Sustainable Development and Biodiversity 15

Dinesh K. Maheshwari *Editor*

Endophytes: Biology and Biotechnology

Volume 1

Springer

Sustainable Development and Biodiversity

Volume 15

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Endophytes: Biology and Biotechnology

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ISSN 2352-474X ISSN 2352-4758 (electronic)
Sustainable Development and Biodiversity
ISBN 978-3-319-66540-5 ISBN 978-3-319-66541-2 (eBook)
DOI 10.1007/978-3-319-66541-2

Library of Congress Control Number: 2017950013

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

Preface

A two volume set is presented on endophytes under the series "Sustainable development and biodiversity". Endophytes are diverse microbial community comprising of archaeal, bacterial including actinobacteria, fungal and protistic taxa inhabiting in all plants and play major roles in plant growth, fitness and diversification, and this diversity is an integral component of ecology. The microbial world in general and endophytes in particular reflect unique genetic and functional (metabolic) diversity. In the recent scenario, significant attention is being paid to endophytes for metabolites of biotechnological applications for sustainable development. Their diversity varies from genotype to genotype, environment to environment and species to species.

The Volume I "Endophytes: Biology and Diversity" focuses on our current understanding of microbial endophytes such as bacterial endophytes in host colonization, quorum quenching enzymes from endophytes, fungal endophytes for plant and human health, endophytes for agroforestry and biopharmacy, endophytic bacteria and actinobacteria as beneficial partners for intensification of agriculture, genomic features and ecology, diversity and their potential biotechnological applications, promising role of fungal and mycorrhizal endophytes towards eco-friendly green technology and future research. These chapters present a detailed account on the basis for their classification, identification and production of useful metabolites.

This book will be useful to botanists, microbiologists, ecologists, plant pathologists, physiologists, agronomists, molecular biologists, environmentalists, conservationists and NGOs working for the protection of species, loss of genetic material and exploitation of useful endophytes. I am thankful to the contributors of these books for their cooperation and patience in the compilation of this task. I am also thankful for Springer team, particularly Drs. R. Valeria and Takeesha, for their constant support in the publication of this work.

Haridwar, India — Dinesh K. Maheshwari

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Chapter 1
An Introduction to Endophytes

Jaya Arora and K.G. Ramawat

Abstract Endophytic micro-organisms are hidden companions of plants living mutually beneficial life inside the host plant. Though these endophytes are supposed to be associated and evolved with land plants, endophytes are recognised in last century. Beneficial effects of endophytes are attaining importance with the possibility of obtaining novel medicinally important compounds as well as their role in increasing crop productivity because they produce a variety of compounds and interact with other micro-organisms, pathogenic and non-pathogenic. With the development of modern tools and techniques of molecular biology, it has become possible to establish correct identity of these micro-organisms and know the interactions with host and other micro-organisms. In this overview, we present current scenario about endophytes and their use for human welfare.

Keywords Bacterial endophytes · Fungal endophytes · Bioactive metabolites Endophytes in agriculture

1.1 Introduction

Endophytes are organisms living as symptomless colony, maybe during a part of their life cycle, inside the host plants (Stone et al. 2000). The term 'endophyte' was coined by de Bary (1866) to distinguish the epiphytic organisms living on surface of plant. Endophytes belong to diverse taxa such as bacterial, fungal, protistic, archaeal and are generally considered as mutualists. Endophytes are defined as

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_1

organisms isolated from surface-sterilised explants or from within the plant tissue and produce no harm to the host plant (Hallman et al. 2011). Endophytes can be recognised as (1) endophytic Clavicipitaceae; (2) fungal endophytes of dicots; (3) endophytic fungi; (4) other systemic fungal endophytes; (5) fungal endophytes of lichens; (6) endophytic fungi of bryophytes and ferns; (7) endophytic fungi of tree bark; (8) fungal endophytes of xylem; (9) fungal endophytes of root; (10) fungal endophytes of galls and cysts; (11) prokaryotic endophytes of plants (includes endophytic bacteria and actinomycetes) (Stone et al. 2000). They receive protection and nutrition from host plants while providing/facilitating nutrient uptake and protection to the plant against biotic and abiotic stresses and pests. There are evidences that the presence of endophyte may not only influence plant growth, developments, fitness and diversity but also population dynamic, plant community diversity and ecosystem functioning (Saikkonen et al. 1998; Hardoim et al. 2015). Endophytes have been evolved with the plants themselves, and during this long period, they have developed all strategies to live, survive, evolve and refine the relationship with the plant (Chap. 8) (Krings et al. 2007; Yu et al. 2010; Selim et al. 2012; Goyal et al. 2017). Use of the term 'infection' thus should be avoided to describe endophytes in general, except those endophytes involved in diseases as causal agents of disease of the host plant.

Endophytic fungi living asymptomatically in plant tissues may present in almost all plants (Saikkonen et al. 1998). One species of an endophyte may be associated with many plant species, and many species of endophytes may be present in the same species. Some endophytes remain as latent in the host plant, while others may interact with other endophytes, pathogenic or non-pathogenic (Zabalgogeazcoa 2008).

Endophytes have evolved mechanisms to live within the plant by defending themselves against all physical and chemical weapons of the plants, e.g. in plant like *Camptotheca acuminata* produces anticancer compound camptothecin which binds to topoisomerase I to stop cell divisions. The endophytic fungus *Fusarium solani* modified its topoisomerase biding site by alterations in amino acids to escape from harmful effects of camptothecin (Kusari et al. 2011). Therefore, endophytes provide two pronged strategy, one for obtaining novel bioactive secondary metabolites with the help of modern tools of chemistry such as selective high-resolution tandem mass spectrometry [equipped with sources such as electrospray ionisation (ESI), or matrix-assisted laser desorption ionisation (MALDI) and analyser such as quadrupole, time of flight (TOF), magnet, Fourier transform ion cyclotron resonance (FT-ICR)], and secondly, they provide clue about mode of action of these bioactive metabolites.

Mycorrhizal fungi form association with plant roots as ectomycorrhiza or endomycorrhiza and play a key role in ecosystem as they modulate nutrient uptake, carbon cycle and also influence soil structure and consequently ecosystem functionality (Van der Heijdan et al. 2015). Mycorrhiza is not discussed in detail in this article (Chap. 11).

In this brief overview, entire gamut of endophyte–plant relationship in terms of plant physiology (nutrition), plant pathology (interaction-protection), improvement

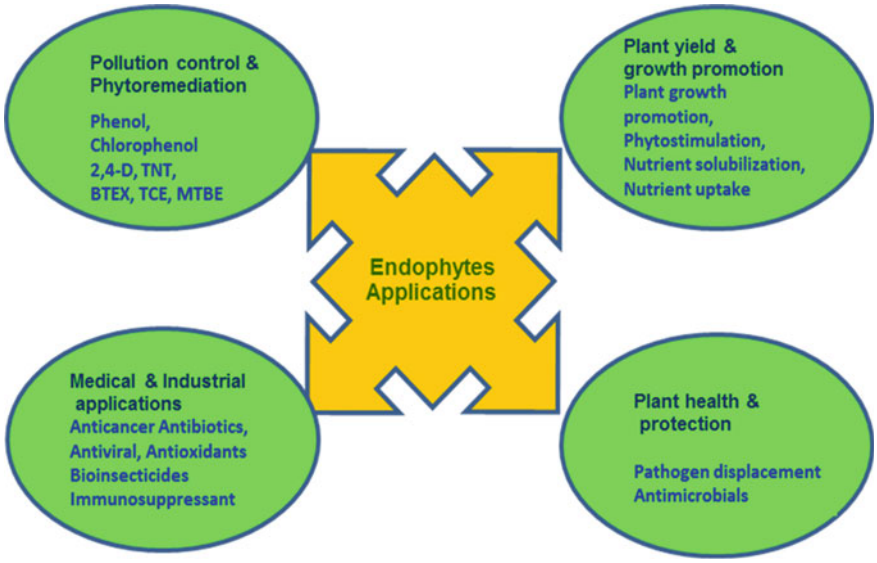

Fig. 1.1 Applications of endophytes in various fields. Examples in each category are symbolic representatives. Pollutant like 2,4-dichlorophenoxyacetic acid (2,4-D) is used as weedicide; petroleum-based products such as benzene–toluene–ethylbenzene–xylene (BTEX), methyl tertiary-butyl ether (MTBE); explosives such as trinitrotoluene used in mining, road and dam making (TNT); trichloroethylene (TCE) is a common solvent

in crop production, pollution control and industrial applications (bioactive molecules) is presented to provide an outlook (Fig. 1.1) of this book. We have tried to summarise these salient applications of endophytes in this brief introduction with the aim that details are presented in various chapters in the book; hence, details of these steps are omitted.

1.2 Origin and Evolution of Endophytes

It is believed that early terrestrial plants evolved in mutualistic association with mycorrhizal fungi which has shaped the plant's life during evolution (Pirozynski and Malloch 1975; Plett and Martin 2015). Fossil record shows that endophytes were associated with land plants for >400 million years ago (Krings et al. 2007). During evolutionary process, plants change habitat from aquatic (oceanic) to terrestrial and were encountered with atmosphere with high carbon dioxide, soil poor in nutrients and fluctuations in temperature and water availability. Under such circumstances, fungi provided endurance to plants to fight with odd conditions and establish themselves on soil (Selosse and Tacon 1998; Bonfante and Selosse 2010). During the same evolutionary period, endophytes have adapted themselves to the

plant microenvironment by genetic variation including uptake of some plant's DNA (Germaine et al. 2004). Due to this adaptation and genetic material uptake, endophytes started producing plant metabolites or their precursors (Stierle et al. 1993, Zhang et al. 2006). Now, endophytes are known to occur in all short of habitats and in different plants such as mosses, ferns, lichens, shrubs, grasses and deciduous and coniferous trees (Sun and Guo 2012). Therefore, they are important part of the ecosystem.

