PksA. ACS Chem Biol 10:1443–1449

Hunter S, Jones P et al (2012) InterPro in 2011: new developments in the family and domain prediction database. Nucleic Acids Res 40:4725–4725

Isaka M, Tanticharoen M, Thebtaranonth Y (2000) Cordyanhydrides A and B. Two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. Tetrahedron Lett 41:1657–1660

Jenni S, Leibundgut M et al (2007) Structure of fungal fatty acid synthase and implications for iterative substrate shuttling. Science 316:254–261

Jung H, Kim C et al (2003) Color characteristics of Monascus pigments derived by fermentation with various amino acids. J Agric Food Chem 51:1302–1306

Kakule TB, Lin Z, Schmidt EW (2014) Combinatorialization of fungal polyketide synthase–peptide synthetase hybrid proteins. J Am Chem Soc 136:17882–17890

Khaldi N, Seifuddin FT et al (2010) SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol 47:736–741

Khayatt BI, Overmars L et al (2013) Classification of the adenylation and acyl-transferase activity of NRPS and PKS systems using ensembles of substrate specific hidden Markov models. PLoS One 8

Kroken S, Glass NL et al (2003) Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. Proc Natl Acad Sci USA 100:15670–15675

Kurtz HJ, Mirocha J (1978) Mycotoxic fungi, mycotoxins, mycotoxicoses, vol 2. Marcel Dekker, New York, NY

Lee TV, Johnson RD et al (2015) Prediction of the substrate for nonribosomal peptide synthetase (NRPS) adenylation domains by virtual screening. Proteins Struct Funct Bioinformatics 83:2052–2066

Li Y, Chooi YH et al (2011) Comparative characterization of fungal anthracenone and naphthacenedione biosynthetic pathways reveals an α-hydroxylation-dependent claisen-like cyclization catalyzed by a dimanganese thioesterase. J Am Chem Soc 133:15773–15785

Li YF, Tsai KJS et al (2016) Comprehensive curation and analysis of fungal biosynthetic gene clusters of published natural products. Fungal Genet Biol 89:18–28

Liu X, Walsh CT (2009) Cyclopiazonic acid biosynthesis in *Aspergillus sp.*: characterization of a reductase-like R* domain in cyclopiazonate synthetase that forms and releases cyclo-AcetoacetylL-tryptophan. Biochemistry 48:8746–8757

Liu T, Chiang YM et al (2011) Engineering of an "unnatural" natural product by swapping polyketide synthase domains in *Aspergillus nidulans*. J Am Chem Soc 133:13314–13316

Liu T, Sanchez JF et al (2014) Rational domain swaps reveal insights about chain length control by ketosynthase domains in fungal nonreducing polyketide synthases. Org Lett 16:1676–1679

Liu L, Zhang Z et al (2015) Bioinformatical analysis of the sequences, structures and functions of fungal polyketide synthase product template domains. Sci Rep 5(10463)

Ma SM, Li JW et al (2009) Complete reconstitution of a highly reducing iterative polyketide synthase. Science 326:589–592

Maier T, Leibundgut M, Ban N (2008) The crystal structure of a mammalian fatty acid synthase. Science 321:1315–1322

Makioka A, Kumagai M et al (2004) Different effects of cytochalasins on the growth and differentiation of *Entamoeba invadens*. Parasitol Res 93:68–71

Malpartida F, Hopwood DA (1984) Molecular cloning of the whole biosynthetic pathway of a *Streptomyces* antibiotic and its expression in a heterologous host. Nature 309:462–464

Mandala SM, Thornton RA et al (1997) Viridiofungins, novel inhibitors of sphingolipid synthesis. J Antibiot 50:339–343

Marasas WF, Kellerman TS et al (1988) Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. J Vet Res 55:197–203

Mayorga ME, Timberlake WE (1990) Isolation and molecular characterization of the *Aspergillus nidulans wA* gene. Genetics 126:73–79

Mayorga ME, Timberlake WE (1992) The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid synthases. Mol Gen Genet 235:205–212

McCapra F, Scott AI et al (1964) The constitution of monorden, an antibiotic with tranquilising action. Tetrahedron Lett 15:869–875

Medema MH, Blin K et al (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39:W339–W346

Medema MH, Kottmann R et al (2015) Minimum information about a biosynthetic gene cluster. Chem Biol 11:625–631

Minowa Y, Araki M, Kanehisa M (2007) Comprehensive analysis of distinctive polyketide and nonribosomal peptide structural motifs encoded in microbial genomes. J Mol Biol 368:1500–1517

Moriguchi T, Ebizuka YY, Fujii I (2008) Domain-domain interactions in the iterative type I polyketide synthase ATX from *Aspergillus terreus*. ChemBioChem 9:1207–1212

Nakayashiki H, Hanada S, Quoc NB, Kadotani N, Tosa Y, Mayama S (2005) RNA silencing as a tool for exploring gene function in ascomycete fungi. Fungal Genet Biol 42:275–283

Newman AG, Vagstad AL, Belecki K, Scheerer JR, Townsend CA (2012) Analysis of the cercosporin polyketide synthase CTB1 reveals a new fungal thioesterase function. Chem Commun 48:11772–11774

Newman AG, Vagstad AL, Storm PA, Townsend CA (2014) Systematic domain swaps of iterative, nonreducing polyketide synthases provide a mechanistic understanding and rationale for catalytic reprogramming. J Am Chem Soc 136:7348–7362

Nicholson TP, Rudd BAM, Dawson M, Lazarus CM, Simpson TJ, Cox RJ (2001) Design and utility of oligonucleotide gene probes for fungal polyketide synthases. Chem Biol 8:157–178

Niehaus E-M, Kleigrewe K, Wiemann P, Studt L, Sieber CMK, Connolly LR, Freitag M, Güldener U, Tudzynski B, Humpf H-U (2013) Genetic manipulation of the *Fusarium fujikuroi* fusarin gene cluster yields insight into the complex regulation and fusarin biosynthetic pathway. Chem Biol 20:1055–1066

Nordberg H, Cantor M, Dusheyko S, Hua S, Poliakov A, Shabalov I, Smirnova T, Grigoriev IV, Dubchak I (2014) The genome portal of the Department of Energy Joint Genome Institute: 2014 updates. Nucleic Acids Res 42:D26–D31

Patten PC (1981) Aflatoxins and disease. Am J Trop Med Hyg 30:422–425

Peterson JR, Mitchison TJ (2002) Small molecules, big impact: a history of chemical inhibitors and the cytoskeleton. Chem Biol 9:1275–1285

Rampal AL, Pinkofsky HB, Jung CY (1998) Structure of cytochalasins and cytochalasin-B binding-sites in human-erythrocyte membranes. Biochemistry 19:679–683

Rees DO, Bushby N, Cox RJ, Harding JR, Simpson TJ, Willis CL (2007) Synthesis of [1,2-C-13(2), N-15]-L-homoserine and its incorporation by the PKS-NRPS system of *Fusarium moniliforme* into the mycotoxin fusarin C. ChemBioChem 8:46–50

Roettig M, Medema MH, Blin K, Weber T, Rausch C, Kohlbacher O (2011) NRPSpredictor2-a web server for predicting NRPS adenylation domain specificity. Nucleic Acids Res 39:W362–W367

Schofield JG (1971) Cytochalasin-B and release of growth hormone. Nat New Biol 234:215–216

Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, Neckers LM (1998) Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones 3:100–108

Seshime Y, Juvvadi PR, Fujii I, Kitamoto K (2005) Discovery of a novel superfamily of type III polyketide synthases in *Aspergillus oryzae*. Biochem Biophys Res Commun 331:253–260

Sharma SV, Agatsuma T, Nakano H (1998) Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. Oncogene 16:2639–2645

Simpson TJ (1998) Application of isotopic methods to secondary metabolic pathways. Top Curr Chem 195:1–48

Solovyev V, Kosarev P, Seledsov I, Vorobyev D (2006) Automatic annotation of eukaryotic genes, pseudogenes and promoters. Genome Biol 7(suppl 1): S10

Song Z, Cox RJ, Lazarus CM, Simpson TJ (2004) Fusarin C biosynthesis in *Fusarium moniliforme* and *Fusarium venenatum*. ChemBioChem 5:1196–1203

Squire RA (1981) Ranking animal carcinogens - a proposed regulatory approach. Science 214:877–880

Stachelhaus T, Mootz HD, Marahiel MA (1999) The specificity-conferring code of adenylation domains in nonribosomal peptide synthetases. Chem Biol 6:493–505

Sun H, Ho CL, Ding F, Soehano I, Liu X-W, Liang Z-X (2012) Synthesis of (R)-mellein by a partially reducing iterative polyketide synthase. J Am Chem Soc 134:11924–11927

Sydenham EW, Shephard GS, Thiel PG, Marasas WFO, Stockenstrom S (1991) Fumonisin contamination of commercial corn-based human foodstuffs. J Agric Food Chem 39:2014–2018

Szwalbe AJ, Williams K, O'Flynn DE, Bailey AM, Mulholland NP, Vincent JL, Willis CL, Cox RJ, Simpson TJ (2015) Novel nonadride, heptadride and maleic acid metabolites from the byssochlamic acid producer *Byssochlamys fulva* IMI 40021-an insight into the biosynthesis of maleidrides. Chem Commun 51:17088–17091

Tilburn J, Roussel F, Scazzocchio C (1990) Insertional inactivation and cloning of the wA-gene of *Aspergillus nidulans*. Genetics 126:81–90

Udwary DW, Merski M, Townsend CA (2002) A method for prediction of the locations of linker regions within large multifunctional proteins, and application to a type I polyketide synthase. J Mol Biol 323:585–598

Ugai T, Minami A, Fujii R, Tanaka M, Oguri H, Gomi K, Oikawa H (2015) Heterologous expression of highly reducing polyketide synthase involved in betaenone biosynthesis. Chem Commun 51:1878–1188

Van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C (2014) Ten years of next-generation sequencing technology. Trends Genet 30:418–426

van Egmond HP (1989) Aflatoxin M1: occurrence, toxicity, regulation. In: van Egmond HP (ed) Mycotoxins in dairy products. Elsevier Applied Science, London, pp 11–55

Warr GA, Veitch JA, Walsh AW, Hesler GA, Pirnik DM, Leet JE, Lin P-FM, Medina IA, McBrien KD, Forenza S, Clark JM, Lam KS (1996) BMS-182123, a fungal metabolite that inhibits the production of TNF-alpha by macrophages and monocytes. J Antibiot 49:234–240

Wasil Z, Pahirulzaman KAK, Butts C, Simpson TJ, Lazarus CM, Cox RJ (2013) One pathway, many compounds: heterologous expression of a fungal biosynthetic pathway reveals its intrinsic potential for diversity. Chem Sci 4:3845–3856

Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Mueller R, Wohlleben W, Breitling R, Takano E, Medema MH (2015) antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243

Weld RJ, Plummer KM, Carpenter MA, Ridgway HJ (2006) Approaches to functional genomics in filamentous fungi. Cell Res 16:31–44

Williams JA, Wolff J (1971) Cytochalasin B inhibits thyroid secretion. Biochem Biophys Res Commun 44:422–425

Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr 80:106–1122

Williams K, Szwalbe AJ, Mulholland NP, Vincent JL, Bailey AM, Willis CL, Simpson TJ, Cox RJ (2016) Heterologous production of fungal maleidrides reveals the cryptic cyclization involved in their biosynthesis. Angew Chem Int Ed 55:6784–6788

Winssinger N, Barluenga S (2007) Chemistry and biology of resorcylic acid lactones. Chem Commun 7:22–36

Woo PCY, Lam C-W, Tam EWT, Leung CKF, Wong SSY, Lau SKP, Yuen K-W (2012) First discovery of two polyketide synthase genes for mitorubrinic acid and mitorubrinol yellow pigment biosynthesis and implications in virulence of *Penicillium marneffei*. PLoS Neglect Trop Dis 6:e1871

Xu Y, Zhou T, Zhou Z, Su S, Roberts SA, Montfort WR, Zeng J, Chen M, Zhang W, Lin M, Zhan J, Molnar I (2013a) Rational reprogramming of fungal polyketide first-ring cyclization. Proc Natl Acad Sci USA 110:5398–5403

Xu Y, Zhou T, Zhang S, Xuan L-J, Zhan J, Molnar I (2013b) Thioesterase domains of fungal nonreducing polyketide synthases act as decision gates during combinatorial biosynthesis. J Am Chem Soc 135:10783–10791

Xu Y, Zhou T, Zhang S, Espinosa-Artiles P, Wang L, Zhang W, Lin M, Gunatilaka AAL, Zhan J, Molnar I (2014) Diversity-oriented combinatorial biosynthesis of benzenediol lactone scaffolds by subunit shuffling of fungal polyketide synthases. Proc Natl Acad Sci USA 111:12354–12359