Bacterial endophytes may originate from rhizosphere and phyllosphere microflora and penetrated through roots to reach the xylem tissues (Sturz and Nowak 2000). Preferable site of attachment may be apical root zone with thin-walled cells and basal root zone. Micro-organisms enter the basal root zone through cuts, wounds and other natural opening or made their entry by dissolving cell wall by enzymes such as cellulase and pectinase (Fig. 1.2). Bacteria form small colonies, and cellulase helps in breaking β 1-4 linkage bond of cellulose. Besides cellulase, endophytes produce pectinase, lipoidase, proteinase, phenoloxidase and lignin catabolic enzymes to establish themselves (Wang and Dai 2011). Generally, nitrogen-fixing bacteria (Rhizobia) produce morphological changes in the roots by forming root nodules; otherwise, endophytes remain silent without any morphological change in the system (Malfanova et al. 2013). Only a few bacteria cross the endodermal barrier and enter the xylem tissues. From xylem, bacteria spread to all tissues and organs including reproductive organs and thus penetrates in the developing seeds. Endophytic bacterial density decreases with increasing distance from roots, the rich source of nutrients. In case of fungal endophytes, growth of mycelium is generally along the longitudinal axis of the organ. Endophytes are transferred from generation to generation through seeds (vertical transmission) or may be transferred to allied species through plant part decay/soil (horizontal transmission) (Zabalgogeazcoa 2008; Herrera et al. 2016). This is evident by the fact that generally, meristems are considered free from pathogens, but unique symbiotic *Methylobacterium* endophyte has been reported in Scott pine seedlings which influences functioning of many genes related to growth and development (Pirttila et al. 2008). Therefore, endophytes were associated with plants during their evolution as land plants from very beginning having a mutual relationship. Selected common endophytes and their host are presented in Table 1.1. It is evident from the data presented in the table that diverse plants such as monocots, dicots, trees, gymnosperms and bryophytes contain endophytes.

1.3 Endophyte Diversity

The presence of asymptomatic endophytic fungi in plants was known since nineteenth century (Guerin 1898). It is estimated that more than 1 million endophytic fungal species exist compared to the existence of number of vascular plant species in ratio of 1:4–5 fungi per plant (Sun and Guo 2012). Bacterial endophytes from more than 200 bacterial genera from 16 phyla of both culturable and unculturable

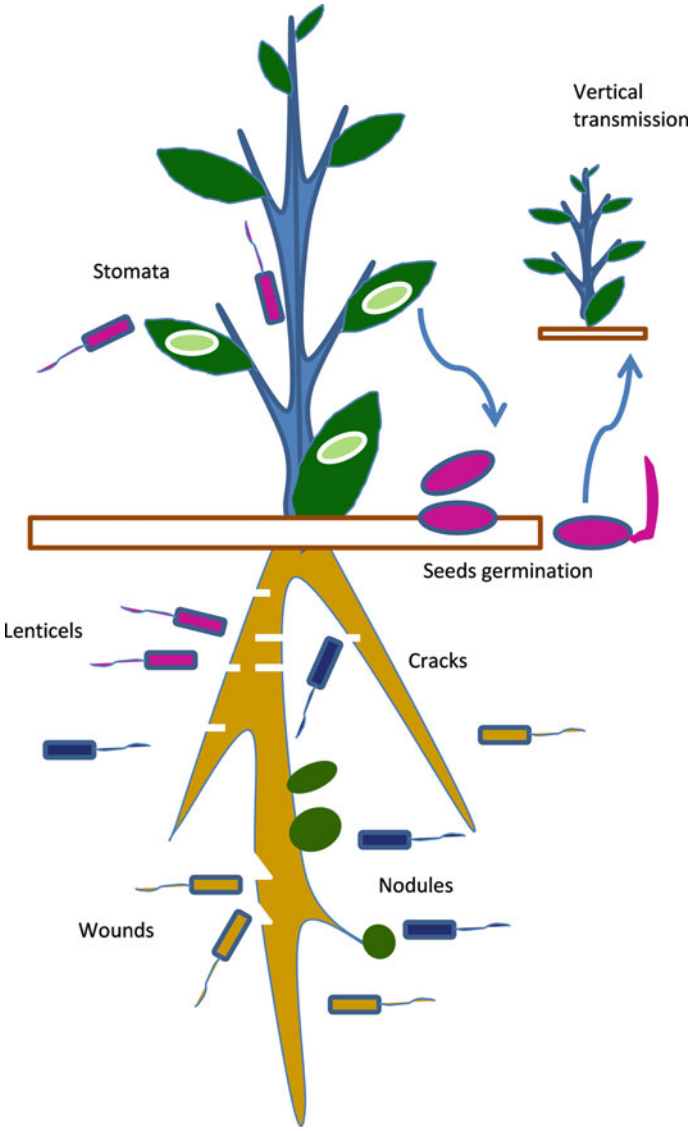

Fig. 1.2 Infection of host plant and transmission of endophytes from generation to generation (*vertical*) through infection of reproductive parts and seeds and allied plants (*horizontal*) through movement in soil. Endophytes enter through cuts, wounds and natural openings like stomata

bacteria belonging to *Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Cholorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes* and *Verrucomicrobiae* have been reported (Hallmann et al. 2011; Sun

Table 1.1 Common endophytes of plants

Endophyte	Plant species	References
Fungal endophytes		
Acremonium sp.	*Taxus chinensis* *Huperzia serrata*	Liu et al. (2009) Glienke-Blanco et al. (2002)
Aspergillus sp.	*Datura stramonium* *Moringa olifera* *Prosopis chilensis*	Mahdi et al. (2014)
Cladosporium sp. *C. herbarum*	*Opuntia ficus indica* *Cinnamomum camphora* *Lycopersicum esculentum* Mill. *Triticum aestivum*	Bezerra et al. (2012) He et al. (2012) Larran et al. (2001) Larran et al. (2002)
Colletotrichum sp. *C. gloeosporiodes*	*Triticum aestivum* *Citrus plants* *Cinnamomum camphora* *Pasania edulis* *Ginkgo biloba* L. *Tectona grandis and Samanea saman* *Huperzia serrata* *Cinnamomum camphora* *Lycopersicum esculentum* Mill.	Larran et al. (2002) Glienke-Blanco et al. (2002) He et al. (2012) Hata and Sone (2008) Thongsandee et al. (2012) Chareprasert et al. (2006) Wang et al. (2011) He el al. (2012) Larran et al. (2001)
Curvularia sp.	*Datura stramonium* *Moringa olifera*	Mahdi et al. (2014)
Penicillium sp.	*Lycopersicum esculentum* Mill. *Huperzia serrata*	Larran et al. (2001) Wang et al. (2011)
Phyllosticta sp.	*Citrus sp.* *Pasania edulis* *Coffea arabica* *Quercus variabilis* *Centella asiatica* *Panax quinquefolium* *Ginkgo biloba* L.	Glienke-Blanco et al. (2002) Hata and Sone (2008) Santamaria and Bayman (2005) Wang et al. (2007) Rakotoniriana et al. (2008) Xing et al. (2010) Thongsandee et al. (2012)
Phomopsis sp.	*Pasania edulis* *Ginkgo biloba* L. *Tectona grandis and Samanea saman* *Taxus chinensis*	Hata and Sone (2008) Thongsandee et al. (2012) Chareprasert et al. (2006) Liu et al. (2009)
Stemphylium globuliferum	*Avicennia marina*	Moussa et al. (2016)
Bacterial endophytes		
Bacillus megatarium	*Medicago satavia,*	Stajkovic et al. (2009)
B. thuringiensis, B. subtilis subsp *subtilis*	*Musa sp.*	Souza et al. (2014)
Burkholderia cepacia	*Lupinus luteus*	Barac et al. (2004)
Enterobacter asburiae	*Ipomoea batatas*	Asis and Adachi (2003)

(continued)

Table 1.1 (continued)

Endophyte	Plant species	References
Erwinia sp.	*Glycine max*	Kuklinsky-Sobral et al. (2004)
Citrobacter	*Musa sp.*	Martinez et al. (2003)
Microbacterium sps.	*Pogonatherum paniceum*	Koskimaki et al. (2010)
Pantoea	*Soyabean (bot name)*	Kuklinsky-Sobral et al. (2004)
Pseudomonas saponiphilia	*Dendrobium candidum*	Wu et al. (2016)
Pseudomonas sp.	*Piper nigrum*	Arvind et al. (2009)
Rhizobium radiobacter	*Daucus carota*	Surette et al. (2003)
Staphylococcus saprophyticus	*Carrot*	Surette et al. (2003)
Micrococcus	*Oryza sativa*	Mbai et al. (2013)
Sporosarcina aquimarina	*Avicennia marina*	Rylo Sona Janarthine et al. (2011)
Other endophytes		
Nostoc	*Leiosporoceros dussii* (Anthocerophyta) *Anthoceros fusiformis and Blasia pusilla*	Villarreal and Renzaglia (2006) Costa et al. (2001)
Oscillatoria	*Alternanthera sessilis*	Keshri and Chatterjee (2010)

and Guo 2012; Sessitsch et al. 2012; Malfonova et al. 2013). Nevertheless, the most prime endophytes belong to three major phyla (Actinobacteria, Proteobacteria and Firmicutes) and include members of *Azoarcus*, *Acetobacter* (renamed as *Gluconobacter*), *Bacillus*, *Enterobacter*, *Burkholderia*, *Herbaspirillum*, *Pseudomonas*, *Serratia*, *Stenotrophomonas* and *Streptomyces* (Malfonova et al. 2013). However, the actual identified numbers of endophytes are very less.

Endophytes gain importance in recent past for their commercial and industrial exploitation. It was after landmark discovery of toxicosis caused by *Neotyphodium coenophialum* (Family Clavicipitaceae) in cattle eating the grass, *Festuca arundinacea* (Bacon et al. 1977). It was recorded that the grass was systemically infected by the fungus without apparent symptoms and that is why escaped from noticing the diseased leaves. The fungus produces several toxic alkaloids which were the actual cause of toxicosis in cattle. This is one example of a fungal endophyte causing toxicity, but a plethora of endophytes may inhabit grasses, and some may remain latent (Zabalgogeazcoa 2008). Due to adaptation and evolution, endophytes of cultivated plants and their wild relatives may differ significantly (Ofek-Lalzar et al. 2016).