Yadav G, Gokhale RS, Mohanty B (2003) Computational approach for prediction of domain organization and substrate specificity of modular polyketide synthases. J Mol Biol 328:335–363

Yadav G, Gokhale RS, Mohanty B (2009) Towards prediction of metabolic products of polyketide synthases: an in silico analysis. PLoS Comput Biol 5(e1000351):1–14

Yalpani N, Altier AJ, Barbour E, Cigan AL, Scelonge CJ (2001) Production of 6-methylsalicyclic acid by expression of a fungal polyketide synthase activates disease resistance in tobacco. Plant Cell 13:1401–1409

Yeh H-H, Chang S-L, Chiang Y-M, Bruno KS, Oakley BR, T-K W, Wang CCC (2013) Engineering fungal nonreducing polyketide synthase by heterologous expression and domain swapping. Org Lett 15:756–759

Zabala AO, Chooi Y-H, Choi MS, Lin H-C, Tang Y (2014) Fungal polyketide synthase product chain-length control by partnering thiohydrolase. ACS Chem Biol 9:1576–1586

Aspects of the Occurrence, Genetics, and Regulation of Biosynthesis of the Three Food Relevant *Penicillium* Mycotoxins: Ochratoxin A, Citrinin, and Patulin

Rolf Geisen[1], Markus Schmidt-Heydt[1], Dominic Stoll[1], Najim Touhami[1]

CONTENTS

I. Introduction

With respect to the production of toxic secondary metabolites, the genus *Penicillium* is important especially in geographic areas with a moderate climate, because most species of this genus have growth optima at temperatures between 15 and 25 °C. The genus *Penicillium* currently consists of 354 species (Visagie et al. 2014), which comprise various ubiquitously occurring species or species adapted to certain environments. Several of the species are contaminants or spoilage organisms in various, mainly plant type, foods. The contamination and spoilage of a food is one important point of food safety. It is assumed that about 20–25% of the world's annual harvest of plant-derived foods is spoiled by fungi (Bhatnagar et al. 2002). The other important aspect in terms of food safety is the capability of fungi to produce toxic secondary metabolites, the mycotoxins. According to published genome sequences, Penicillia can contain between 40 and 50 potential secondary metabolite gene clusters. However, not all of them code for mycotoxins or are active under normal growth conditions. Mycotoxins are a group of diverse secondary metabolites with toxic effects against humans or animals. The Penicillia are important food-relevant mycotoxin producing fungi, able to produce ochratoxin A, citrinin, or patulin, among others. The biological reason for the biosynthesis of secondary metabolites by fungi is not always clear. Several hypotheses are described in this respect, such as their activity as defense metabolites against competing microorganisms, their production of secondary metabolites as storage compounds for carbon or nitrogen, their potential activity as communication metabolites, or their potential activity as metabolites, which support the adaptation of the fungus to a specific environment. The last aspect, especially, seems to be true for ochratoxin A, citrinin, and patulin biosynthesis by *Penicillium*. It was shown that ochratoxin A increases the adaptation to salt-rich environments that citrinin apparently has protective functions and that patulin increases the pathogenicity of *P. expansum* with respect to infection of the plant host. New results concerning the regulation and the simultaneous occurrence of ochratoxin, citrinin, and patulin-producing Penicillia in certain habitats will be presented.

[1] Max Rubner-Institut, Karlsruhe, Germany; e-mail: rolf.geisen@mri.bund.de; markus.schmidt-heydt@mri.bund.de; dominic.stoll@mri.bund.de; najim.touhami@mri.bund.de

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

II. Importance of the Three Mycotoxins

Fungi can produce a great variety of mycotoxins. Several of these mycotoxins are highly toxic and potentially carcinogenic, like the aflatoxins (produced mainly by *A. flavus* and *A. parasiticus*), ochratoxin A (produced by *Penicillium* and *Aspergillus*), and the fumonisins (produced by *Fusarium* and *Aspergillus*). For other mycotoxins, like patulin or citrinin (produced by Aspergilli and Penicillia), carcinogenicity is not ultimately shown, but they exhibit definitive toxicological activities. Ochratoxin and citrinin are structurally related (Fig. 1) and both have nephrotoxic activities. If they occur in parallel in a food commodity, they can exhibit synergistic toxic activities (Braunberg et al. 1994). Ochratoxin A is regarded as a class B carcinogen (Petzinger and Weidenbach 2002), meaning that nephro-carcinogenesis was demonstrated in animal models, but no epidemiological data concerning humans are available (in contrast to class A carcinogens, like the aflatoxins from *A. flavus*/*A. parasiticus*). Some publications relate the endemically occurring nephropathy in some regions of the Balkan (BEN, Balkan endemic nephropathy) to elevated uptake of ochratoxin A-contaminated cereals and cereal products (Pfohl-Leszkowicz et al. 2002). At the molecular level, it has been shown that ochratoxin is an inhibiting competitor of the phenylalanine synthetase. Ochratoxin A itself carries a phenylalanine in its molecule and binds competitively to the phenylalanine synthetase (Höhler 1998). In any case, because of its potential carcinogenicity and because of its potential occurrence in important staple foods, like cereals and cereal products, governmental limits have been set to reduce the intake of this toxin by humans and animals (Bayman and Baker 2006). Citrinin also exhibits nephrotoxicity; however, much less data about its toxicological activity are available. Citrinin can co-occur with ochratoxin A and is often produced at higher amounts than ochratoxin A (Wawrzyniak and Waskiewicz 2014). Citrinin is related to the "yellow rice syndrome" (Udagawa and Tatsuno 2004), which leads to beriberi-like syndromes, a thiamine deficiency disease. However, because of the insufficient data available, no governmental limit has been set yet (EFSA 2012).

Patulin possesses strong antibiotic activities. It was first isolated as an antibiotic from *P. patulum* (Berk et al. 1984), until its toxic activities have been recognized. Patulin exhibits a multifactorial toxicity, resulting, among others, in potential neurotoxic, immunotoxic, or teratogenic effects (Moake et al. 2005). On the molecular level, inactivation of transcription and DNA synthesis is described (Moake et al. 2005). Obviously, it has a low genotoxicity and seems not to be carcinogenic in humans (Puel et al. 2010). Because of its characterized toxicity and its high and frequent occurrence in certain food commodities, regulatory limits have been set for this toxin (Bonerba et al. 2010).

The structures of the three mycotoxins are shown in Fig. 1.

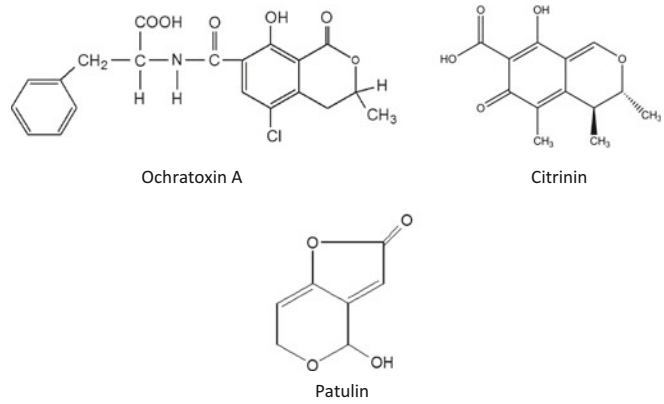


Fig. 1 Structural formulas of ochratoxin A, citrinin and patulin

III. *Penicillium* and Other Species Able to Produce Ochratoxin, Citrinin, and Patulin

Ochratoxin, citrinin, and patulin are the most important *Penicillium* mycotoxins. All three of them however can also be produced by Aspergilli. The ability to produce ochratoxin A is currently described for three related *Penicillium* species, e.g., *P. verrucosum*, *P. nordicum*, and *P. thymicola*. Citrinin can be produced by several *Penicillium* species; however, *P. verrucosum*, *P. expansum*, and *P. citrinum* are the most important species. Finally, patulin can also be produced by different *Penicillium* species but also by a range of species from other genera. In this respect *P. expansum* is the most important species of the genus *Penicillium* (Moake et al. 2005).

All three mycotoxins are polyketides. In fact, the chemical structure of the polyketide part between ochratoxin and citrinin is very similar (Fig. 1). In both cases it is a dihydroisocoumarin derivative. Synonyms of the official names for ochratoxin A are (*R*)-*N*- [(5-Chloro- 3,4-dihydro- 8-hydroxy- 3-methyl- 1-oxo- 1H-2-benzopyran-7-yl) -carbonyl]- *L*-phenylalanine and that for citrinin (3R,4S)-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid. The major differences between the structures of ochratoxin A and citrinin are the facts that ochratoxin A carries the amino acid phenylalanine via a peptide linkage and chlorine as a ligand at position 5 of the polyketide part. The non-chlorinated form, ochratoxin B, has a much lower toxicity, by a factor of about 10 (Stander et al. 2000) than ochratoxin A. Ochratoxin B is produced as a main compound under certain conditions by the fungus (Schmidt-Heydt et al. 2010). Patulin is a polyketide, which is synthesized by a MSAS-type (methyl salicylic acid) polyketide synthase (Beck et al. 1990). In contrast to other fully reducing polyketide synthases, the MSAS-type polyketide synthases are partially reducing polyketide synthases. The official name of patulin is 4-hydroxy-4H-furo [3,2-c]pyran-2(6H)-one.

P. verrucosum and *P. nordicum* are the most important *Penicillium* species, able to produce ochratoxin A. Both species are morphologically closely related and were formerly grouped in a single species, that is, *P. verrucosum* (Pitt 1987). However, based on the secondary metabolites produced (Larsen et al. 2001) and on molecular typing data (Castella et al. 2002), *P. nordicum* could clearly be separated from *P. verrucosum* as a distinct species. This phylogenetic difference is also reflected at the level of the genes responsible for ochratoxin A biosynthesis, which is described later. *P. verrucosum* mainly occurs on cereals, like wheat, and is responsible for the occurrence of ochratoxin A and citrinin in these products. In contrast *P. nordicum* is almost never found on cereals. It is however adapted to NaCl-rich environments like hypersaline waters (Butinar et al. 2011) or with respect to foods to NaCl-rich products like cheeses or dry-cured meats (Larsen et al. 2001). Occasionally also *P. verrucosum* can be found in this environment. *P. verrucosum* generally produces moderate amounts of OTA, whereas *P. nordicum* is a strong and consistent OTA producer. This different capacity of the two species to produce OTA is owed to the different habitats and reflects an adaptation mechanism, which will be described later.

Beside the Penicillia also different Aspergilli are able to produce ochratoxin A. The most important are *Aspergillus niger*, a ubiquitous fungus, responsible for the occurrence of ochratoxin in certain fruits, grapes, and coffee (Taniwaki et al. 2003) and *A. carbonarius* mainly responsible for the occurrence of ochratoxin in grapes (Battilani et al. 2003). Both are related black Aspergilli belonging to the section Nigri. Interestingly also in this case, the capacity to produce ochratoxin A differs. *A. niger* is a moderate ochratoxin A-producing fungus, and only about 6–10% of the naturally isolated strains are able to produce ochratoxin A (Nielsen et al. 2009). In contrast to *A. niger*, *A. carbonarius* is a very consistent and strong ochratoxin A-producing organism (Nielsen et al. 2009). Almost all strains isolated from its natural habitat are able to produce ochratoxin A. Only recently, Cabañes et al. (2015) identified and characterized a first *A. carbonarius* strain which was not able to produce ochratoxin A. The group showed that this was due to mutations in the ochratoxin polyketide

synthase gene. Perhaps also this different capability to produce ochratoxin may be related to the different natural environments of the two species. Whereas *A. niger* is an ubiquitous occurring species, *A. carbonarius* is mainly exclusively found as a contaminant on grapes. High and consistent ochratoxin A biosynthesis may facilitate the colonization of grapes by *A. carbonarius*. Beside these species from section Nigri, species from section Circumdati are also able to produce ochratoxin A. In fact, *A. ochraceus* is the type species from which ochratoxin was first isolated and described (van der Merwe et al. 1965). Recently two species were separated from *A. ochraceus* based on chemotaxonomic differences (Frisvad et al. 2004), e.g., *A. westerdijkiae* and *A. steynii*. Both are morphologically very similar to *A. ochraceus*, but they are able to produce a different set of secondary metabolites. In fact, as is the case with the Penicillia or the *Aspergillus* section Nigri species, also in the section Circumdati, an increasing capacity to produce ochratoxin A can be observed. *A. ochraceus* is generally a low to moderately and non-consistent ochratoxin A-producing fungus. In contrast *A. westerdijkiae* produces moderate to high amount of the secondary metabolite, whereas *A. steynii* is a very consistent and high-producing species (Gil-Serna et al. 2010). *Aspergillus* species from the section Circumdati are seldom occurring on grapes but more on tropical products like coffee or cocoa (Morello et al. 2007).

As described above citrinin is a polyketide, which is structurally very much related to the polyketide part of ochratoxin A. The main differences are the facts that citrinin is not bound to the amino acid phenylalanine and that citrinin never carries chlorine in its structure. Important citrinin-producing organisms are *P. citrinum*, *P. expansum*, *P. verrucosum*, *Monascus purpureus*, *M. aurantiacus*, and *M. ruber* (Ostry et al. 2013). *M. purpureus* and *M. ruber* are food relevant species occurring, for example, in soya, sorghum, rice, and oats. *M. purpureus* is known for its biotechnological use in Asian countries. With the aid of this fungus, fermented red rice is produced, which is used as a functional food to reduce high cholesterol levels. This cholesterol-reducing activity is due to the production of monacolin (Lee et al. 2007), a lovastatin-like secondary metabolite. However, beside monacolin, several species of the genus *Monascus*, like *M. purpureus*, *M. ruber*, and others (Chen et al. 2008), are able to produce the nephrotoxin citrinin. Citrinin was first described from *P. citrinum* by Hetherington and Raistrik (1931). Citrinin is a yellow substance, which can be visualized in high-producing strains after growth on citrinin supporting media. *P. citrinum* is an ubiquitously occurring fungus which can be identified on many substrates, like cereals, rice, nuts, fruits, fermented meats, etc. (Pitt and Hocking 1999). According to the occurrence data described in the literature, *P. citrinum* does not seem to have a typical habitat, in contrast to the other Penicillia treated here. *P. citrinum* is a very consistent citrinin-producing organism, able to synthesize this toxin in high amounts. *P. verrucosum* is a further important citrinin-producing fungus. This species possesses an inconsistent and only moderate ability to produce citrinin compared to *P. citrinum*. Moreover, in *P. verrcosum* citrinin, biosynthesis is regulated in a complex manner in response to the regulation of ochratoxin A. This seems to be an adaptation to specific different environmental conditions, which will be described later. As described above important habitats of *P. verrucosum* are cereals and cereal products; however, this species can also occur on salt-rich foods, like dry-cured meats and cheeses, and is obviously be responsible for the co-occurrence of ochratoxin A and citrinin in these types of products (Markov et al. 2013). A third important citrinin-producing species is *P. expansum*. *P. expansum* is responsible for the green rot of certain fruits. It is a severe postharvest pathogen, colonizing mainly apples but also other fruits (Reddy et al. 2010). Strains of *P. expansum* are variable in their ability to produce citrinin. Some produce high amounts, however, generally not that consistently as *P. citrinum*. Strains of *P. expansum* also can occur on salt-rich environments, beside fruits as its typical habitat.

Patulin is produced by a variety of species like *Byssochlamys nivea*, *B. fulva*, *A. giganteus*, *A. terreus*, *A. clavatus*, *Paecilomyces* sp.,

P. patulum, *P. crustosum*, *P. roqueforti*, and *P. expansum* (Moake et al. 2005). From this list of fungi, *P. expansum* is by far the most important one, because it regularly occurs in certain habitats described above and is therefore responsible for the occurrence of patulin in these plant-derived types of food. As mentioned above the maximum amount of patulin in foods is governmentally regulated.

Like *P. verrucosum*, which is able to produce a pair of two important mycotoxins, e.g., ochratoxin A and citrinin, *P. expansum* produces citrinin and patulin. However, whereas the biosynthesis of ochratoxin and citrinin is tightly linked at the genetic and regulatory level in *P. verrucosum*, which results in mutual regulation of both toxins, this linkage is not that pronounced for the synthesis of patulin and citrinin by *P. expansum*. Nevertheless both toxins are produced under different conditions, depending on the growth parameters, and seem, like in *P. verrucosum*, be responsible for the adaptation to certain environments.

IV. Common Features and Common Occurrence of the Treated Penicillia

Despite the fact of the different typical habitats of the treated Penicillia, they share some common features. For example, they all can be found in fungal populations of a somewhat more challenging habitat, e.g., high-salt (NaCl) and lower water activity environments. Butinar et al. (2011), for example, described the occurrence of *P. citrinum*, *P. nordicum*, and *P. expansum* in various hypersaline waters, indicating a common resistance against increased NaCl concentrations. Certain foods like fermented meats, cheeses, or salted vegetables like olives also contain quite high amounts of NaCl. Also in these habitats, all treated *Penicillium* species can regularly be found. For example, Comi et al. (2004) isolated *P. verrucosum*, *P. citrinum*, *P. chrysogenum*, *P. commune*, and *P. expansum* from Istrian fermented meats. Núnez et al. (1996) identified *P. citrinum*, *P. expansum*, *P. chrysogenum*, and *P. viridicatum* (*P. verrucosum*) from Iberian fermented meats. Battilani et al. (2007) isolated *P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. nalgiovense*, and *P. nordicum* from Italian fermented meats. Furthermore, Asefa et al. (2009) described *P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. solitum*, and *P. nalgiovense* from these types of products. Also Rodriguez et al. (2014) identified *P. verrucosum* on these products at a quite high frequency. This shared occurrence of these ochratoxin-, citrinin-, and patulin-producing Penicillia suggest similar ecological constraints and evolutionary relationships. In none of these environments, ochratoxin, citrinin, or patulin is produced by Aspergilli, indicating that irrespective of the production of the similar secondary metabolites, the ecology and the biological importance of the treated secondary metabolites are completely different.

V. The Gene Clusters of the Treated Mycotoxins

Until now no complete cluster in case of ochratoxin A biosynthesis in *Penicillium* and *Aspergillus* is known. For both genera only certain genes, mainly polyketide synthases (pks) and non-ribosomal peptide synthetases (nrps) are described. In their genome analysis of *A. niger*, Pel et al. (2007) mention the identification of a putative ochratoxin A biosynthesis gene cluster, but this putative cluster is not described in detail. For *P. nordicum* mainly a partial gene cluster consisting of a polyketide synthase (*otapks*PN), a non-ribosomal peptide synthase (*otanps*PN) and an adjacent alkaline serine protease (*asp*PN) have been described (Geisen et al. 2006). Expression of the *otapks*PN gene strongly coincides with ochratoxin biosynthesis (Bogs et al. 2006; Schmidt-Heydt et al. 2012a; Rodriguez et al. 2014; Ferrara et al. 2016). Early PCR results with primers generated from the sequences of the ochratoxin biosynthesis genes revealed that most described genes of *P. nordicum* (*otanps*PN, *asp*PN) are also present in *P. verrucosum*, except the polyketide synthase gene *otapks*PN (Geisen et al. 2006). That was already an indication that the ochratoxin polyketide synthase of *P. verrucosum* might be

different from that of *P. nordicum*. Recently O'Callaghan et al. (2013) identified and sequenced a polyketide synthase gene from *P. verrucosum* and inactivated the gene by integrative transformation. The resulting transformant was not able to produce ochratoxin A, in contrast to the wild type, indicating that this pks gene (*otapks*PV) is involved in ochratoxin A biosynthesis in *P. verrucosum*. The authors could show that the *otapks*PV gene has high homology to the citrinin polyketide synthase gene (*pks*CT) of *Monascus*, which supports the view that the citrinin polyketide synthase apparently is responsible for ochratoxin biosynthesis in *P. verrucosum*. This view is further supported by the fact that adjacent to this *pks*CT (*otapks*PV now called *pks*CT), two genes are located, which have high homology to two further genes of the citrinin biosynthesis gene cluster of *Monascus* (O'Callaghan et al. 2013). Further information on the differences between *P. verrucosum* and *P. nordicum* comes from the recent sequencing of *P. verrucosum* (Gene bank accession number LAKW00000000.1) and *P. nordicum* (Gene bank accession number PRJNA239658). As already shown earlier, the *P. verrucosum* genome contains the *asp*PN and *otanps*PN genes. However, in case of the *otapks*PN gene, *P. verrucosum* contains only a shortened gene with low homology to *otapks*PN gene of *P. nordicum*. The results described by O'Callaghan et al. (2013) suggest that the polyketide part of ochratoxin in *P. verrucosum* is synthesized by the citrinin polyketide synthase (*pks*CT). Information about the involvement of further biosynthetic enzymes in ochratoxin biosynthesis, either from the citrinin biosynthesis pathway or from outside of this pathway (e.g., the homologue to the *otanps*PN gene of *P. nordicum*) is not known to date. Interestingly also *P. nordicum* contains a homologue of the *pks*CT gene. However, the *pks*CT homologous sequence of *P. nordicum* does not have an open reading frame, suggesting that no functional *pks*CT gene is present in this species (Fig. 2).

Recently, Nguyen et al. (2016) identified *P. thymicola* as a further *Penicillium* species able to produce ochratoxin A. The genome of this species was determined and screened for known ochratoxin genes. No homologues to the known ochratoxin polyketide synthase genes from *P. nordicum* and *P. verrucosum* could be found. The authors found however a pks and nrps gene with homology to OTA biosynthetic enzymes of *A. carbonarius* and *A. ochraceus*. Very interestingly, the analyzed *P. thymicola* strain was isolated from cheese that is also a salt-rich environment and thereby a similar habitat as the one described above.

Various ochratoxin polyketide synthase genes from Aspergilli have been described. O'Callaghan et al. (2003) isolated a part of a pks gene from *A. ochraceus*. Because the transcription of that gene correlated to the production of ochratoxin A, the authors concluded its involvement in the biosynthesis of the toxin. Bacha et al. (2009) described the identification of an ochratoxin A polyketide synthase gene from *A. westerdijkiae*. The transcription of this gene also coincides with ochratoxin biosynthesis and a disruption of this gene abolished ochratoxin A biosynthesis. Recently Gil-Serna et al. (2015) described part of a gene cluster of *A. steynii* consisting of a polyketide synthase gene (*pksste*), a non-ribosomal peptide synthase gene (*nrpsste*), and a p450 cytochrome oxidase gene (*p450ste*), suggested to be involved in ochratoxin A biosynthesis. Gallo et al. (2014) identified an ochratoxin polyketide synthase gene and a non-ribosomal peptide synthase gene (Gallo et al. 2012) from *A. carbonarius*. An interesting observation is given by Wang et al. (2015). These authors recently identified two polyketide synthase genes, *AoOTApks*-1 and *AoOTApks*-2, in *A. ochraceus*. This is also supposed by results from Bacha et al. (2009), who also described two polyketide synthase genes to be involved in ochratoxin biosynthesis in *A. westerdijkiae*. Wang et al. (2015) deleted both polyketide synthases and could show that a deletion of *AoOTApks*-1 results in complete, whereas a deletion of *AoOTApks*-2 results in a partial reduction in the biosynthesis of ochratoxin A. Based on these results, they concluded that both pks genes are involved in ochratoxin A biosynthesis but that *AoOTApks*-2 might have an accessory function. According to the sequence homology, *AoOTApks*-1 resembles the pks gene of *A. carbonar-*