Conventionally, micro-organisms are identified on the basis of morphological characters, but in case of bacteria, it is difficult to characterise them on the basis of morphological characters because of their small size. Hence, some physiological

characters of growth and nutrition are added for identification. Modern tools of molecular biology and genetics are helpful in clearly establishing their identity, and genetic bar coding is one of them (Diaz et al. 2012; Sun and Guo 2012). Bar coding of plants and animals is already done to characterise the species, and it is now used for the micro-organisms. Genomic characterisation of living organisms is lead by the Consortium for the Barcode of Life (CBoL; http://barcodeoflife.org/). This information is used for taxonomic classification of the organisms; thus, morphological characters have become of secondary importance. Instead of mitochondrial DNA used for animals and algae, for plants and fungi, ribosomal DNA is used for taxonomy, phylogeny and identification purposes (Rodriguez et al. 2009) because mitochondrial DNA in these organisms has not changed much during evolution. Internal transcribed spacer (ITS) is the most commonly used DNA barcode in molecular identification of endophytes (Sun and Guo 2012) in ecological and diversity studies. Modern techniques of molecular biology are helpful in identification of endophytes, and availability of such facilities in more laboratories associated with microscopic techniques will help in proper characterisation of large number endophytes and will establish their diversity (Chap. 7).

1.4 Isolation of Endophytes

Criteria for isolation of endophytes are closely related to isolation of bioactive molecules, e.g. importance of the plant and its bioactive molecule, rarity of compound, endemic nature of the plant and its environment (Tiwari 2015). Generally, endophytes are isolated from surface-disinfectant tissues grown on a synthetic medium and may or may not containing extracts of host tissues (Galney and Newcombe 2006; Hata and Sone 2008). But synthetic medium may not support the growth of obligate parasites resulting in not getting information about such endophytes. Endophytes have been isolated from almost all the plant parts including leaves, scales, roots, stem and resin canals and even from meristems (Pirttila et al. 2008). Identification of endophytic fungi is done as used for fungi using morphological characters of colony, vegetative hyphae and asexual/sexual spores (conidial development, size, shape, conidia, attachment of conidia and shape of conidial head) (Nagamani et al. 2006). With the advent of tools and techniques of molecular biology, it has become feasible to characterise these micro-organisms on the basis of their molecular markers and establish identity. It was only after the use of tools of molecular biology that many more endophytes could be identified (Duong et al. 2006). These tools are gaining importance in establishing phylogenetic relationship between different taxa also (Duong et al. 2006; Sun and Guo 2012).

1.5 Endophytes and Plant Protection

Endophytes are known to provide various types of protections to their host plant, viz. endurance to grow in hot springs, deter herbivores by producing toxic alkaloids in grasses and provide protection from pests in dicots (Zhang et al. 2006). Endophytes share everything with an invading pathogen in the host plant. Increasing evidences suggest that endophytes interact with the pathogen in different ways in different hosts, and resultantly, altered physiology may suppress the growth of the pathogen, alter nutrient balance in favour of endophyte or stimulate the plant's defence mechanism (Zabalgogeazcoa 2008; Bushby et al. 2016). Many endophytic species produce antibiotics and antifungal compounds (Istifadah and McGee 2006) and provide protection against pathogen with reduced severity (Zabalgogeazcoa 2008). Colonisation of plants by fungal endophyte provides a better protection against plant nematodes. This is a complex phenomenon, and mechanism of this antagonism is poorly understood (Schouten 2016). Thus, endophytes influence functioning of pathosystem and consequently plant's survival, diversity and conservation (Bushby et al. 2016).

About 1000 insect pathogenic fungi ranging from class Chytridiomycetes to Basidiomycetes are known to occur as endophyte, which are closely related to grass endophytic fungi such as *Claviceps* and *Epichloë* (Moonjely et al. 2016). The process of cross-protection is well established in case of viruses. Similar to cross-protection, endophytes provide protection to various pests and herbivores and there is need to understand mechanism underlying this process to exploit it for crop protection (Chap. 4).

1.6 Endophytes and Metabolites

Several important medicines are obtained from plants such as vincristine, vinblastine, camptothecin, quinine and taxol (Ramawat et al. 2009), while more than 8500 bioactive metabolites of fungal origin are known (Demain and Sanchez 2009; Goyal et al. 2017). Association of an endophytic fungi *Taxomyces adreanae* present in *Taxus baccata* to taxol biosynthesis fuelled the search for endophytic fungi associated with promising bioactive molecules and their derivatives (Nicoletti and Fiorentino 2015). This has two repercussions: one the complex evolutionary insight about the microbes and the host plants and second, the possibility of obtaining new bioactive compounds. As we are discussing in different parts of this chapter, isolation and identification of endophytes is still a challenging task, and subsequently, establishment of correlation with the bioactive molecule production is another important task. The challenges to produce them commercially are many (Kusari and Spiteller 2011). Endophytes may produce diverse chemicals as illustrated by classic example of gibberellin production by *Fusarium oxysporum* causing foolish seedling disease of rice. The other classes of compounds include alkaloids, essential oils,

terpenes, azadirachtins, coumarins, flavonoids, lignans and several others (Nicoletti and Fiorentino 2015). A large number of secondary metabolites of potential therapeutic value in cancer, as antioxidants and antimicrobials such as azadirachtin A, B, camptothecin, citrinal B, cytochalasin N, diosgenin, gliotoxin, germacrane-type sesquiterpenes, ginkgolide-B, huperzine A, penicillide derivatives and α-pyrone analogues, piperine, podophyllotoxin, taxol (Paclitaxel), have been isolated from endophytes, and some of the selected examples for bioactive molecules produced by endophytic fungi (Fig. 1.3) and their host plants are presented in Table 1.2. Besides their production, biotransformation of secondary metabolites has been successfully attempted by using endophytes (Pimentel et al. 2011; Wang and Dia 2011). Biotransformation can be defined as the chemical alteration of an exogenous substance by or in a biological system (Wang and Dia 2011). It has been observed

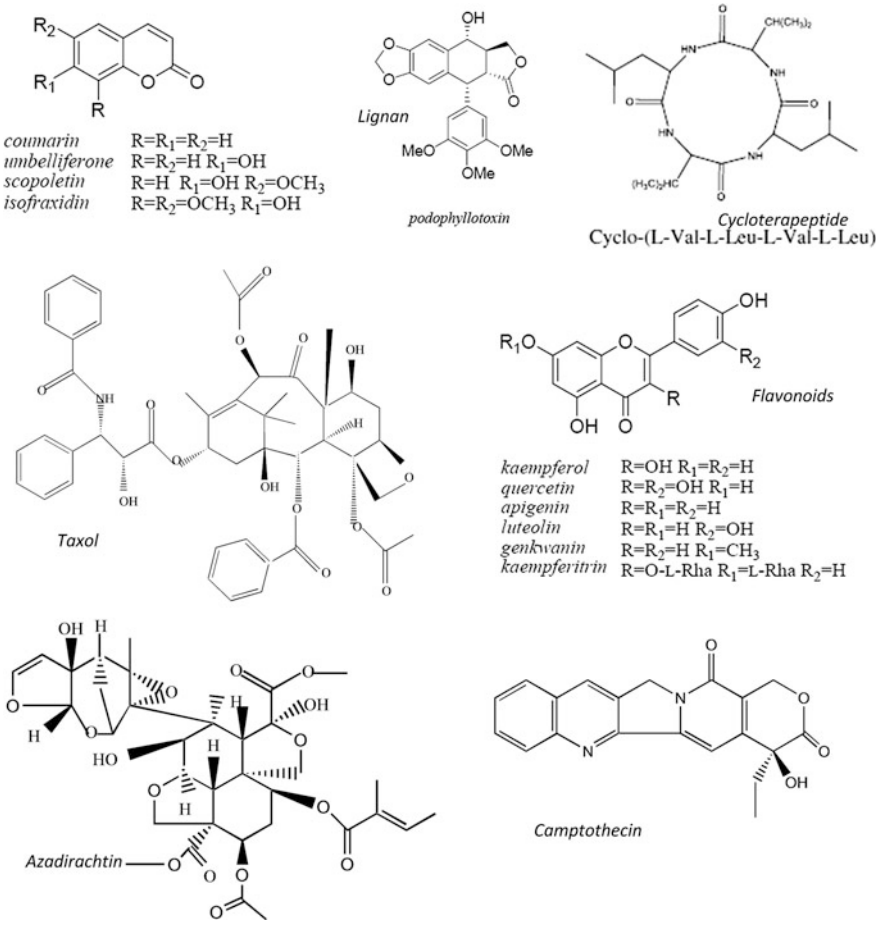

Fig. 1.3 Selected bioactive molecules associated with endophytes and their hosts

Table 1.2 Selected examples of bioactive metabolites produced by endophytes

Compound (Metabolite)	Bioactivity	Endophyte	Plant species	References
(-)-(1R,4R)-1,4-(2,3)-indolmethane-1-methyl-2,4-dihydro-1H-pyrazino-[2,1-b]-quinazoline-3,6-dione	Antifungal and cytotoxic	*Penicillium vinaceum*	*Crocus sativus*	Zheng et al. (2012)
2,3-dihydro,2,2-dimethyl-4 (1H)-quinazolinone	Cytotoxic activity	Actinobacteria-*Streptomyces*	*Lychnophora ericoides*	Conti et al. (2016)
β-sitosterol	Antifungal	*Phoma sp.*	*Arisaema erubescens*	Wang et al. (2012)
Azadirachtin A and B	Insecticidal	*Eupenicillium parvum*	*Azadirachta indica*	Kusari et al. (2012)
Bacctatin III	Anticancer	*Diaporthe phaseolorum, Trichoderma sp.*	Taxus wallichiana var. mairei	Zaiyou et al. (2013), Li et al. (2015)
Campyridones A-D (pyridone alkaloids)	Cytotoxic against Hela cells	*Campylocarpon sp.*	*Sonneratia caseolaris*	Zhu et al. (2016)
Camptothecin	Anticancer	*Fusarium solani*	*Camptotheca accuminata, Apodytes dimidiata*	Shweta et al. (2010), Kusari et al. (2009)
Caryolanes	Anticancer	*Streptomyces sp.*	*Bruguiera gymnorrhiza*	Ding et al. (2015)
Citrinal B	Cytotoxic	*Colletotrichum capsici*	*Capsicum sp.*	Wang et al. (2016)
Cryptocin	antimycotic	*Cryptosporiopsiscf. quercina*	*Tripterygium wilfordii.*	Li et al. (2000)
Cytochalasin N, Cytochalasin H and Epoxycytochalasin H	Antifungal	*Phomopsis sp.*	*Gossypium hirsutum*	Fu et al. (2011)
Diosgenin	Steroidal drugs, oestrogenic effects, antispasmodic	*Cephalosporium sp.*	*Paris pollyphylla*	Cao et al. (2007)

(continued)

Table 1.2 (continued)

Compound (Metabolite)	Bioactivity	Endophyte	Plant species	References
Epipolythiodioxopiperazine and Gliotoxin	Antifungal	*Chaetomium globosum*	*Ginkgo biloba*	Li et al. (2011)
Extracellular enzymes and Auxins (IAA)	Plant growth promoting activity	*Penicillum citrinum, Preussia sp., Aureobasidium*	*Boswellia sacra*	Khan et al. (2016)
Germacrane-type sesquiterpenes	Treatment of cardiovascular disease and cancer	*Streptomyces griseus subsp.*	*Kandelia candel.*	Guan et al. (2005)
Ginkgolide-B	Antiallergic and anti-inflammatory	*Fusarium oxysporum*	*Ginkgo biloba*	Cui et al. (2012)
Huperzine A	Treatment of Alzheimer's disease. Memory enhancement	*Penicillium chrysogenum Penicillium* sp.	*Lycopodium serratum Huperza seretta*	Zhou et al. (2009)
Hypericin	Antidepressant. Mood enhancing, antiviral	*Chaetomium globosum*	*Hypericum perforatum*	Kusari et al. (2008)
Lipopeptides	Antifungal	*Bacillus amyloliquefaciens*	Ornamental plants	Zouari et al. (2016)
Peimisine and imperialine-3-β-D glucoside	Antitumor and antitussive	*Fusarium redolens*	*Fritillaria unibracteata*	Pan et al. (2015)
Penicillide derivatives and α-pyrone analogues	Proteasome inhibitory activity	*Pestalotiopsis sydowiana*	*Phragmites communis*	Xia et al. (2016)
Phomenone, Phaseolinone	Antifungal	*Xylaria sp.*	*Piper aduncum*	Silva et al. (2010)
Piperine	Antimicrobial, antidepressant, anticancer	*Colletotrichum gloeosporioides*	*Piper nigrum*	Chithra et al. (2014)
Podophyllotoxin	Anticancer, antimicrobial, antirheumatic	*Phialocephala fortinii*	*Podophyllum peltatum*	Eyberger et al. (2006)
Taxol (Paclitaxel)	Anticancer	*Taxomyces andreanae* and other several sp.	*Taxus brevifolia*	Kusari et al. (2014)

(continued)

Table 1.2 (continued)

Compound (Metabolite)	Bioactivity	Endophyte	Plant species	References
Thiodiketopiperazine derivatives	Antimicrobial activity (*Staphylococcus aureus*, *S. pyrogenes*)	*Phoma sp.*	*Glycyrrhiza glabra*	Arora et al. (2016)
Resveratrol	Anticancer Lifespan increasing activity	*Aspergillus niger*	*Wine grape* *Carbernet Sauvignon*	Liu et al. (2016)
Vinblastine	Anticancer	*Alternaria*	*Catharanthus roseus*	Guo et al. (1998)
Vincristine	Anticancer	*Fusarium oxysporum*	*Catharanthus roseus*	Zhang et al. (2000)
Vindoline	Antimitotic activity	*Curvularia sp.*, *Choanephora infundibuliphera*	*Catharanthus roseus*	Pandey et al. (2016)

that alterations in the basic molecule may result in a more potent physiologically active compound; e.g., semisynthetic compounds developed from taxol and podophyllotoxin are more potent than the basic molecule (Ramawat et al. 2009). It is evident that several compounds important in medicine, agriculture and industry are produced by endophytes (Chap. 12).

Details of secondary metabolites and other useful metabolites can be found in recent reviews on endophytes (Pimentel et al. 2011; Tiwari 2015; Nisa et al. 2015; Venugopalan and Srivastava 2015; Rehman 2016). Because endophytes influence the growth and metabolism of host plant by influencing nutrients uptake and endurance, they also influence the production of bioactive secondary metabolites of these host plants (Jia et al. 2016). Production of secondary metabolites by endophyte will follow the same course as a plant or fungal metabolites. Once endophyte is isolated and production of metabolites is established, then strategies can be used for its large-scale production using biosynthetic pathway manipulation and other

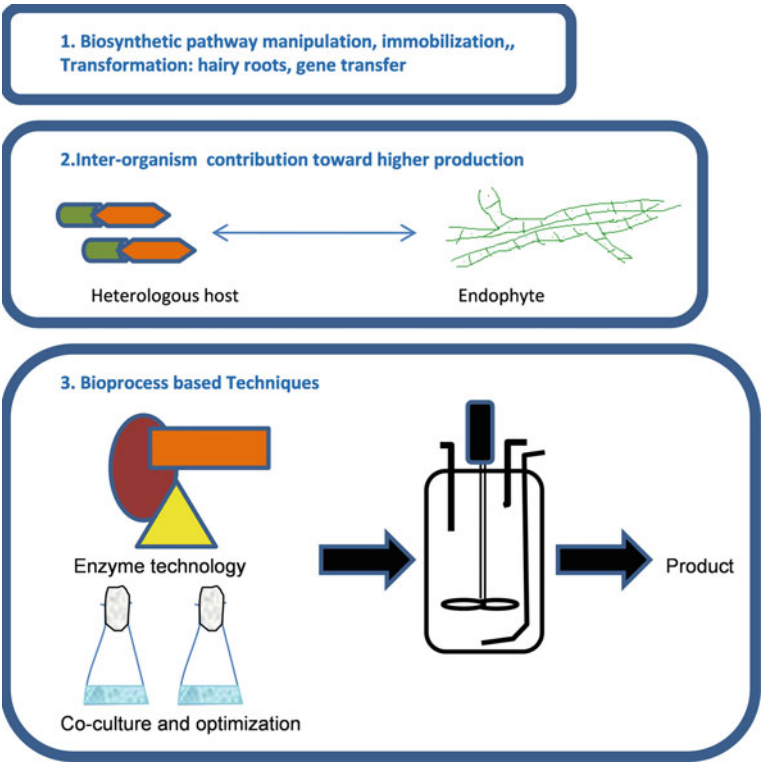

Fig. 1.4 Possible strategies for obtaining secondary metabolites using endophytes. Biosynthetic pathway manipulation and genetic transformation using Agrobacterium species are well-established techniques for plant cells. Several products are produced using heterologous expression system. Scale-up production technology and downstream processing of selected metabolites require optimisation of production system

techniques of biotechnology (Fig. 1.4). Use of heterologous expression system and scale-up production are useful steps towards industrial production of secondary metabolites (Suthar and Ramawat 2010; Goyal et al. 2011, 2015).

Polysaccharides and enzyme production are commonly associated with bacterial endophytes. Due to this, process of gummosis is considered as a result of endophyte association in most of the gum-yielding trees (Arora and Ramawat 2014). Besides enzymes (which are proteins), several other proteins have been isolated and characterised from bacterial endophytes. In recent past, cyclic and non-cyclic peptides have been isolated and characterised from several endophytes showing potential applications such as anticancer, immunosuppressant, antifungal and other activities (Abdalla and Matasyoh 2014). It is evident from the above account that a wide variety of useful metabolites are produced by endophytes. There is a need to integrate available different technologies such as tools of molecular biology for their identification, use of tools of chemistry for identification of bioactive metabolites and biotechnology for scale-up production of metabolites to explore and exploit the potentiality of endophytes for human welfare.

1.7 Useful Biological Activities of Endophytes

Endophytes producing toxic substances protect host from insects and herbivores. *Neotyphodium* and *Epichloë* are an example of host beneficial endophytes which not only provide antiherbivore defence but also better nutrient uptake and drought tolerance to host plant (Schard et al. 2004). Other species of similar functions of defence and growth promotion are *Piriformospora indica* (Waller et al. 2005), *Acremonium strictum* (Hol et al. 2007) and some *Stagonospora* species (Ernst et al. 2003). In case of banana, endophytic bacteria (*Bacillus amyloliquefaciens*, *B. subtilis* subsp *subtilis* and *B. thuringiensis*) provide protection against fungal (*Fusarium oxysporum* f. sp *cubense* and *Colletotrichum guaranicola)* pathogens (Souza et al. 2014). Endophytic fungi isolated from different plants (Fig. 1.5) have shown antifungal activity.