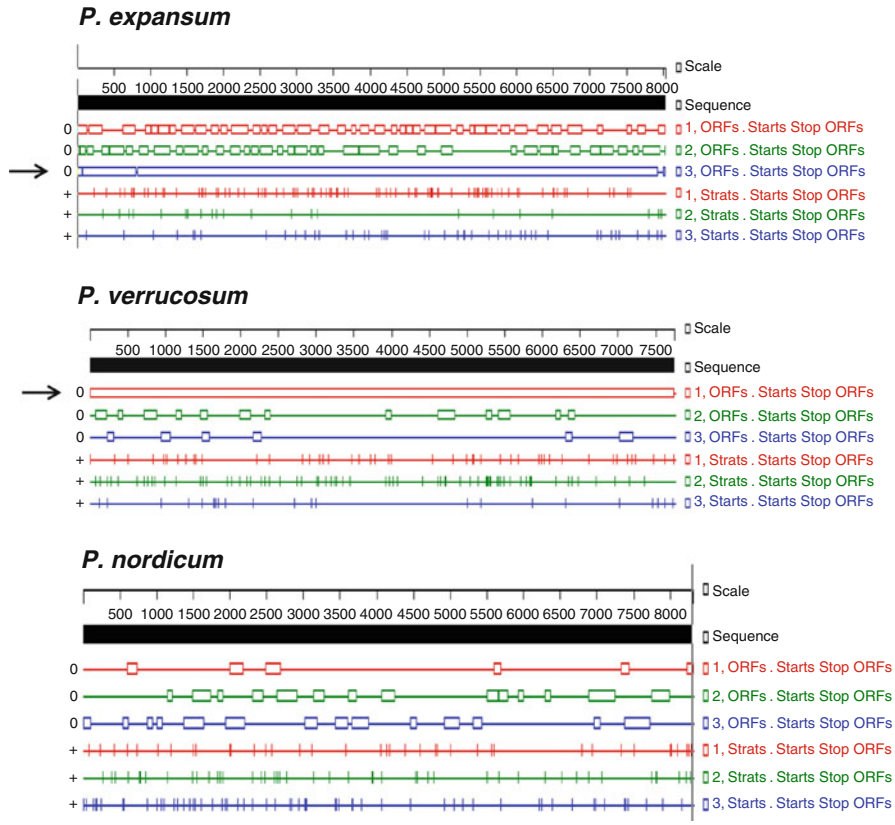


Fig. 2 Analysis of the presence of open reading frames using the sequence of the *pks*CT gene of *P. expansum, P. verrucosum* and *P. nordicum*. Only for *P. expansum* and *P. verrucosum* a long open reading frame (arrow) could be identified by analyzing the respective sequences with the DNA Star software package. The sequence of *P. nordicum* contains several stop codons in all reading frames

ius and *A. niger* described above, whereas the *AoOTApks*-2 has the characteristics of a partially reducing polyketide synthase and has structural similarities to the *P. nordicum otapks*PN gene. Because of the recent availability of the genome of *P. verrucosum*, it was screened for the presence of *Aspergillus* ochratoxin pks genes. Interestingly sequence regions with homology to the *A. steynii* ochratoxin polyketide synthase gene cluster (Gil-Serna et al. 2015) could be identified. Because of the new genome data and the inactivation data of the *pks*CT gene (O'Callaghan et al. 2013), the situation in Penicillia might be similar to the situation in *A. ochraceus* as described by Wang et al. (2015) meaning that two polyketide synthases might be involved in the biosynthesis of ochratoxin A. The *Aspergillus* homologues could also be found in *P. nordicum* and *P. thymicola*. Preliminary results with *P. nordicum* show, that under certain environmental conditions, transcription of the *A. steynii* homologues fit to ochratoxin biosynthesis, whereas under other conditions the expression of the *otapks*PN gene parallels ochratoxin A biosynthesis.

The citrinin biosynthesis genes were first characterized from *Monascus* as an important citrinin-producing organism. Shimizu et al. (2005) identified the citrinin polyketide synthase gene (*pks*CT) from *M. purpureus*. They showed that the expression of the gene was correlated to the production of citrinin. After disruption of the *pks*CT gene, the mutant strain was no longer able to produce citrinin, indicating its involvement in the biosynthesis. The same group identified open reading frames in the vicinity of *pks*CT. The ORFs had homo-

logies to a dehydrogenase, a transcriptional regulator, an oxygenase, an oxidoreductase, and a transport protein. The inactivation of *ctn*A, the transcriptional regulator, resulted in a decrease in transcription of the *pks*CT gene. Additionally, Li et al. (2012) described the complete citrinin gene cluster from *M. aurantiacus*. Ballester et al. (2015) described the genome and transcriptome of *P. expansum*. During this work, the putative citrinin cluster of *P. expansum* was identified based on the sequences of the *Monascus* genes. In order to analyze gene function, the transcription of the *pks*CT gene was analyzed. A clear correlation between transcription and citrinin biosynthesis could be identified in *P. expansum*. For further characterization, the *pks*CT gene was inactivated by replacement. The mutant strains had similar growth rates than the wild type on PDA plates; however, the replacement mutants were unable to produce citrinin, confirming the involvement of the *pks*CT gene in citrinin biosynthesis in this fungus. The own sequencing of the genomes of *P. verrucosum* (GeneBank accession number: LAKW00000000.1) and *P. citrinum* (GeneBank accession number: LKUP00000000.1) enabled the identification of the citrinin biosynthesis gene clusters and their comparison with the clusters of *M. purpureus* and *P. expansum* (Fig. 3). Except for difference of the orf1 and *ctn*R genes of *P. verrucosum*, which might be due to the status of the available shotgun sequence, irrespective of its 60x coverage, the gene clusters look very similar between the species. Within the Penicillia there exist a sequence similarity of about 90% for the *pks*CT gene and 50–70% for the whole citrinin cluster. This similarity and the high synteny of the clusters between the species suggest a common origin of the clusters or the possibility of horizontal gene transfer (HGT). In any case an evolutionary constraint for the maintenance of the cluster seems to be present.

The *pks*CT (*otapks*PV) gene in *P. verrucosum* was inactivated by O'Callaghan et al. (2013). These authors showed, as mentioned above, that this inactivation leads to a strain, which was not able to produce ochratoxin. Unfortunately, no information about the capability of this mutant to produce citrinin was given. Recently He and Cox (2016) showed that the *pks*CT of *M. purpureus* is a non-reducing pks. The *pks*CT from *P. expansum* was inactivated by Ballester et al. (2015). During this work it could also be demonstrated that *pks*CT is responsible for citrinin biosynthesis in *P. expansum*. Touhami et al. (unpublished) furthermore inactivated the *pks*CT gene from *P. citrinum*. It could be shown that the resulting mutant strain again was not able to produce citrinin. These results identified the *pks*CT gene as the key gene in citrinin biosynthesis in *Penicillium*. Also *P. nordicum*, a species which is usually not able to produce citrinin, carries homologues of citrinin biosynthesis genes;

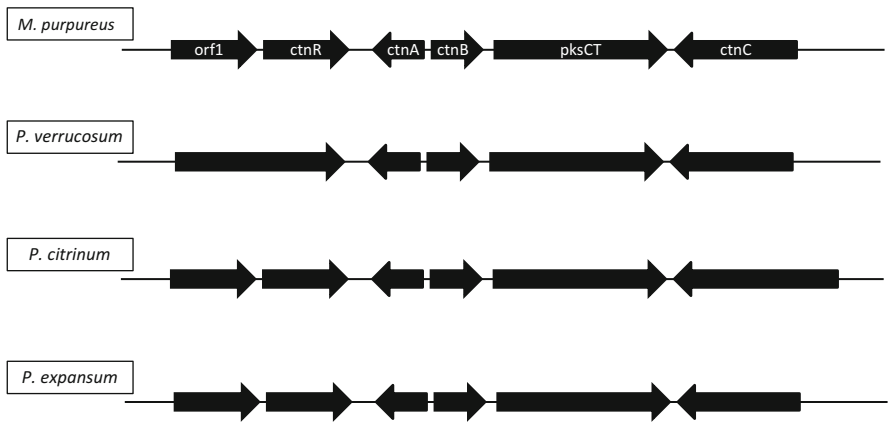


Fig. 3 Comparison of part the citrinin clusters of *M. purpureus, P. verrucosum, P. citrinum, P. expansum*

however most of the genes have mutations and do not represent complete ORFs.

The high homology of the various citrinin clusters and their distribution suggest that the gene clusters stem from a common ancestor (Gallo et al. 2013). However, the fact that all mentioned Penicillia occur in a limited common environment (NaCl-rich habitat) also opens the possibility of the distribution of this cluster by HGT, as it is described for other fungi (Proctor et al. 2013). HGT events are also suggested for ochratoxin A-producing Aspergilli (Davolos and Pietrangeli 2014). Genetic exchange in a specialized *Penicillium* population from cheeses (also a NaCl-rich environment) was recently described (Cheeseman et al. 2013; Ropars et al. 2015). These authors identified a 575-kb-long genomic fragment in *P. roqueforti*, called Wallaby, which could also be found in *P. camemberti* and *P. rubens*, however, at different genomic positions. Both latter species are commensals of *P. roqueforti* in cheeses. A further element, called CheesyTer, could also be identified. Both elements obviously constitute large genomic islands, which are easily transferable. Wallaby, for example, contains about 250 genes. Strains, which carry this element, are better adapted to the cheese environment compared to strains of the same species, which do not carry this genomic island. Interestingly the fragment was only found in *Penicillium* species obtained from the food environment. This suggests that the close proximity or the specific conditions in the food environment favor HGT events (Ropars et al. 2015).

As mentioned above, the first and near parallel isolation of a gene of the patulin biosynthesis pathway was achieved by Beck et al. (1990) characterizing the MSAS-type polyketide synthase gene from *P. patulum*, respectively, the description of the same gene from *P. urticae* (synonym *P. griseofulvum*) by Wang et al. (1991). White et al. (2006) cloned the *idh* gene, the gene for the isoepoxydon dehydrogenase from *P. expansum*. This is a pathway gene for patulin biosynthesis. Furthermore, these authors isolated two putative cytochrome oxidase genes. All genes were co-regulated under conditions supportive for patulin biosynthesis. For this reason, the authors conclude that the gene products were involved in patulin biosynthesis. This was confirmed by Artigot et al. (2009) who also described two cytochrome oxidase genes involved in patulin biosynthesis. One of the gene products was responsible for the hydroxylation of m-cresol, whereas the other catalyzed the hydroxylation of m-hydroxybenzyl alcohol. Dombrink-Kurtzman (2006) showed by an analysis of a partial fragment of the idh gene that there were clear sequence differences between the genes of *P. griseofulvum* (*P. urticae*) and *P. expansum*. The importance of the *idh* gene for the capability of *B. nivea* to produce patulin was already demonstrated earlier by Dombrink-Kurtzman and Engberg (2006). The same author (Dombrink-Kurtzman 2007) also analyzed variances in the *idh* gene in different *Penicillium* species and could show that the sequences of this gene in strains of *P. expansum* and *P. griseofulvum*, the two most important producing *Penicillium* species, differ. Snini et al. (2014) characterized the enzymatic activity of *pat*G, which was shown to code for a 6-methylsalicylic acid decarboxylase. The authors could show that *pat*G was strongly expressed during patulin biosynthesis.

The first complete description of the gene cluster of *P. expansum* was done by Tannous et al. (2014). The cluster consists of 15 genes on an approximately 40 kb fragment. During this and other analyses (Ballester et al. 2015), it was found that incomplete clusters were present in several *Penicillium* species. Tannous et al. (2014) compared the complete cluster from *P. expansum* with the incomplete clusters of *P. digitatum* and *P. chrysogenum*. The genes of the cluster of *P. expansum* shares 60–70% of identity with genes of a putative patulin gene cluster from *A. clavatus* but 70–90% with *P. digitatum* and *P. expansum*. The similarity of the gene sequences to *P. digitatum* was higher than that to *P. chrysogenum*. Interestingly the gene order in the *P. expansum* patulin gene cluster differed to the putative *A. clavatus* gene cluster (Fig. 4) (Tannous et al. 2014). Ballester et al. (2015) also, like Tannous et al. (2014), identified further *Penicillium* species carrying nonfunctional, incomplete clusters,

PAT gene order →

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B	8	9	10	11	12	13	14	15	3	2	1	4	7	6	5
C	2	3	4	9	10	11	13	14							
D	2	1	14	15											
E	1	2	3	4	5	6	7	8	10	11	14	15			

A = *P. expansum*
B = *A. clavatus*
C = *P. digitatum*
D = *P. italicum*
E = *P. roqueforti*

Fig. 4 Visualization of the different order of the *pat* genes and the occurrence of incomplete clusters in different *Penicillium* and *Aspergillus* species compared to *P. expansum* [adapted according to Ballester et al. (2015) and Tannous et al. (2014)]

e.g., *P. roqueforti*, *P. camemberti*, *P. digitatum*, and *P. italicum*, and relate this fact to the possibility that the production of patulin is an ancestral trait. According to sequence comparisons, *P. verrucosum* does not contain a patulin cluster; however *P. verrucosum* as well as *P. nordicum* contain MSAS-like polyketide synthase genes. According to Ballester et al. (2015), the current situation concerning the dysfunction and variability of the putative gene clusters in the nonproducing species could be a result of differential loss, as assumed in the case of patulin or due to HGT events.