1.8 Endophytes in Agriculture

Agriculture is major economic activity and livelihood of millions of people particularly in developing countries. Increasing population needs to be fed by increasing the production and productivity of agricultural produce, and novel strategies are required. Endophytes are gaining importance because of their role in plant growth stimulation, protection against biotic and abiotic stresses and pests via modulation of growth hormone signalling, higher seed yield and plant growth hormones (Miliute et al. 2015). Consequently, this has profound effects on agricultural traits of crop plants (Fig. 1.5) which hold promises for eco-friendly and

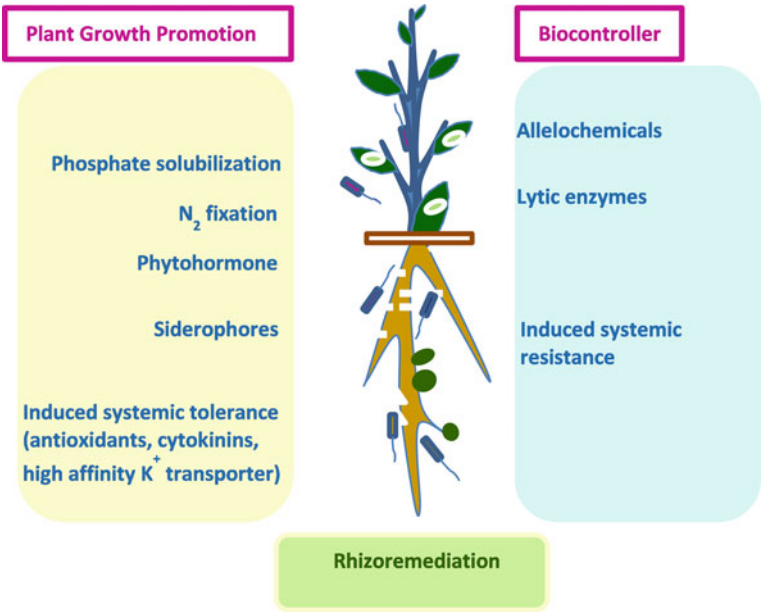

Fig. 1.5 Application of associative bacteria for sustainable agriculture, producing substances for plant growth and also suppressing the growth of pathogens and competitive plants

economically sustainable agriculture (Hallman et al. 2011; Rai et al. 2014). The wild relatives of wheat (*Triticum dicoccoides* and *Aegilops sharonensis*) harbour many useful endophytes of diverse taxonomic groups which are absent in cultivated modern-day wheat (*T. aestivum*) (Ofek-Lalzar et al. 2016). Use of modern agricultural practices such as fertiliser and chemicals to control pathogens and pests alters the balance between endophytes and its host (cultivated plant) as well as structure and function of soil. Such chemical environment is absent for wild relatives and endophytes thrive well in the system (Minz et al. 2011). Similarly, modern breeding methods cause changes in genotype of cultivated plant making them free from several insects, pests and endophytes. These changes have profound effect of agricultural traits and association of endophytes (Ofek-Lalzar et al. 2016). Therefore, bacterial endophytes hold a great promise for sustainable agriculture production along with health and nutritive values (Chap. 9).

1.9 Conclusions

Research on endophytes has gained momentum in last three decades as evident by >31,400 publications (primary research papers and reviews) on Google Scholar and data about their beneficial properties. Sustainable agriculture requires

self-contained functioning and low-cost eco-friendly inputs. To meet the ever-increasing food demand, biological nature-dependent developments are welcomed. Endophytes play an important role in plant physiology and functioning of agroecosystem. Application of tools and techniques of molecular biology has provided insight into their diversity and genomic structure. This book is a timely compilation of state of technology developed towards better understanding these micro-organisms. Better isolation techniques, faster genomic data mining and sequence matching will be helpful in the identification of endophytes and knowing their diversity as well as usefulness. Production of various useful drugs in large quantity is still a challenge as biosynthesis involves several genes. If some useful genes are identified on endophyte genome, it will be helpful in elucidating the pathway and consequently biosynthesis of secondary metabolites of choice in desired quantities.

References

Abdalla MA, Matasyoh JC (2014) Endophytes as producers of peptides: an overview about the recently discovered peptides from endophytic microbes. Nat Prod Bioprospect 4:257–270

Aravind R, Kumar A, Eapen SJ, Ramana KV (2009) Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici*. Lett Appl Microbiol 48(1):58–64

Arora J, Ramawat KG (2014) Biology and biotechnology of gum yielding indian trees. In: Ramawat KG, Merillon JM, Ahuja MR (eds) Tree Biotechnology. CRC Press, Boca Raton NY, pp 125–150

Arora P, Wani ZA, Nalli Y, Ali A, Hassan RU (2016) Antimicrobial potential of Thiodiketopiperazine derivatives produced by *Phoma* sp. An endophyte of *Glycyrrhiza glabra* Linn. Microb Ecol Jun 29 [Epub ahead of print]

Asis CA, Adachi K (2003) Isolation of endophytic diazotroph *Pantoea agglomerans* and nondiazotroph *Enterobacter asburiae* from sweet potato stem in Japan. Lett Appl Microbiol 38:19–23

Bacon CW, Porter JK, Robins JD, Lutrell EJ (1977) *Epichloë typhina* from toxic tall fescue grass. Appl Env Microbiol 34:576–581

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, Vander lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Bezerra JDP, Santos MGS, Svedese VM (2012) Richness of endophytic fungi isolated from Opuntiaficus-indica Mill. (Cactaceae) and preliminary screening for enzyme production. World J Microbiol Biotechnol 28(5):1989–1995

Bonfante P, Selosse MA (2010) A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo. New Phytol 186:267–270

Busby PE, Ridout M, Newcombe G (2016) Fungal endophytes: modifiers of plant disease. Plant Mol Biol 90:645–655

Cao X, Zhou L, Xu L, Li J, Zhao J (2007) Determination of diosgenin content of the endophytic fungi from *Paris polyphylla* var. *yunnanensis* by using an optimum ELISA. Natl Prod Res Develop 19:1020–1023

Chareprasert S, Piapukiew J, Thienhirun S, Whalley AJS, Sihanonth P (2006) Endophytic fungi of teak leaves *Tectona grandis* L. and rain tree leaves *Samanea saman* Merr. World J Microbiol Biotechnol 22(5):481–486

Chithra S, Jasim B, Sachidanandan P, Jyothis M, Radhakrishnan EK (2014) Piperin production by endophytic fungus *Colletotrichum gloeosporioides* isolated from *Piper nigrum*. Phytomedicine 21:534–540

Conti R, Chagas FO, Caraballo-Rodriguez AM, Melo WG, do Nascimento AM, Cavalcanti BC, de Moraes MO, Pessoa C, Costa-Lotufo LV, Krogh R, Andricopulo AD, Lopes NP, Pupo MT (2016) Endophytic action bacteria from the Brazilian medicinal plant *Lychnophora ericoides* Mart. and the Biological potential of their secondary metabolites. Chem Biodivers 13(6):727–36

Costa JL, Paulsrud P, Rikkinen J, Lindblad P (2001) Genetic diversity of *Nostoc* Symbionts Endophytically associated with two bryophyte species. Appl Environ Microbiol 67(9):4393–4396

Cui Y, Yi D, Bai X, Sun B, Zhao Y, Zhang Y (2012) Ginkgolide B produced endophytic fungus (*Fusariumoxysporum)* isolated from *Ginkgo biloba*. Fitoterapia 83:913–920

De Bary A (1866) Morphologie und Physiologie Pilze, Flechten, und myxomyceten. Hofmeister's Handbook of Physiological Botany. Vol. 2. Leipzig

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot (Tokyo) 62:5–16

Diaz PL, Hennell JR, Sucher NJ (2012) Genomic DNA extraction and barcoding of endophytic fungi. In: Sucher et al (eds) Plant DNA fingerprinting and barcoding: methods and protocol, methods in molecular biology. Springer Science+Business Media 862(1):171–179

Ding L, Goerls H, Dornblut K, Lin W, Maier A, Fiebig HH, Hertweck C (2015) Bacaryolanes A −C, rare bacterial caryolanes from a mangrove endophyte. J Nat Prod 78:2963–2967

Duong LM, Jeewon R, Lumyong S, Hyde KD (2006) DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal endophytes. Fung Divers 23:121–138

Ernst M, Menden KW, Wirsel SGR (2003) Endophytic fungal mutualists:seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosmos. MIPMI 16:580–587

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produces podophyllotoxin. J Nat Prod 69:1121–1124

Fu J, Zhou Y, Li HF, Ye YH, Guo JH (2011) Antifungal metabolites from Phomopsis sp. By254, an endophytic fungus in *Gossypium hirsutum*. Afri J Microbiol Res 5(10):1231–1236

Ganley RJ, Newcombe G (2006) Fungal endophytes in seeds and needles of *Pinus monticola*. Mycol Res 110(3):318–327

Germaine K, Keogh E, Garcia-Cabellos G, Borremans B, Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell CD, Ryan D, Dowling DN (2004) Colonization of poplar tree by GFP expressing bacterial endophytes. FEMS Microbiol Ecol 48:109–118

Glienke-Blanco C, Aguilar-Vildoso CI, Vieira MLC, Barroso PAV, Azevedo JL (2002) Genetic variability in the endophytic fungus *Guignardia citricarpa* isolated from citrus plants. G enet Mol Biol 25(2):251–255

Goyal S, Sharma V, Ramawat KG (2011) Marked effect of *Cuscuta* on puerarin accumulation in cell cultures of *Pueraria tuberosa* grown in shake flasks and bioreactor. Plant Biotech Rep 5:121–12

Goyal S, Sharma V, Ramawat KG (2015) A review of biotechnological approaches to conservation and sustainable utilization of medicinal lianas in India. In: Parthasarathy N (ed) Biodiversity of Lianas, sustainable development and biodiversity. Springer International Publishing, Switzerland, pp 179–210

Goyal S, Ramawat KG, Mérillon JM (2017) Different shades of fungal metabolites: An Overview. In: Mérillon JM, Ramawat KG (eds) Fungal metabolites. Springer International Publishing, Switzerland, DOI 10.1007/978-3-319-19456-1_34-1

Guan S, Grabley S, Groth I, Lin W, Christner A, Guo D, Sattler I (2005) Structure determination of germacrane-type sesquiterpene alcohols from an endophyte *Streptomyces griseus* subsp. Magn Reson Chem 43(12):1028–1031

Guerin P (1898) Sur la présence d'un champignon dans l'ivraie. J Botanique 12:230–238 [In French]

Guo B, Li H, Zhang L (1998) Isolation of the fungus producing vinblastine. J Yunnan Univ (Natural Science Edition) 20:214–215

Hallmann JA, Von-Quadt A, Mahaffee WF, Kloepper JW (2011) Endophytic bacteria in agricultural crops. Can J Microbiol 43(10):895–914

Hardoim PR, van Overbeek LS, Berg G, Pirtilla AM, Compant S, Campisano A, Dorind M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations f or defining functioning of microbial endophytes. Microbial Mol Biol Rev 79(3):293–320

Hata K, Sone K (2008) Isolation of endophytes from leaves of *Neolitsea sericea*in broad leaf and conifer stands. Mycoscience 49(4):229–232

He X, Han G, Lin Y et al (2012) Diversity and decomposition potential of endophytes in leaves of a *Cinnamomum camphora* plantation in China. Ecol Res 27(2):273–284

Herrera SD, Grossi C, Zawoznik M, Groppaa MD (2016) Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. Microbiol Res 186–187:37–43