VI. Environmental Regulation of the Biosynthesis of the Three Secondary Metabolites

It is well known that the productions of most secondary metabolites are highly regulated according to environmental conditions. Most important parameters with an influence on mycotoxin regulations are the composition of the substrate, the temperature, the water activity (a_w), and the pH. For example, the temperature plays a prominent role for the geographic distribution of mycotoxin-producing fungi. Ochratoxin A-producing Penicillia predominantly occur in geographic regions with a moderate climate, like European and North European countries. Generally, *P. verrucosum* is responsible for the occurrence of ochratoxin A and citrinin in cereals from these regions. Ochratoxigenic Aspergilli can almost never be found under these conditions (Lund and Frisvad 2003). On the other hand, products from warmer geographical areas or from tropical countries, like grapes, coffee, cocoa, or spices, are never described to be contaminated by ochratoxin A through the presence of Penicillia (Bragulat et al. 2008) but through *A. ochraceus*, *A. westerdijkiae*, and *A. steynii* (Taniwaki et al. 2003; Leong et al. 2007). However, not only the occurrence of toxicogenic fungi is influenced by the environmental conditions but also the biosynthesis of the secondary metabolite himself. *P. nordicum*, for example, produces ochratoxin A in a pH- and temperature-dependent manner on YES medium (Geisen 2004). A temperature of 25 °C was the optimum for ochratoxin A biosynthesis, whereas only low amounts of ochratoxin A were produced at 15 or 30 °C. Concerning pH, cultures grown on YES medium with a pH in the range of 6–8 produced the highest amounts of ochratoxin in contrast to cultures below pH 5. Interestingly the addition of 20 g/L NaCl significantly increased ochratoxin A biosynthesis compared to non-

substituted medium. A very detailed analysis about the conditions which support ochratoxin A biosynthesis by *P. nordicum* in its natural habitat was performed by Battilani et al. (2010). These authors found in agreement to the results above that temperature and time are significant parameters influencing ochratoxin biosynthesis on dry-cured meats. The increase of the temperature from 15 to 20 °C with respect to the same incubation time resulted in an increase in ochratoxin A biosynthesis by a factor of 10.

The influence of the external parameters on ochratoxin biosynthesis acts on the level of transcription. Rodriguez et al. (2014), for example, showed that the *otapks*PN and the *otanps*PN gene expression were influenced by the NaCl concentration of the medium. In a wild-type strain, isolated from a natural environment, expressions of both genes were higher at 22% NaCl than at 10% NaCl. This influence of NaCl concentration on the expression of the *otapks*PN gene and on ochratoxin A biosynthesis is in agreement with results described by Schmidt-Heydt et al. (2012a). In the latter work, it was also shown that ochratoxin A biosynthesis was highest in media containing 20 g/L NaCl. This was also paralleled by the expression of the *otapks*PN gene. Generally, the expression of this gene increases with increasing NaCl concentrations. Also at the transcriptional level, the optimum was at 20 g/L NaCl. As mentioned above *P. nordicum* is adapted to higher NaCl concentrations, so the increase of ochratoxin A under these conditions might be an adaptation to this environment. Ferrara et al. (2016) recently showed that the kinetics of *otapks*PN expression in *P. nordicum*, grown on dry-cured meats, preceded and thereby completely coincides with the phenotypic ochratoxin biosynthesis in this habitat.

Cairns-Fuller et al. (2005) analyzed the influence of water activity and temperature on growth and ochratoxin A biosynthesis by a strain of *P. verrucosum* and demonstrated the influence of these parameters on the phenotypical level. With respect to water activity and the influence of NaCl concentrations, *P. verrucosum* resembles *P. nordicum*. However, in contrast to the latter, *P. verrucosum* can also produce citrinin, beside ochratoxin. The biosynthesis of both secondary metabolites are mutually regulated (Schmidt-Heydt et al. 2012a). That means that depending on the environmental conditions, either higher amounts of ochratoxin A or higher amounts of citrinin are produced. Interestingly high NaCl conditions shift the biosynthesis toward ochratoxin A (Schmidt-Heydt et al. 2012a), whereas conditions which increase oxidative stress shift the biosynthesis toward citrinin (Schmidt-Heydt et al. 2015), which is apparently again an adaptation mechanism toward different environments.

For *P. expansum* mainly the influence of the pH as an important factor in the apple environment has been analyzed. According to the results of Zong et al. (2015), patulin biosynthesis is increased at acidic pH values by a factor of more than 3 compared to neutral conditions. That is feasible because of the fact that apples, which are the main habitat of *P. expansum*, generally have a pH value in the acidic range and in particular in the range of pH 4 and pH 5 an optimum of patulin biosynthesis could be found. Also in this case, differences in patulin biosynthesis are regulated at the level of transcription. The expression of the *pat*L gene, a gene of the biosynthetic pathway of patulin, showed an optimum at pH 4, which is congruent with the phenotypic biosynthesis of patulin. Welke et al. (2011) even found a high production of patulin by *P. expansum* at pH values between 2.5 and 3.5 after growth of the fungus in apples. This was confirmed by results of Morales et al. (2008). In this work highest amount of patulin was produced in apples at a pH of 3.5. According to Barad et al. (2014), *P. expansum* itself reduces the pH of the habitat (apples) by production of acidic metabolites like D-gluconic acid. These authors suggest that the dynamic change in pH by the production of D-gluconic acid, rather than the preformed low pH of apple tissue, is responsible for the induction of patulin biosynthesis. The authors could show that an inhibition of D-gluconic acid production is paralleled by an impairment of the expression of the *idh* gene, a gene of the patulin biosynthesis pathway.

Much less is known about the regulation of citrinin biosynthesis by *P. expansum*. Patulin and citrin biosynthesis does not seem to be genetically linked, as it is the case with ochratoxin and citrinin in *P. verrucosum*, but the regulation of both toxins differ. According to an analysis of Abramson et al. (2009), 83% of several isolated *P. expansum* isolates produced patulin, whereas 91% were able to produce citrinin. That is in agreement with results obtained by Touhami et al. (2018) and shows that the ability to produce patulin and citrinin is a common feature in *P. expansum*. In a time-dependent production kinetic experiment of patulin and citrinin, it could be shown that patulin was produced on PDA medium after 3–6 days. In contrast citrinin was produced later after 6–14 days. Moreover, patulin was shown to be produced at low pH (4–6), whereas citrinin was produced by *P. expansum* at higher pH values ranging from pH 6 to 8 (Touhami et al. 2018).

Even less is known about the regulation of citrinin in *P. citrinum*. Comerio et al. (1998) showed that *P. citrinum* was not able to produce citrinin below an a_w of 0.8 however a high production at a_w 0.885 could be determined. This demonstrates that citrinin can be produced by *P. citrinum* at very harsh conditions. This is confirmed by results of Touhami (unpublished) which clearly shows that citrinin biosynthesis in *P. citrinum* is much less regulated compared to *P. verrucosum* and *P. expansum*. *P. citrinum* generally produces high amounts of citrinin, and reduction is only possible under very extreme conditions indicating a much higher independence from regulatory mechanisms compared to the situation in *P. verrucosum* and *P. expansum*. Table 1 shows an overview about the influence of environmental factors on the biosynthesis of the three mycotoxins.

VII. Signal Transduction Pathways Involved in the Regulation of the Treated Mycotoxins

Changes in the environment, which influence mycotoxin production at the transcriptional level, as was shown for mycotoxin biosynthesis genes, must be transmitted from the external environment to the internal transcription machinery. This is ensured via signal transduction pathways like HOG MAP kinase, GTP/cAMP/Pka, or pacC pathways. HOG signaling pathways (high osmolarity glycerol) are often involved in sensing changes in external osmotic

Table 1 Influence of environmental factors on the biosynthesis of citrinin, ochratoxin, and citrinin

NaCl			
P. nordicum	High	High	Ochratoxin
P. verrucosum	Low	High	Ochratoxin
P. verrucosum	High	Low	Citrinin
P. expansum	Low	High	Citrinin
P. expansum	High	Low	Patulin
P. citrinum	High	High	Citrinin
NaCl concentration	**Low**	**High**	
pH			
P. expansum	High	Low	Patulin
P. expansum	Low	High	Citrinin
P. citrinum	High	High	Citrinin
pH	**Acidic**	**Neutral–alkaline**	
Oxidative stress			
P. verrucosum	High	Low	Ochratoxin
P. verrucosum	Low	High	Citrinin
P. expansum	Low	High	Citrinin
P. citrinum	High	High	Citrinin
Oxidative stress	**Low**	**High**	

conditions, which subsequently leads to adaptation of the expression of certain genes (Rispail et al. 2009). However, it can also be involved in responses toward changes in the oxidative status of the fungal cell induced by external challenges. In addition, certain GTP/cAMP/PKA signaling pathways are involved in responses to changes of the oxidative status of the cell (García-Rico et al. 2008). Changes in external pH are sensed and transmitted to the transcriptional level by the pacC transduction pathway (Merhej et al. 2011). The pacC cascade is activated under alkaline conditions and represses acid regulated genes while inducing alkaline regulated genes (Penalva and Arst 2002). In several experiments, it has been shown that also the transcription of the secondary metabolite biosynthetic gene clusters is under the control of signal transduction pathways. However, this control is apparently species and mycotoxin specific and may act either positive or negative, depending on the specific conditions. For example, Kohut et al. (2009) could show that the HOG pathway is involved in the activation of fumonisin biosynthesis under nitrogen starvation stress in *F. proliferatum*. On the other hand, the biosynthesis of tenuazonic acid, a mycotoxin produced by *Alternaria alternata*, is inhibited by HOG. Yun et al. (2015) showed that HOG gene inactivated mutants produced more tenuazonic acid than the wild type. A heterotrimeric G-protein/PKA pathway is involved in sterigmatocystin biosynthesis in *A. nidulans*, respectively, in aflatoxin biosynthesis in *A. flavus*. This signal cascade positively regulates *afl*R expression. The gene *afl*R codes for the transcription regulator of the sterigmatocystin or aflatoxin gene clusters (Georgianna and Payne 2009). Shimizu and Keller (2001) showed that downstream from the heterotrimeric G-protein, a cAMP-dependent protein kinase A (PKA), is responsible for further signal transduction. When the *pka* gene is overexpressed, the expression of the *afl*R gene is negatively influenced in *A. nidulans*, and sterigmatocystin biosynthesis is reduced. Roze et al. (2004) further showed that the addition of cAMP to solid growth medium induced *afl*R transcription and thereby aflatoxin biosynthesis by *A. parasiticus*. Hong et al. (2013) demonstrated that cAMP/PKA pathways can be activated by increased oxidative stress conditions, which leads to increased aflatoxin biosynthesis.

Also the pH-responsive pacC signal cascade can be involved in the regulation of certain mycotoxins. Merhej et al. (2011) showed that pacC played a role in the regulation of TRI gene expression and trichothecene biosynthesis in *F. graminearum*. The fungus only produces trichothecenes under acidic pH conditions. A *pac*C deletion mutant produced trichothecenes earlier, compared to the wild type. A mutant with a constitutive, nonregulated *pac*C gene showed strong reduction of the expression of the TRI genes, indicating that *pac*C obviously has a negative regulatory function upon trichothecene biosynthesis. An influence of the *pac*C gene on alternariol biosynthesis by *A. alternata* was also shown by Graf et al. (2012). The wild type of *A. alternaria* produces alternariol over a wide pH range from about 4 to 10. A mutant in the *pac*C gene however was only able to produce alternariol up to pH 7, but not under alkaline conditions, indicating the involvement of *pac*C in the regulation of alternariol biosynthesis.

In case of ochratoxin A biosynthesis by *P. nordicum*, it could be shown, as mentioned above, that increasing NaCl concentrations, increase ochratoxin A biosynthesis (Schmidt-Heydt et al. 2012a). Also for *P. verrucosum* NaCl induces ochratoxin biosynthesis. On YES medium with no additional NaCl, *P. verrucosum* usually produces moderate amounts of citrinin, but no or low amounts of ochratoxin. However, at increasing NaCl concentrations, the biosynthesis is shifted toward ochratoxin biosynthesis. This indicates that under high NaCl conditions, the production of ochratoxin A is preferred for both *Penicillium* species. In order to analyze if a HOG MAP kinase regulation is involved in these processes, Stoll et al. (2013) performed Western blotting experiments in order to monitor differences in HOG phosphorylation. The HOG protein is the last kinase of the HOG MAP kinase pathway, and the phosphorylated form of HOG is the active form, which regulates transcription. According to the obtained results, there is a clear difference in the phosphorylation of HOG between the two species. In *P. nordicum*, which pro-

duces ochratoxin in a wide NaCl concentration range (0–100 g/L), HOG was phosphorylated over the whole concentration range. In case of *P. verrucosum*, in which ochratoxin A biosynthesis is predominately produced at high NaCl concentrations, HOG was not or very low phosphorylated at low NaCl concentrations but was increasingly phosphorylated at enhanced NaCl concentrations. This suggests that the enhanced phosphorylation of HOG is a signal for the shift of the biosynthesis from citrinin toward ochratoxin in this species (Schmidt-Heydt et al. 2013).