Hol WHG, La PeñaE De, Moens M, Cook R (2007) Interaction between a fungal endophyte and root herbivores of *Ammophila arenaria*. Basic Appl Ecol 8:500–509

Istifadah N, Mcgee PA (2006) Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. Aust Plant Path 35:411–418

Jia M, Chen L, Xin H-L, Zheng C-J, Rahman K, Han T, Qin L-P (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Front Microbiol 7:906. doi:10.3389/fmicb.2016.00906

Keshri JP, Chatterjee S (2010) First record of two cyanoprokaryotes *Oscillatoria*(Oscillatoriales) and *Nostoc*(Nostocales) endophytic within the angiosperm *Alternanthera sessilis* (Amaranthaceae) from India. Algol Stud 135:83–88

Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M et al (2016) Endophytic fungi from *Frankincense* tree improves host growth and produces extracellular enzymes and indole acetic acid. PLoS ONE 11(6):e0158207. doi:10.1371/journal.pone.0158207

Koskimaki JJ, Hankala E, Suorsa M, Nylund S, Pirttila AM (2010) Mycobacteria are hidden endophytes in the shoots of rock plant [*Pogonatherum paniceum* (Lam.) Hack.] (Poaceae). Environ Microbiol Rep 2(4):619–24

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007) Fungal endophytes in a 400-million-yr-old land plant:infection pathways, spatial distribution, and host responses. New Phytol 174:648–657

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kusari S, Spiteller M (2011) Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes? Nat Prod Rep 28:1203–1207

Kusari S, LamshoftM Zuhlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. J Nat Prod 71:159–162

Kusari S, Zuhlke S, Spiteller M (2009) An endophytic fungus from *Camptotheca acuminate* that produces camptothecin and analogues. J Nat Prod 72:2–7

Kusari S, Kosuth J, Cellarova E, Spiteller M (2011) Survival-strategies of endophytic *Fusarium solani* against indigenous camptothecin biosynthesis. Fungal Ecol 4:219–223

Kusari S, Verma VC, Lamsho¨ ft M, Spiteller M (2012) An endophytic fungus from *Azadirachta indica* A. Juss. That produces azadirachtin. World J Microbiol Biotechnol 28:1287–1294

Kusari S, Singh S, Jayabaskaran C (2014) Rethinking production of Taxol (Paclitaxel) using endophyte biotechnology. Trends Biotechnol 32(6):304–311

Larran S, M´onaco C, Alippi HE (2001) Endophytic fungi in leaves of *Lycopersiconesculentum*Mill. World J Microbiol Biotechnol 17(2):181–184

Larran S, Perell´o A, Sim´on MR, Moreno V (2002) Isolation and analysis of endophytic microorganisms in wheat (*Triticumaestivum*L.) Leaves. World J Microbiolog Biotechnol 18(7):683–686

Li JY, Strobel G, Harper J, Lobkovsky E, Clardy J (2000) Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina*. Org Lett 2(6):767–770

Li HQ, Li XJ, Wang YL, Zhang Q, Zhang AL, Gao JM, Zhang XC (2011) Antifungal metabolites from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*. Biochemi Syst Ecol 39:876–879

Li Y, Yang J, Zhou X, Zhao W, Jian Z (2015) Isolation and identification of a 10-deacetyl baccatin-III-producing endophyte from *Taxus wallichiana*. Appl Biochem Biotechnol 175 (4):2224–2231

Liu K, Ding X, Deng B, Chen W (2009) Isolation and characterization of endophytictaxol-producing fungi from *Taxus chinensis*. J Ind Microbiol Biotechnol 36 (9):1171–1177

Liu Y, Nan L, Yan h, Zhang D, Han X (2016) Isolation and identification of resveratrol producing endophytes from wine grape *Cabernet sauvignon*. SpringerPlus 5(1):1029 doi:10.1186/s40064-016-2571-0. eCollection

Mahdi T, Mohamed L, Yagi S (2014) Endophytic fungal communities associated with ethno-medicinal plants from Sudan and their antimicrobial and antioxidant prospective. J Forest Prod & Ind 3(6):248–256

Malfanova N, Lugtenberg B, and Berg G (2013) Bacterial endophytes: who and where, and what are they doing there? In: Frans J. de Bruijn (ed) Molecular microbial ecology of the rhizosphere. Wiley-Blackwell, pp 15–37

Martínez L, Caballero J, Orozco J, Martínez-Romero E (2003) Diazotrophic bacteria associated with banana (*Musa* spp.). Plant Soil 257:35–47

Mbai FN, Magiri EN, Matiru VN, Nganga J, Nyambati VCS (2013) Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. Am Int J Contemp Res 3(4):25–40

Miliute I, Buzaite O, Baniulis D, Stanys V (2015) Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste-Agriculture 102(4):465–478

Minz D, Ofek M, Hadar Y (2011) Plant rhizosphere microbial communities. In: Rosenberg E, DeLong EF, Lory S et al (eds) The prokaryotes, prokaryotic communities and ecophysiology, 4th edn. Springer Press, New York, pp 57–74

Moonjely S, Barelli L, Bidochka MK (2016) Insect pathogenic fungi as endophytes. Adv Genet 94:107–135

Moussa M, Ebrahim W, El-Neketi M, Mándi A, Kurtán T, Hartmann R, Lin W, Liu Z, Proksch P (2016) Tetrahydroanthraquinone derivatives from the mangrove-derived endophytic fungus *Stemphyliumglobuliferum*. Tetrahedron Lett 57(36):4074–4078

Nagamani A, Kunwar IK, Manoharachary C (2006) Hand book of soil fungi. I K International, New Delhi

Nicoletti R, Fiorentino A (2015) Plant bioactive metabolites and drugs produced by endophytic fungi of spermatophyta. Agriculture 5:918–970

Nisa H, Kamili AN, Nawchoo IA, Shana S, Shameen N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: A review. Microb Pathog 82:50–59

Ofek-Lalzar M, Gur Y, ben-Moshe S, Sharon O, Kosman E, Mochli E, and Sharon A (2016) Diversity of fungal endophytes in recent and ancient wheat ancestors *Triticum dicoccoides* and *Aegilo pssharonensis*. FEMS Microbiol Ecol 92(10) fiw152, doi:10.1093/femsec/fiw152

Pan BF, Su X, Hu B, Yang N, Chen Q, Wu W (2015) *Fusarium redolens* 6WBY3, an endophytic fungus isolated from *Fritillaria unibracteata* var. *wabuensis*, produce speimisine and imperialine-3b -d-glucoside. Fitoterapia 103:213–221

Pandey SS, Singh S, Babu CS, Shanker K, Srivastava NK, Shukla AK, Kalra A (2016) Fungal endophytes of *Catharanthus roseus* enhance vindoline content by modulating structural and regulatory genes related to terpenoidindole alkaloid biosynthesis. Sci Rep 25(6):265–283

Pimentel MR, Molina G, Dionisio AP et al (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Inter Article ID 576286, 11 pages. doi:10.4061/2011/576286

Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotrophism. Biosystems 6:153–164
Pirttilä AM, Podolich O, Koskimäki JJ, Hohtola E, Hohtola A (2008) Role of origin and endophyte infection in browning of bud-derived tissue cultures of Scots pine (*Pinus sylvestris* L.). Plant Cell, Tissue Organ Cult 95(1):47–55
Plett JM, Martin F (2015) Reconsidering mutualistic plant–fungal interactions through the lens of effector biology. Curr Opin Plant Biol 26:45–50
Rai M, Agarkar G (2014) Rathod D (2014) Multiple applications of endophytic *Colletotrichum* species occurring in medicinal plants. In: Gurib-Fakim A (ed) Novel plant bioresources: applications in food, medicine and cosmetics. Wiley, Chichester, pp 227–236
Rakotoniriana EF, Munaut F, Decock C et al (2008) Endophytic fungi from leaves of *Centella asiatica*: occurrence and potential interactions within leaves. Antonie Van Leeuwenhoek 93(1–2):27–36
Ramawat KG, Dass S, Mathur M (2009) The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Ramawat KG (ed) Herbal drugs: ethnomedicine to modern medicine. Springer Verlag, Heidelberg, Germany, pp 7–32
Rehman S (2016) Endophytes: the producers of important functional metabolites. Int J Curr Microbiol App Sci 5(5):377–391
Rodriguez RJ, White JF, Arnold AE et al (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330
RylosonaJanarthine S, Eganathan P, Balasubramanian T, Vijayalakshmi S (2011) Endophytic bacteria isolated from the pneumatophores of *Avicennia marina*. Afr J Microbiol Res 5:4455–4466
Saikkonen K, Faeth SH, Heander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Ann Rev EcolSyst 29:319–343
Santamar´ıa J, Bayman P (2005) Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). Microbial Ecol 50(1):1–8
Schard CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seed borne fungal endophytes. Ann Rev Plant Biol 55:315–340
Schouten A (2016) Mechanisms involved in nematode control by endophytic Fungi. Ann Rev Phytopath 54:121–142
Selim KA, El-Beih AA, AbdEl-Rahman TM, El-Diwany AI (2012) Biology of endophytic Fungi. Curr Res Environ Appl Mycol 2(1):31–82
Selosse MA, Le Tacon F (1998) The land flora: a phototroph–fungus partnership? Tree 13:15–20
Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B et al (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant-Microbe Interact 25:28–36
Shweta S, Zuehlke S, Ramesha BT, Priti V, MohanaKunar P, Ravikanth G, Spiteller M, Vasudeva R, Shaanker RU (2010) Endophytic fungal strains of *Fusarium solani* from *Apodytes dimidiate* E. Mey. exArn (Icacinaceae) produce camptothecin,10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochem 71:117–122
Silva GH, de Oliveira CM, Teles HL, Pauletti PM, Castro-Gamboa I, Silva DHS, Bolzani VS, Young MCM, Costa-Neto CM, Pfenning LH, Berlinck RGH, Araujo AR (2010) Sesquiterpenes from Xylaria sp., an endophytic fungus associated with Piper aduncum (Piperaceae). Phytochem Let 3(3)164–167
Souza A, Cruz JC, Sousa NR, ProcópioARL Silva GF (2014) Endophytic bacteria from banana cultivars and their antifungal activity. Genet Mol Res 13(4):8661–8670
Stajkovic O, De Meyer S, Milicic B, Willems A, Delic D (2009) Isolation and characterization of endophytic nonrhizobial bacteria from root nodules of alfalfa (*Medicago sativa* L.). Botanica Serbica 33(1):107–114
Stierle A, Strobel GA, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae* an endophytic fungus of Pacific yew. Science 260(5105):214–216
Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: Endophytism defined. In: Bacon CW, White JF (eds) Microbial endophytes. M Dekker Inc, New York, pp 3–5