As shown above, the activation of HOG, by challenging osmotic conditions, leads to an increase in ochratoxin A biosynthesis in *P. verrucosum*, however, at the expense of the biosynthesis of citrinin. In contrast, by choosing conditions which increase oxidative stresses, *P. verrucosum* shifts ochratoxin A biosynthesis toward citrinin. This was shown by increasing the concentration of Cu_2SO_4 (0–40 mg/L) in the medium. Increasing concentrations gradually increases citrinin biosynthesis. Already above a concentration of 10 mg/L, no ochratoxin A was produced by *P. verrucosum* (Schmidt-Heydt et al. 2015). Cu^{2+} ions increase oxidative stress conditions (Gaetke and Chow 2003) in biological systems. Because changes in oxidative stress conditions in the environment are often targeted to the transcriptional level by GTP/cAMP/PKA pathways (García-Rico et al. 2008), the influence of cAMP on the regulation of citrinin biosynthesis was analyzed. Interestingly increased amounts of externally added cAMP decreased citrinin biosynthesis significantly. Moreover, the measured ROS level (reactive oxygen species), which is an indication for elevated cellular stress conditions, increases in a fungal strain not able to produce citrinin at increasing Cu^{2+} concentrations, whereas it stays constant, respectively, and even decreases in a *P. verrucosum* strain able to produce citrinin, indicating a protective, antioxidative effect of this mycotoxin.

Not that much is known about the involvement of signal transduction pathways in *P. expansum* and *P. citrinum*. Barad et al. (2014) reported that pacC has an influence on patulin biosynthesis by *P. expansum*.

VIII. Biological Importance of Mycotoxin Biosynthesis

For most mycotoxins or secondary metabolites, the biological reason for their biosynthesis is not known. Several different possibilities are being discussed as ecological reasons for mycotoxin biosynthesis. For example, certain secondary metabolites and mycotoxins serve as antibiotics to act against competing microorganisms. For patulin and citrinin, strong antibiotic activities have been described (Puel et al. 2010; Heller and Röschenthaler 1978). Other potential reasons for the biosynthesis of mycotoxins, which are debated, are their possible activity as storage components for certain primary metabolites, like, for example, acetyl or malonyl units in case of polyketides. In fact the concentration of the mycotoxin produced is not static, but the mycotoxin can be degraded down to quite low levels in a later life phase of the fungus (Abrunhosa et al. 2010). Moreover, as was shown for ochratoxin biosynthesis, the secondary metabolite concentration in the stationary phase may also oscillate around a certain concentration. An almost daily oscillation was shown for ochratoxin biosynthesis by *P. nordicum* (Schmidt-Heydt et al. 2010). This oscillation was partly coupled to the circadian rhythm. Ochratoxin A was degraded under light conditions and resynthesized under dark conditions. In fact for *A. niger*, an ochratoxinase, a putative amidase gene, which is able to degrade ochratoxin A, was described (Dobritzsch et al. 2014). Abrunhosa and Venancio (2007) also isolated an enzyme from *A. niger*, which could degrade ochratoxin A into ochratoxin α and phenylalanine. Ochratoxin α is the chlorinated polyketide part of the ochratoxin A molecule. In this regard, it is interesting to note that in the vicinity of the *otapks*PN gene, an alkaline serine protease is located. In ochratoxin, phenylalanine is coupled via peptide linkage to the polyketide part of the molecule.

The view that the production of mycotoxins (secondary metabolites) is an adaptation to a specific environment is an intriguing hypothesis as a biological reason for the production of these substances (Roze et al. 2011, 2013) and

might be one of the reasons why mutants in mycotoxin biosynthesis often grow equally well compared to the wild type under laboratory conditions. However, viability differences under certain more stressful conditions might be found. Certain abiotic stresses, for example, induces mycotoxin biosynthesis (Jayashree and Subramanyam 2000; Schmidt-Heydt et al. 2008; Jurado et al. 2008). This induction in mycotoxin biosynthesis by abiotic stresses might increase the fitness of the producing organism under these specific conditions.

This situation seems to be the case, for example, for ochratoxin A biosynthesis in case of *P. nordicum* and *P. verrucosum*. As mentioned above *P. nordicum* occurs regularly in fermented foods with high concentration of NaCl. High NaCl concentrations pose a specific stress toward the fungus. Samapundo et al. (2010), for example, showed that chloride salts, like NaCl or $MgCl_2$, had a higher inhibitory activity on growth of fungi than other salts, like, for example, $MgSO_4$. As mentioned above higher NaCl concentrations either increase the biosynthesis of ochratoxin A in *P. nordicum* or shift the secondary metabolism from citrinin to ochratoxin in case of *P. verrucosum*. In this context, it is interesting to note that ochratoxin A carries a chloride in its molecule and that it is permanently synthesized and excreted due to the oscillating mechanism described above. According to Atoui et al. (2007), ochratoxin A is rapidly excreted into the medium, which overall obviously increases the viability of these fungi in NaCl-rich environments (Schmidt-Heydt et al. 2012a).

On the other hand, elevated citrinin biosynthesis in Penicillia is observed under increasing conditions of oxidative stress (Touhami et al. 2018). Indeed, Heider et al. (2006) showed that the citrinin molecule has antioxidative properties. Interestingly, in fungi, high NaCl concentrations can increase internal oxidative stress due to increased oxidative respiration as a secondary function (Petrovic 2006), and beside ochratoxin, the production of citrinin might therefore be a further response to increased NaCl concentrations, in case the fungus is not able to produce ochratoxin (like, e.g., *P. citrinum*). In this respect, it is interesting to note that beside ochratoxinogenic species, many citrinin-producing species occur in salt-rich environments. Butinar et al. (2011) identified *P. citrinum* from hypersaline waters. Lu et al. (2008) identified citrinin derivatives from a halotolerant *P. citrinum* strain. Heperkan et al. (2009) found *P. citrinum* in salted olives, indicating the apparent halotolerance of *P. citrinum*. It could be shown that *P. citrinum* which produces high amounts of citrinin even further increase the citrinin biosynthesis at increasing NaCl concentrations (Touhami, unpublished).

Furthermore, it was shown that citrinin-producing fungi are more resistant against shortwave light and even UV light compared to nonproducing or low-producing fungi (Schmidt-Heydt et al. 2015). Light is also an inductor for oxidative stresses. Citrinin has an absorption optimum at low wavelength (Schmidt-Heydt et al. 2012b). Already Stormer et al. (1998) questioned if citrinin might have a light protectant function, which could be confirmed by Schmidt-Heydt et al. (2015). In these experiments *P. citrinum*, as a strong citrinin-producing fungal species, were most resistant against UV light, in comparison to the other species. Huang et al. (2012) showed that *P. citrinum* spores are very resistant against treatments with ozone, which further supports the hypothesis of citrinin protection against oxidative stress conditions. Eventually because of the protective function of citrinin and because of its capability to produce high amounts of citrinin in a near constitutive fashion, *P. citrinum* is a ubiquitously occurring resistant fungus, and its conidiospores are one of the most frequent ones found in the air (Nayar and Jothish 2013).

Beside adaptation to certain stressful environments, the production of some mycotoxins has been shown to be important with respect to pathogenicity for plant colonizing species. For example, Desjardins et al. (1992) showed by disruption of the Tri5 gene, a key gene in trichothecene biosynthesis of *F. graminearum*, that the resulting mutants were, in contrast to the wild type, no longer able to colonize host

plants (parsnip roots). For saprophytic strains of *A. alternata*, it was shown that the production of alternariol supports the postharvest colonization of tomatoes (Graf et al. 2012). In contrast to the wild type, a mutant in alternariol biosynthesis was no longer able to colonize healthy tomato tissue. However, after addition of external purified alternariol, the mutant could grow on tomatoes in a concentration-dependent manner. Also for the production of patulin by *P. expansum*, a function during the colonization of apples has been described by Sanzani et al. (2012). These authors inactivated the MSAS-type PKS gene of *P. expansum*. The mutants were leaky and still were able to produce patulin, however, at an either 33 or 41% lower concentration compared to the wild type. During colonization experiments on apples, it could be shown that the mutants had a reduced capacity to colonize apples compared to the wild type. This however was not confirmed by the work of Ballester et al. (2015). These authors deleted patulin or citrinin biosynthesis pathway genes. All respective *P. expansum* mutants were not able to produce either patulin or citrinin. However, during their infection experiment, neither patulin nor citrinin mutants differed in their colonization of apples compared to the wild type. From these results the authors concluded that neither patulin nor citrinin plays roles as pathogenicity factors. This was confirmed by results described by Li et al. (2015). Also these authors found, by gene inactivation of *pat*K and *pat*L, no evidence that patulin is involved in the pathogenicity of *P. expansum*. The discrepancy in the published results could be clarified by Snini et al. (2015). They showed by inactivation of the *pat*L gene that patulin may play a role as pathogenicity factor but that this role is dependent on the apple variety analyzed. Barad et al. (2014) also demonstrated an effect of patulin as pathogenicity factor; however they showed that there is an interplay with D-gluconic acid as a further pathogenicity factor. So it can be concluded, and this fits with the adaptation hypothesis described above, that the mycotoxin patulin is not a pathogenicity factor per se but supports colonization (adaptation) under certain (perhaps stressful) conditions. In comparison to patulin, which seems to be an important colonization factor for plant tissues, citrinin might be supportive for *P. expansum* to adapt to NaCl-rich environments. Experiments performed by Touhami et al. (2018) revealed that patulin is produced early during the infection process of apples by *P. expansum*, whereas citrinin is produced late. Again, these experiments revealed that citrinin has protective antioxidative activities, which support the infection process.

IX. Conclusions

Despite the otherwise diverse distribution of *Penicillium nordicum*, *P. verrucosum*, *P.expansum*, and *P. citrinum*, they all can be found in NaCl-rich habitats, like salterns, saline waters, and salt-rich foods. According to results of Nguyen et al. (2016), that is also true for *P. thymicola*. All of them produce either ochratoxin A or citrinin or both. All of them carry a citrinin cluster (not known yet for *P. thymicola*), either functional or apparently inactive, and most of them contain MSAS-like polyketide synthases, indicating that HGT might also exist in this limited habitat. The production of ochratoxin A and citrinin may support the viability of these fungi under NaCl-rich and/or oxidative conditions. Patulin seems to be a pathogenicity factor in a specific environment. The switch between the biosynthesis of different mycotoxins may support the adaptation to the different habitats.

P. nordicum produces high amounts of ochratoxin. Ochratoxin A apparently acts as a chlorine exporter and increases viability under high NaCl conditions. *P. nordicum* is well adapted and specified to high NaCl environments.

P. citrinum produces high amounts of citrinin. Citrinin apparently acts antioxidative and may serve as a protectant. *P. citrinum* shows no preference to a certain habitat. It occurs ubiquitously and can occur under extreme conditions, including high NaCl-rich habitats.

P. verrucosum can shift ochratoxin and citrinin biosynthesis depending on the exter-

nal conditions. The biosynthesis of ochratoxin and citrinin is interconnected. It generally occurs in cereals but can be found in NaCl-rich habitats. Under the latter conditions rather, ochratoxin A than citrinin biosynthesis is apparently favorable (Schmidt-Heydt et al. 2015).

P. expansum can shift citrinin and patulin biosynthesis in relation to the growth conditions. Both biosynthetic pathways are apparently independent. The fungus generally occurs in apples and other fruits, where the production of patulin is important (Sanzani et al. 2012; Snini et al. 2015) but also can be found NaCl-rich habitats. Its capacity to produce citrinin may support the adaption of this species to the latter habitat.