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing association with crops. Appl Soil Ecol 15(2):183–190

Sun X, Guo LD (2012) Endophytic fungal diversity: review of traditional and molecular techniques. Mycology 3(1):65–76

Surette MA, Sturz AV, Lada RR, Nowak J (2003) Bacterial endophytes in processing carrots (*Daucuscarota* L. var. *sativus*): their localization, population density, biodiversity and their effects on plant growth. Plant Soil 253:381–390

Suthar S, Ramawat KG (2010) Growth retardants stimulate guggulsterone production in the presence of fungal elicitor in fed-batch cultures of *Commiphora wightii*. Plant Biotechnol Rep 4:9–13

Thongsandee W, Matsuda Y, Ito S (2012) Temporal variations in endophytic fungal assemblages of *Ginkgo biloba* L. J For Res 17(2):213–218

Tiwari K (2015) The future products: endophytic fungal metabolites. J Biodivers Biopros Dev 2:145. doi:10.4172/2376-0214.1000145

Van der Heijdan MGA, Martin FM, Selosse MA, Sanders IR (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol 205:1406–1423

Venugopalan A, Srivastava S (2015) Endophytes as in vitro production platforms of high value plant secondary metabolites. Biotechnol Adv 33:873–887

Villarrea AJC, Renzaglia KS (2006) Structure and development of *Nostoc* strands in *Leiosporo cerosdussii* (Anthocerotophyta): a novel symbiosis in land plants. Am J Bot 93:693–705

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Hückelhoven R, Neumann C, Von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformspora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. PNAS USA 102:13386–13391

Wang Y, Dai CC (2011) Endophytes: a potential resource for biosynthesis, biotransformation, and biodegradation. Ann Microbiol 61:207–215

Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC (2007) Antimicrobial potentials of endophytic fungi residing in *Quercus variabilis* and brefeldin A obtained from *Cladosporium* sp. World J Microbiol Biotechnol 23(1):79–83

Wang Y, Zeng QG, Zhang ZB, Yan RM, Wang LY, Zhu D (2011) Isolation and characterization of endophytic huperzine A-producing fungi from *Huperzia serrate*. J Ind Microbiol Biotechnol 38(9):1267–1278

Wang LW, Xu BG, Wang JY, Su ZZ, Lin FC, Zhang CL, Kubicek CP (2012) Bioactive metabolites from *Phoma* species, an endophytic fungus from the Chinese medicinal plant *Arisaema erubescens* App Microb Biotechnol 93(3):1231–1239

Wang F, Zhu H, Ma H, Jiang J, Sun W, Cheng L, Zhang G, Zhang Y (2016) Citrinal B, a new secondary metabolite from endophytic fungus *Colletotrichum capsici* and structure revision of citrinal A. Tetrahedron Lett 57(37):4250–4253

Wu L, Shang H, Wang Q, Gu H, Liu G, Yang S (2016) Isolation and characterisation of antagonistic endophytes from *Dendrobium candidum* Wall ex Lindl. And the biofertilizing potential of a novel *Pseudomonas saponiphila* strain. Appl Soil Ecol 105:101–108

Xia X, Kim S, Liu C, Shim SH (2016) Secondary metabolites produced by an endophytic fungus *Pestalotiopsis sydowiana* and their 20S Proteasome Inhibitory Activities. Molecules 21:944. doi:10.3390/molecules21070944

Xing X, Guo S, Fu J (2010) Biodiversity and distribution of endophytic fungi associated with *Panax quinquefolium* L. cultivated in a forest reserve. Symbiosis 51(2):161–166

Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, Sun P, Qin L (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165:437–449

Zabalgogeazcoa I (2008) Review: Fungal endophytes and their interaction with plant pathogens. Span J Agric Res 6 (Special issue):138–146

Zaiyou J, Li M, Guifang X, Xiuren Z (2013) Isolation of an endophytic fungus producing baccatin III from *Taxus wallichiana* var. *mairei*. J Ind Microbiol Biotechnol 40(11):1297–1302

Zhang L, Guo B, Li H, Zeng S, Shao H, Gu S, Wei R (2000) Preliminary study on the isolation of endophytic fungus of *Catharanthus roseus* and its fermentation to produce products of therapeutic value. Chin Tradit Herbal Drugs 31:805–807

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zheng CJ, Li L, Han T, Qin LP (2012) Identification of a quinazoline alkaloid produced by *Penicillium vinaceum*, an endophytic fungus from *Crocus sativus*. Pharm Biol 50(2):129–133

Zhou S, Yang F, Lan S, Xu N, Hong Y (2009) A producing conditions from endophytic fungus in SHB *Huperzia serrata*. J Microbiol 29:32–36

Zhu M, Zhang X, Feng H, Che Q, Zhu T, Gu Q, Li D (2016) Campyridones A-D, pyridone alkaloids from a mangrove endophytic fungus Campylo carpon sp. HDN13-307. Tetrahedron 72(37):5679–5683

Zouari I, Jlaiel L, Tounsi S, Trigui M (2016) Biocontrol activity of the endophytic *Bacillus amyloliquefaciens* strains CEIZ-11 against *Pythium aphanidermatum* and purification of its bioactive compounds. Biol Control 100:54–62

Chapter 2
Bacterial Endophytes of Plants: Diversity, Invasion Mechanisms and Effects on the Host

Fernando Ibáñez, María Laura Tonelli, Vanina Muñoz, María Soledad Figueredo and Adriana Fabra

Abstract Plant inner tissues are colonized by bacterial organisms known as endophytes. The relatively recent application of culture independent and molecular high throughput techniques allowed the description of a large diversity of endophytic bacterial taxa. These microorganisms can be found in any plant organ, including fruits and legume nodules. Some endophytic bacteria benefit the host by several mechanisms, and their application to economically important crops represents an interesting alternative to the use of agrochemicals. However, more studies are required to clearly assess their effects on the hosts (especially in co-inoculation with other beneficial bacteria) and the molecular events that lead to the interaction between plants and endophytic microorganisms. In this chapter, we focus on bacterial endophytes from legumes and non-legumes plants, analyzing their diversity and effects on the hosts. We also discuss the endophytic colonization of legume nodules, with emphasis on the endophytic bacterial diversity, the mechanisms involved in the nodule invasion and their effects on the hosts.

Keywords Endophytes · Biocontrol · Symbiosis · Rhizobia · Legumes

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_2

2.1 Introduction

Plants are known by their ability to interact with a large number of diverse microorganisms. In fact, it is thought that this ability constitutes one of the main innovations that allowed the algal ancestor of plants to colonize land (Delaux et al. 2015). Microorganisms interacting with plants include prokaryotic and eukaryotic taxa and can colonize the surface or internal parts of the host. Those prokaryotic microorganisms that can be detected within the tissues of apparently healthy plant host are considered as endophytic bacteria (Schulz and Boyle 2006). Although this definition is arbitrarily limited to non-pathogenic bacteria, its functional nature is useful for the purpose of this chapter. Here, we will use the term "endophyte" to refer to those bacteria detected by molecular methods or isolated from inside tissues that cause no visible harm to the plant. Indeed, some endophytes are able to benefit the host in several ways such as conferring biotic and abiotic stresses resistance and tolerance, enhancing nutrient availability, degrading toxic substances, and producing phytohormones (Wilson 1995; Hardoim et al. 2008; Doty 2011; Gaiero et al. 2013; Kandel et al. 2015).

Years ago, analysis of endophytic microorganism diversity relied on the identification of those that can be recovered in rich culture media from surface sterilized plant organs. However, culture-dependent methods confer selective advantage to some bacteria and do not allow a complete overview of the endophytic population (Bhattacharjee et al. 2008). Recently, the use of molecular approaches (including high throughput techniques) allowed the description of a larger diversity of plant endophytes.

2.2 Rhizobial and Non-Rhizobial Endophytes of Non-Legume Plants

Endophytic bacteria have been recovered from a wide array of plant species, suggesting a ubiquitous presence in nearly all higher plants (Luo et al. 2012). The structure of these communities depends on soil biotic and abiotic factors affecting bacterial survival, host factors that allow colonization and microbial determinants that shape the ability of the endophytes to survive and compete within the plant hosts (Gaiero et al. 2013). Microorganisms can reach the plants through a variety of sources, such as soil (Hallmann et al. 1997), water from precipitation or irrigation, the fall of atmospheric dust or wind (Agrios 1997; Morris et al. 2010; Savage et al. 2012), animals that can carry microorganisms (Villate et al. 2012), seeds, seedlings, plants from distant areas (Agrios 1997; Dobbelaere et al. 2001; Alabouvette et al. 2006; Truyens et al. 2014), and plant remnants (litter, crop residues) (Leplat et al. 2013). Moreover, seed endophytes can be vertically transmitted from generation to generation in plants that are propagated vegetatively (Moënne-Loccoz et al. 2015).

Application of new tools such as next generation sequencing technologies to study the plants endophytic community has shown that its composition is highly underestimated. Hardoim et al. (2015) constructed and analyzed a database of all currently 16S rDNA sequences assigned to endophytes, including cultured and uncultured microorganisms, and found that, although the sequences belong to 23 different bacterial Phyla, 4 of them (Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes) encompass for 96% of the total number of endophytic prokaryotic sequences. Among them, Proteobacteria includes more than 50% of the sequences in the database. Within this phylum, isolates from the Gammaproteobacteria subclass are the most commonly found as endophytes, including genera such as *Pseudomonas*, *Enterobacter*, *Pantoea*, *Stenotrophomonas*, *Acinetobacter,* and *Serratia*. On the other hand, genera *Streptomyces*, *Microbacterium*, *Mycobacterium*, *Arthrobacter* (within Actinobacteria) as well as *Bacillus*, *Paenibacillus,* and *Staphylococcus* (Firmicutes) are also well represented among the endophytic microorganisms (Hardoim et al. 2015). As species from all these genera are common in soils, it has been suggested that the endophytic microbial community constitutes a subpopulation of the rhizospheric bacteria (Germida et al. 1998; Marquez-Santacruz et al. 2010; Santoyo et al. 2016). However, how the plants manage to select a certain group of endophytes is still not fully understood.