References

Abramson D, Lombaert G, Clear RM, Sholberg P, Trelka R, Rosin E (2009) Production of patulin and citrinin by *Penicillium expansum* from British Columbia (Canada) apples. Mycotoxin Res 25(2):85–88

Abrunhosa L, Venancio A (2007) Isolation and purification of an enzyme hydrolysing ochratoxin A from *Aspergillus niger*. Biotechnol Lett 29:1909–1914

Abrunhosa L, Paterson RRM, Venancio A (2010) Biodegradation of ochratoxin A for food and feed decontamination. Toxins 2:1078–1099

Artigot MP, Loiseau N, Lafitte J, Mas-Reguieg L, Tadrist S, Oswald IP, Puel O (2009) Molecular cloning and functional characterization of two CYP619 cytochrome P450s involved in the biosynthesis of patulin in *Aspergillus clavatus*. Microbiology 155:1738–1747

Asefa DT, Gjerde RO, Sidhu MS, Langsrud S, Kure CF, Nesbakken T, Skaar I (2009) Moulds contaminants on Norwegian dry-cured meat products. Int J Food Microbiol 128:435–439

Atoui A, Mitchell D, Mathieu F, Magan N, Lebrihi A (2007) Partitioning of ochratoxin A in mycelium and conidia of *Aspergillus carbonarius* and the impact on toxin contamination of grapes and wine. J Appl Microbiol 103:961–968

Bacha N, Atoui A, Mathieu F, Liboz T, Lebrihi A (2009) *Aspergillus westerdijkiae* polyketide synthase gene "aoks1" is involved in the biosynthesis of ochratoxin A. Fungal Genet Biol 46:77–84

Ballester AR, Marcet-Houben M, Levin E, Sela N, Selma-Lázaro C, Carmona L, Wisniewski M, Droby S, Gonzáles-Candelas L, Gabaldón T (2015) Genome, transcriptome, and functional analyses of *Penicillium expansum* provide new insights into secondary metabolism and pathogenicity. Mol Plant Microbe Interact 28:232–248

Barad S, Horowitz SB, Kobiler I, Sherman A, Prusky D (2014) Accumulation of the mycotoxin patulin in the presence of gluconic acid contributes to pathogenicity of *Penicillium expansum*. Mol Plant Microbe Interact 27:66–77

Battilani P, Giorni P, Pietri A (2003) Epidemiology of toxin-producing fungi and ochratoxin A occurrence in grape. Eur J Plant Pathol 109:715–722

Battilani P, Pietri A, Giorni P, Formenti S, Bertuzzi T, Toscani T, Virgili R, Kozakiewicz Z (2007) *Penicillium* populations in dry-cured ham manufacturing plants. J Food Protect 70:975–980

Battilani P, Formenti S, Toscani T, Virgili R (2010) Influence of abiotic parameters on ochratoxin A production by a *Penicillium nordicum* strain in dry-cured meat model systems. Food Control 21:1739–1744

Bayman P, Baker JL (2006) Ochratoxins: a global perspective. Mycopathologia 162:215–223

Beck J, Ripka S, Siegner A, Schiltz E, Schweizer E (1990) The multifunctional 6-methylsalicylic acid synthase gene of *Penicillium patulum*. Eur J Biochem 192:487–498

Berk D, Behie LA, Jones A, Lesser BH, Gaucher G (1984) The production of the antibiotic patulin in a three phase fluidized bed reactor I. Effect of medium composition. Can J Chem Eng 62:112–119

Bhatnagar D, Yu J, Ehrlich KC (2002) Toxins of filamentous fungi. In: Breitenbach M, Crameri R, Lehrer SB (eds) Fungal allergy and pathogenicity, vol 81. Karger, Basel, pp 167–206

Bogs C, Battilani P, Geisen R (2006) Development of a molecular detection and differentiation system for ochratoxin A producing *Penicillium* species and its application to analyse the occurrence of *Penicillium nordicum* in cured meats. Int J Food Microbiol 107:39–47

Bonerba E, Ceci E, Conte R, Tantillo G (2010) Survey of the presence of patulin in fruit juices. Food Addit Contam 3:114–119

Bragulat MR, Abarca ML, Cabanes FJ (2008) Low occurrence of patulin- and citrinin-producing species isolated from grapes. Lett Appl Microbiol 47:286–289

Braunberg RC, Barton CN, Gantt OO, Friedman L (1994) Interaction of citrinin and ochratoxin A. Nat Toxins 2:124–131

Butinar L, Frisvad JC, Gunde-Cimerman N (2011) Hypersaline waters - a potential source of foodborne toxigenic Aspergilli and Penicillia. FEMS Microbiol Ecol 77:186–199

Cabañes FJ, Sanserverino W, Castellá G, Bragulat MR, Cigliano RA, Sanchez A (2015) Rapid genome resequencing of an atoxigenic strain of *Aspergillus carbonarius*. Nat Sci Rep. https://doi.org/10.1038/srep09086

Cairns-Fuller V, Aldred D, Magan N (2005) Water, temperature and gas composition interactions

affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. J Appl Microbiol 99:1215–1221

Castella G, Larsen TO, Cabanes J, Schmidt H, Alboresi A, Niessen L, Färber P, Geisen R (2002) Molecular characterization of ochratoxin A producing strains of the genus *Penicillium*. Syst Appl Microbiol 25:74–83

Cheeseman K, Ropars J, Renault P, Dupont J, Gouzy J, Branca A, Abraham AL, Ceppi M, Conseiller E, Debuchy R, Malagnac F, Goarin A, Silar P, Lacoste S, Sallet E, Bensimon A, Giraud T, Brygoo Y (2013) Multiple recent horizontal transfers of a large genomic region in cheese making fungi. Nat Commun. https://doi.org/10.1038/ncomms3876

Chen YP, Tseng CP, Chien IL, Wang WY, Liaw LL, Yuan GF (2008) Exploring the distribution of citrinin biosynthesis related genes among *Monascus* species. J Agric Food Chem 56:11767–11772

Comerio R, Fernández Pinto VE, Vaamonde G (1998) Influence of water activity on Penicillium citrinum growth and kinetics of citrinin accumulation in wheat. Int J Food Microbiol 42(3):219–223

Comi G, Orlic S, Redzepovic S, Urso R, Iacumin L (2004) Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. Int J Food Microbiol 96:29–34

Davolos D, Pietrangeli B (2014) A molecular and bioinformatic study on the ochratoxin A (OTA)-producing *Aspergillus affinis* (Section Circumdati). Mycotoxin Res 30:113–122

Desjardins AE, Hohn TM, McCormick SP (1992) Effect of gene disruption of trichodiene synthase on the virulence of *Gibberella pulicaris*. Mol Plant Microbe Interact 5:214–222

Dobritzsch D, Wang H, Schneider G, Yu S (2014) Structural and functional chracaterization of ochratoxinase, a novel mycotoxin-degrading enzyme. Biochem J 462:441–452

Dombrink-Kurtzman MA (2006) The isoepoxydon dehydrogenase gene of the patulin metabolic pathway differs for *Penicillium griseofulvum* and *Penicillium expansum*. Antonie van Leeuwenhoek 89:1–8

Dombrink-Kurtzman MA (2007) The sequence of the isoepoxydon dehydrogenase gene of the patulin biosynthestic pathway in *Penicillium* species. Antonie van Leeuwenhoek 91:179–189

Dombrink-Kurtzman MA, Engberg AE (2006) *Byssochlamys nivea* with patulin-producing capability has an isoepoxydon dehydrogenase gene (*idh*) with sequence homology to *Penicillium expansum* and *P. griseofulvum*. Mycol Res 110:1111–1118

European Food Safety Authority (2012) Scientific opinion on the risks for public and animal health related to the presence of citrinin in food and feed. EFSA J 10:2605

Ferrara M, Magista D, Epifani F, Cervellieri S, Lippolis V, Gallo A, Perrone G, Susca A (2016) Study of gene expression and OTA production by *Penicillium nordicum* during a small-scale seasoning process of salami. Int J Food Microbiol 227:51–55

Frisvad J, Frank JM, Houbraken JAMP, Kuijpers AFA, Samson RA (2004) New ochratoxin A producing species of *Aspergillus* section Circumdati. Stud Mycol 50:23–43

Gaetke LM, Chow CK (2003) Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189:147–163

Gallo A, Bruno K, Solfrizzo M, Perrone G, Mulè G, Visconti A, Baker SE (2012) New insight in the ochratoxin A biosynthetic pathway by deletion of an nrps gene in *Aspergillus carbonarius*. Appl Environ Microbiol 78:8208–8218

Gallo A, Ferrara M, Perrone G (2013) Phylogenetic study of polyketide synthases and nonribosomal peptide synthetases involved in the biosynthesis of mycotoxins. Toxins 5(4):717–742

Gallo A, Knox BP, Bruno KS, Solfrizzo M, Baker SE, Perrone G (2014) Identification and characterization of the polyketide synthase involved in ochratoxin A biosynthesis in *Aspergillus carbonarius*. Int J Food Microbiol 179:10–17

García-Rico RO, Fierro F, Martín JF (2008) Heterotrimeric G protein Pga1 of *Penicillium chrysogenum* controls conidiation mainly by a cAMP-independent mechanism. Biochem Cell Biol 86: 57–69

Geisen R (2004) Molecular monitoring of environmental conditions influencing the induction of ochratoxin A biosynthetic genes in *Penicillium nordicum*. Mol Nutr Food Res 48:532–540

Geisen R, Schmidt-Heydt M, Karolewiez A (2006) A gene cluster of the ochratoxin A biosynthetic genes in *Penicillium*. Mycotoxin Res 22:134–141

Georgianna DR, Payne GA (2009) Genetic regulation of aflatoxin biosynthesis: from gene to genome. Fungal Genet Biol 46:113–125

Gil-Serna J, Vásquez C, Sardinas N, González-Jaén MT (2010) Revision of ochratoxin a production capacity by the main species of *Aspergillus* section Circumdati, *Aspergillus steynii* revealed as the main risk of OTA contamination. Food Control 22:1–3

Gil-Serna J, Vázquez C, González-Jaén MT, Patiño B (2015) Clustered array of ochratoxin A biosynthesis genes in *Aspergillus steynii* and their expression patterns in permissive conditions. Int J Food Microbiol 214:102–108

Graf E, Schmidt-Heydt M, Geisen R (2012) HOG MAP kinase regulation of alternariol biosynthesis in *Alternaria alternata* is important for substrate colonization. Int J Food Microbiol 157:353–359

He Y, Cox RJ (2016) The molecular steps of citrinin biosynthesis in fungi. Chem Sci 7:2119–2127

Heider EM, Harper JK, Grant DM, Hoffmann A, Dugan F, Tomer DP, O'Neil KO (2006) Exploring unusual antioxidant activity in a benzoic acid derivative: a

proposed mechanism for citrinin. Tetrahedron 62: 1199–1208
Heller K, Röschenthaler R (1978) Inhibition of protein synthesis in *Streptococccus faecalis* by ochratoxin A. Can J Microbiol 24:466–472
Heperkan D, Dazkir GS, Kansu DZ, Güler FK (2009) Influence of temperature on citrinin accumulation by *Penicillium citrinum* and *Penicillium verrucosum* in black table olives. Toxin Rev 28:180–186
Hetherington AC, Raistrick H (1931) Studies in the biochemistry of microorganisms. Part XIV. On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by *Penicillium citrinum* Thom. Philos Trans R Soc Lond B 220B:269–295
Höhler D (1998) Ochratoxin A in Lebens- und Futtermitteln: Vorkommen, gesetzliche Regelung und Wirkungsmechanismen. Eur J Nutr 37(1):2–12
Hong SY, Roze LV, Wee J, Linz J (2013) Evidence that a transcription factor regulatory network coordinates oxidative stress response and secondary metabolism in Aspergilli. Microbiology 2:144–160
Huang HL, Lee MG, Tai JH (2012) Controlling indoor bioaerosols using a hybrid system of ozone and catalysts. Aerosol Air Qual Res 12:73–82
Jayashree T, Subramanyam C (2000) Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus*. Free Radic Biol Med 29:981–985
Jurado M, Marín P, Magan N, González-Jaén MT (2008) Relationship between solute and matric potential stress, temperature, growth, and FUM1 gene expression in two *Fusarium verticillioides* strains from Spain. Appl Environ Microbiol 74:2032–2036
Kohut G, Ádám AL, Fazekas B, Hornok L (2009) N-starvation stress induced FUM gene expression and fumonisin production is mediated via the HOG-type MAPK pathway in *Fusarium proliferatum*. Int J Food Microbiol 130:65–69
Larsen TO, Svendsen A, Smedsgaard J (2001) Biochemical characterization of ochratoxin A-producing strains of the genus *Penicillium*. Appl Environ Microbiol 67:3630–3635
Lee CL, Hung HK, Wang JJ, Pan TM (2007) Improving the ratio of monacolin K to citrinin production of *Monascus purpureus* NTU 568 under dioscorea medium through the mediation of pH value and ethanol addition. J Agric Food Chem 55:6493–6502
Leong SL, Hien LT, An TV, Trang NT, Hocking AD, Scott ES (2007) Ochratoxin A-producing Aspergilli in Vietnamese green coffee beans. Lett Appl Microbiol 45:301–306
Li YP, Tang X, Wu X, Xu Y, Huang ZB, He QH (2012) Isolation and characterization of the citrinin biosynthetic gene cluster from *Monascus aurantiacus*. Biotechnol Lett 34:131–136
Li B, Zong Y, Du Z, ChenY ZZ, Qin G, Zhao W, Tian S (2015) Genomic characterization reveals insights into patulin biosynthesis and pathogenicity in *Penicillium* species. Mol Plant Microbe Interact 28:635–647
Lu ZY, Lin ZJ, Wang WL, Du L, Zhu TJ, Fang YC, QQ G, Zhu WM (2008) Citrinin dimers from the halotolerant fungus *Penicillium citrinum* B-57. J Nat Prod 71:543–546
Lund F, Frisvad JC (2003) *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. J Appl Microbiol 95:1117–1123
Markov K, Pleadin J, Bevardi M, Vahcic N, Sokolic-Mihalak D, Frece J (2013) Natural occurrence of aflatoxin B1, ochratoxin and citrinin in Croatian fermented meat products. Food Control 34: 312–317
Merhej J, Richard-Forget F, Barreau C (2011) The pH regulatory factor Pac1 regulates Tri gene expression and trichothecene production in *Fusarium graminearum*. Fungal Genet Biol 48:275–284
Moake MM, Padilla-Zakour OI, Worobo RW (2005) Comprehensive review of patulin control methods in foods. Compr Rev Food Sci Food Safety 1:8–21
Morales H, Barros G, Marín S, Chulze S, Ramos AJ, Sanchis V (2008) Effects of apple and pear varieties and pH on patulin accumulation by *Penicillium expansum*. J Sci Food Agr 88(15):2738–2743
Morello LG, Sartori D, de Oliveira Martinez AL, Vieira MLC, Taniwaki MH, Fungaro MHP (2007) Detection and quantification of *Aspergillus westerdijkiae* in coffee beans based on selective amplification of b-tubulin gene by using real-time PCR. Int J Food Microbiol 119:270–276
Nayar TS, Jothish PS (2013) An assessment of the air quality in indoor and outdoor air with reference to fungal spores and pollen grains in four working environments in Kerala, India. Aerobiologia 29(1): 131–152
Nguyen HDT, McMullin DR, Ponomareva E, Riley R, Pomraning KR, Baker SB, Seifert KA (2016) Ochratoxin A production by *Penicillium thymicola*. Fungal Biol. https://doi.org/10.1016/j.funbio.2016.04.002
Nielsen KF, Mogensen JM, Johansen M, Larsen TO, Frisvad JC (2009) Review of secondary metabolites and mycotoxins form the *Aspergillus niger* group. Anal Bioanal Chem 395:1225–1242
Núnez F, Rodriguez MM, Bermúdez ME, Córdoba JJ, Asensio MA (1996) Composition and toxigenic potential of the mould population on dry-cured Iberian ham. Int J Food Microbiol 32:185–197
O'Callaghan J, Caddick MX, Dobson ADW (2003) A polyketide synthase gene required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. Micro biology 149:3485–3491
O'Callaghan J, Coghlan A, Abbas A, Garcia-Estrada C, Martin JF, Dobson ADW (2013) Functional characterization of the polyketide synthase gene required for ochratoxin A biosynthesis in *Penicillium verrucosum*. Int J Food Microbiol 161: 172–181
Ostry V, Malir F, Ruprich J (2013) Producers and important dietary sources of ochratoxin A and citrinin. Toxins 5:1574–1586

Pel HJ, de Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, de Vries RP, Albang R, Albermann K, Andersen MR, Bendtsen JD, Benen JAE, van den Berg M, Breestraat S, Caddick MX, Contreras R, Cornell M, Coutinho PM, Danchin EGJ, Debets AJM, Dekker P, van Dijck PWM, van Dijk A, Dijkhuizen L, Driessen AJM, D'Enfert C, Geysens S, Goosen C, Groot GSP, de Groot PWJ, Guillemette T, Henrissat B, Herweijer M, van den Hombergh JPTW, van den Hondel CAMJJ, van den Heijden RTJM, van der Kaaij RM, Klis FM, Kools HJ, Kubicek CP, van Kuyk PA, Lauber J, Lu X, van der Maarel MJEC, Meulenberg R, Menke H, Mortimer MA, Nielsen J, Oliver SG, Olsthoorn M, Pal K, van Peij NNME, Ram AFJ, Rinas U, Roubos JA, Sagt CMJ, Schmoll M, Sun J, Ussery D, Varga J, Vervecken W, van de Vondervoort PJJ, Wedler H, Wösten HAB, Zeng AP, van Ooyen AJJ, Visser J, Stam H (2007) Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. Nat Biotechnol 25:221–231

Penalva MA, Arst HN (2002) Regulation of gene expression by ambient pH in filamentous fungi and yeasts. Microbiol Mol Biol Rev 66:426–446

Petrovic U (2006) Role of oxidative stress in the extremely salt-tolerant yeast Hortaea werneckii. FEMS Yeast Res. 6:816–822

Petzinger E, Weidenbach A (2002) Mycotoxins in the food chain: the role of ochratoxins. Livestock Prod Sci 76:245–250

Pfohl-Leszkowicz A, Petkova-Bocharova T, Chernozemsky IN, Castegnaro M (2002) Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. Food Addit Contam 19:282–302

Pitt JI (1987) *Penicillium viridicatum, Penicillium verrucosum* and production of ochratoxin A. Appl Environ Microbiol 53:266–269

Pitt JI, Hocking AD (1999) Fungi and food spoilage. Aspen, Gaitherburg, MD

Proctor RH, van Hove F, Susca A, Stea G, Busman M, van der Lee T, Waalwijk C, Moretti A, Ward TJ (2013) Birth, death and horizontal transfer of the fumonisin biosynthetic gene cluster during the evolutionary diversification of *Fusarium*. Mol Microbiol 90:290–306

Puel O, Galtier P, Oswald IP (2010) Biosynthesis and toxicological effects of Patulin. Toxins 2:613–631

Reddy KRN, Spadaro D, Lore A, Gullino ML, Garibaldi A (2010) Potential of patulin production by *Penicillium expansum* strains on various fruits. Mycotoxin Res 26:257–265

Rispail N, Soanes DM, Ant C, Czajkowski R, Grünler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, Valiante V, Yang M, Beffa R, Brakhage AA, Gow NAR, Kahmann R, Lebrun MH, Lenasi H, Perez-Martin J, Talbot NJ, Wendland J, Di Pietro A (2009) Comparative genomics of MAP kinase and calcium-calcineurin signalling compontents in plant and human pathogenic fungi. Fungal Genet Biol 46:287–298

Rodriguez A, Medina A, Cordoba JJ, Magan N (2014) The influence of salt (NaCl) on ochratoxin A biosynthetic genes, growth and ochratoxin A production by three strains of *Penicillium nordicum* on a dry-cured ham-based medium. Int J Food Microbiol 178:113–119

Ropars J, Rodríguez de la Vega RC, López-Villavicencio M, Gouzy J, Sallet B, Dumas E, Lacoste S, Debuchy R, Dupont J, Branca A, Giraud F (2015) Adaptive horizontal gene transfers between multiple cheese-associated fungi. Curr Biol 25:2562–2569

Roze LV, Beaudry RM, Keller NP, Linz JE (2004) Regulation of aflatoxin synthesis by FadA/cAMP/protein kinase A signaling in *Aspergillus parasiticus*. Mycopathologia 158:219–232

Roze LV, Chanda A, Linz JE (2011) Compartmentalization and molecular traffic in secondary metabolism: a new understanding of established cellular processes. Fungal Genet Biol 48:35–48

Roze LV, Hong SY, Linz JE (2013) Aflatoxin biosynthesis: current frontiers. Annu Rev Food Sci Technol 4:293–311

Samapundo S, Deschuyffeleer N, Van Laere D, De Leyn I, Devlieghere F (2010) Effect of NaCl reduction and replacement on the growth of fungi important to the spoilage of bread. Food Microbiol 27(6): 749–756

Sanzani SM, Reverberi M, Punelli M, Ippolito A, Fanelli C (2012) Study on the role of patulin on pathogenicity and virulence of *Penicillium expansum*. Int J Food Microbiol 153:323–331

Schmidt-Heydt M, Magan N, Geisen R (2008) Stress induction of mycotoxin biosynthesis genes by abiotic factors. FEMS Microbiol Lett 284:142–149

Schmidt-Heydt M, Bode H, Raupp F, Geisen R (2010) Influence of light on ochratoxin biosynthesis by *Penicillium*. Mycotoxin Res 26:1–8

Schmidt-Heydt M, Graf E, Stoll D, Geisen R (2012a) The biosynthesis of ochratoxin A by *Penicillium* as one mechanism for adaptation to NaCl rich foods. Food Microbiol 29:233–241

Schmidt-Heydt M, Cramer B, Graf I, Lerch S, Humpf HU, Geisen R (2012b) Wavelength-dependent degradation of ochratoxin and citrinin by light in vitro and in vivo and its implications on *Penicillium*. Toxins 4:1535–1551

Schmidt-Heydt M, Stoll DA, Mrohs J, Geisen R (2013) Intraspecific variability of HOG1 phosphorylation reflects different adaptation levels to salt rich habitats. Int J Food Microbiol 165:246–250

Schmidt-Heydt M, Stoll D, Schütz P, Geisen R (2015) Oxidative stress induces the biosynthesis of citrinin by *Penicillium verrucosum* at the expense of ochratoxin. Int J Food Microbiol 192:1–6

Shimizu K, Keller NP (2001) Genetic involvement of a cAMP-dependent protein kinase in a G protein

signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. Genetics 157:591–600

Shimizu T, Kinoshita H, Ishihara S, Sakai K, Nagai S, Nihira T (2005) Polyketide synthase gene responsible for citrinin biosynthesis in *Monascus purpureus*. Appl Environ Microbiol 71:3453–3457

Snini SP, Tadrist S, Lafitte J, Jamin EL, Oswald IP, Puel O (2014) The gene *pat*G involved in the biosynthesis of patulin, a food-borne mycotoxin, encodes a 6-methylsalycylic acid decarboxylase. Int J Food Microbiol 171:77–83

Snini SP, Tannous J, Heuillard P, Bailly S, Lippi Y, Zehraoui E, Barreau C, Oswald I, Puel O (2015) Patulin is a cultivar-dependent aggressiveness factor favouring the colonization of apples by *Penicillium expansum*. Mol Plant Pathol. https://doi.org/10.1111/mpp.12338

Stander MA, Steyn PS, Lübben A, Miljkovic A, Mantle PG, Marais GJ (2000) Influence of halogen salts on the production of the ochratoxins by *Aspergillus ochraceus* Wilh. J Agric Food Chem 48:1865–1871

Stoll D, Schmidt-Heydt M, Geisen R (2013) Differences in the regulation of ochratoxin A by the HOG pathway in *Penicillium* and *Aspergillus* in response to high osmolar environments. Toxins 5:1282–1298

Stormer FC, Sandven P, Huitfeldt S, Eduard W, Skogstad A (1998) Does the mycotoxin citrinin function as a sun protectant in conidia from *Penicillium verrucosum*? Mycopathologia 142:43–47

Taniwaki MH, Pitt JI, Teixeira AA, Iamanaka BT (2003) The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. Int J Food Microbiol 82:173–179

Tannous J, El Khoury R, Snini SP, Lippi Y, El Khoury A, Atoui A, Lteif R, Oswald IP, Puel O (2014) Sequencing, physical organization and kinetic expression of the patulin biosynthetic gene cluster from *Penicillium expansum*. Int J Food Microbiol 189:51–60

Touhami N, Soukup ST, Schmidt-Heydt M, Kulling SE, Geisen R (2018) Citrinin as an accessory establishment factor of *P. expansum* for the colonization of apples. Int J Food Microbiol 266:224–233

Udagawa S, Tatsuno T (2004) Safety of rice grains and mycotoxins – a historical review of yellow rice mycotoxicoses. Yakushigaku Zasshi 39:321–342

van der Merwe KJ, Steyn PS, Fourie L, Scott DB, Theron JJ (1965) Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature 205(4976):1112–1113

Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CH, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA (2014) Identification and nomenclature of the genus *Penicillium*. Stud Mycol 78:343–371

Wang IK, Reeves C, Gaucher GM (1991) Isolation and sequencing of a genomic DNA clone containing the 3 terminus of the 6-methysalicylic acid polyketide synthetase gene of *Penicillium urticae*. Can J Microbiol 37:86–95

Wang L, Wang A, Wang Q, Liu F, Selvaraj JN, Liu L, Xing F, Zhao Y, Zhou L, Liu Y (2015) Functional characterization of new polyketide synthase genes involved in ochratoxin A biosynthesis in *Aspergillus ochraceus* fc-1. Toxins 7:2723–2738

Wawrzyniak J, Waskiewicz A (2014) Ochratoxin A and citrinin production by *Penicillium verrucosum* on cereal solid substrates. Food Addit Contam 31: 139–148

Welke JE, Hoeltz M, Dottori HA, Noll IB (2011) Patulin accumulation in apples during storage by *Penicillium expansum* and *Penicillium griseofulvum* strains. Braz J Microbiol 42:172–180

White S, O'Callaghan J, ADW D (2006) Cloning and molecular characterization of *Penicillium expansum* genes upregulated under conditions permissive for patulin biosynthesis. FEMS Microbiol Lett 255:17–26

Yun CS, Motoyama T, Osada H (2015) Biosynthesis of the mycotoxin tenuazonic acid by a fungal NRPS-PKS hybrid enzyme. Nat Commun 6:8758

Zong Y, Li B, Tian S (2015) Effects of carbon, nitrogen and ambient pH on patulin production and related gene expression in *Penicillium expansum*. Int J Food Microbiol 206:102–108

Biosystematic Index

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)

B

C

R

Subject Index

Physiology and Genetics, 2nd Edition
The Mycota XV
T. Anke, A. Schüffler (Eds.)