Rhizobia are a diverse group of soil bacteria known for their ability to establish a symbiotic interaction with legumes. They induce in their plant host the development of nodules that house these nitrogen fixing microorganisms. Interestingly, rhizobia have also been found colonizing non-legume plants tissues, but, with the exception of *Parasponia*, induction of nodule formation has never been reported (Yanni et al. 1997, 2001; Prayitno et al. 1999; Biswas et al. 2000a, b; Chaintreuil et al. 2000; Gutierrez-Zamora and Martinez-Romero 2001, Hilali et al. 2001; Peng et al. 2002; Lupwayi et al. 2004). As several studies indicated that endophytic rhizobia promote non-legume plants growth, their application as biofertilizers may represent a useful strategy in sustainable agriculture.

2.2.1 How Endophytes Gain Access to Plant Tissues?

Bacterial endophytes invade and colonize internal plant tissues, using organic plant metabolites for growth and survival, and avoiding host defense responses. The main site for endophytes entry into plants tissues is the root zone (Compant et al. 2005; Meneses et al. 2011; Gaiero et al. 2013), but they can also invade aerial tissues (Chi et al. 2005). Bacteria endophytes can entry through plant's flowers and therefore, they may be found in fruits. Another mode of invasion of the host plant is through infection of seeds, assuring their presence in new plants.

Chemotactic signals play a very important role in the first step of endophytes root surface colonization. Moreover, they can enhance their competitive performance and regulate the expression of genes involved in plant tissue invasion (Bais et al. 2006; Rosenblueth and Martínez-Romero 2006; Compant et al. 2010;

Carvalho et al. 2016). Meanwhile, the host plant recognizes and selects the beneficial bacteria to associate with and as a consequence, root endophytic bacteria communities may differ from bacteria communities in the rhizosphere. Therefore, microbe–microbe and microbe–plant signaling are involved in the plant tissue colonization process. Host plant–potential endophytic bacteria cross talk begins with signaling molecules released by plant roots. Chemical signals and nutrients excreted by the roots modulate and determine the abundance and diversity of bacteria that colonize the root (Bais et al. 2004). For example, flavonoids and some phytohormones were also found to improve *Serratia* spp. rice seedlings endophytic colonization (Balachandar et al. 2006). It has been observed that *Arabidopsis thaliana* selectively recruits the biocontrol agent *Bacillus subtilis* FB17 by secretion of malic acid to prevent pathogenic attack (Rudrappa et al. 2008). Rice and sugarcane plants modify their chemical signals when they interact with beneficial bacteria or pathogenic bacteria (Gaiero et al. 2013).

In addition to plant exudates, the quorum sensing system (QS) of potential endophytes has a main role in plant tissue colonization, since it regulates the expression of bacterial genes involved in this process. The most common QS signals found in Gram-negative bacteria are *N*-acyl homoserine lactones (AHLs) while in Gram-positive bacteria are peptides (Kleerebezem et al. 1997; Gaiero et al. 2013). It is known that plants can positively or negatively affect AHL-dependent QS responses.

Once the potential endophyte is attracted to the plant root, it has to attach to it. Type IV pili are essential for bacterial adherence and colonization of host cell surfaces (Carvalho et al. 2016). Moreover, a mutation in *Azoarcus* sp. pilin, a major component of Type IV pili, reduced its adhesion and colonization of rice roots (Dörr et al. 1998). In addition, Gram-negative bacteria surface components (exopolysaccharides (EPS) and lipopolysaccharides (LPS) are involved in the attachment and colonization. Moreover, plant–bacteria recognition may be modulated by bacterial effectors delivered into the plant cells by a type III protein secretion system (TTSS) (Carvalho et al. 2016).

After the potential endophyte bacteria are attracted to the root and attached to its surface, they multiply and reach a population density that enables them to form biofilms. Biofilm formation allows non-spore-forming soil bacteria to colonize their surrounding habitat. The major components of biofilms are water and bacterial cells. The next most important component is an EPS matrix, which provides a physical barrier against diffusion of defense substances from the host and protection against environmental stressing factors. Minor components include macromolecules such as proteins, DNA, and other products released by cells lysis (Rinaudi and Giordano 2010). Meneses et al. (2011) demonstrated that EPS biosynthesis is required for *Gluconacetobacter diazotrophicus* PAL5 biofilm formation and rice endophytic root colonization, since when they knocked out a gene involved in EPS biosynthesis, mutant bacteria were defective in biofilm formation, root surface attachment, and endophytic colonization.

Bacterial signals recognition by plants is mainly mediated by the plant receptors-like kinases (RLK), such as leucine-rich repeat–receptor-like kinases

(LRR–RLKs), wall-associated kinases (WAK), lectin receptor-like kinases (LecRLKs), Lys-motif receptors (LysM), among others; and by plant small RNAs (sRNA) as miRNA, and small interfering RNA (siRNA) (Carvalho et al. 2016).

After the initial colonization, some endophytes enter roots and gain access to the interior tissues, migrating endophytically upward into the leaf or stem bases. They may pass through root tips (root tip pathway) or through the middle lamella of the epidermal layer (Compant et al. 2005). Three modes of nitrogen fixing organism entry into roots have been described: (a) through wounds particularly where lateral or adventitious roots protrude, (b) through root hairs, (c) between undamaged epidermal cells (Cocking 2003). It has been proposed that cellulolytic and pectinolytic enzymes produced by endophytes are involved in the infection process (Hallmann et al. 1997). The mechanism is known as "crack entry" allows some endophytes to passively gain entry the interior part of plant using epidermal junctions between root hair and adjacent epidermal cells, or disrupted endodermal cell layers resulting from the emergence of developing lateral roots. This mode of entry (often combined with active penetration) has been suggested for different bacterial species such as *Burkholderia* (Compant et al. 2005; Govindarajan et al. 2006), *Bacillus* (Ji et al. 2008), and *Herbaspirillum* (James et al. 2002) among others. It is interesting that this entry route is an ancient strategy also used by rhizobia in the interaction with some legumes to establish a symbiotic relationship (Fabra et al. 2010; Huang et al. 2011). Instead of that, *Pseudomonas* spp. use root hairs as the main entrance for endophytic colonization of olive roots, regardless they have been previously colonized, but well-known root hair morphological changes induced by rhizobia in legumes were not observed (Prieto et al. 2011).

2.2.2 Plant Growth Promotion by Endophytes

Plant endophytes can promote plant growth by fixing atmospheric nitrogen, producing phytohormones, controlling phytopathogens, or by enhancing the uptake of minerals. In this sense, there are many studies demonstrating the beneficial effects of endophytes. For instance, the endophytic diazotrophic bacteria *Gluconacetobacter diazotrophicus* improves sugarcane growth (Cocking 2003). In this plant, as well as in other non-legumes plants, the role of endophytic diazotrophic bacteria in N nutrition has been demonstrated by quantifying ^{15}N (Chalk 2016).

In *Zea mays*, the endophyte *Azospirillum lipoferum* alleviates drought stress symptoms through production of abscisic acid and gibberellins (Cohen et al. 2009). In *Solanum tuberosum* and *Vitis vinifera,* the endophyte *Burkholderia* sp. promotes plant growth by reducing the level of the inhibitory hormone ethylene through production of high levels of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Fommel et al. 1991; Barka et al. 2000). Citrus plants were protected against the pathogen *Xylella fastidiosa* by the endophyte *Curtobacterium flaccumfaciens* (Araujo et al. 2002). The inoculation of *Bacillus* sp in *Arachis hypogaea* plants induced the systemic resistance against *Sclerotium rolfsii* (Tonelli et al. 2011).

Some studies indicated that co-inoculation of endophytes with different ecological niches is a promising alternative to individual PGPR inoculation For example, *Avicennia germinans*, *Laguncurlaria racemosa,* and *Rhizophora mangle* plants co-inoculated with the phosphate solubilizing *Bacillus licheniformis* and the nitrogen-fixing *Phyllobacterium* sp. showed better nitrogen and phosphorous assimilation than plants inoculated individually with the endophytic bacteria (Rojas et al. 2001). It is important to highlight that not always the co-inoculation of beneficial endophytes results in an improved plant growth effect compared to individual inoculation. Bent and Chanway (1998) showed that the plant-growth-promoting ability of some rhizobacteria in *Pinus contorta* can be significantly reduced in the presence of another rhizobacterium, even when individually both strains can benefit plant growth.

2.3 Non-Rhizobial Endophytic Bacteria Within Legume Nodules

Although the interior of any plant organ can be colonized, a particular endophytic colonization takes place in legume root nodules. We refer to nodule endophytic bacteria as the occupants of the nodules unable to induce their formation, therefore excluding compatible rhizobia. At first, nodule endophytic bacteria were considered artifacts derived from a deficient surface disinfection of the root nodules. Later, it was found that they were capable to effectively colonize the interior of nodules induced by compatible rhizobial strains (Bai et al. 2002; Ibáñez et al. 2009). Currently, endophytic colonization of legume nodules is a promising field for identifying bacterial strains with new PGP activities or for optimizing plant growth promoting rhizobacteria (PGPR) inoculation. In fact, these endophytes share the nodule resources with rhizobia and, at least in theory, can positively or negatively affect biological nitrogen fixation. Moreover, nodules offer a controlled and rich in carbon source environment where endophytic bacteria can multiply. Afterward, releasing of bacteria with PGP properties from senescent nodules could represent a new source of inoculum to the soil.

2.3.1 Diversity of Endophytic Bacteria Found Inside Nodules and Their Hosts

As research expands to include new geographic regions or other legume clades, more and more endophytic bacterial groups are described inside nodules. To date, a wide range of bacteria was described as nodule endophytes. They comprise Gram-negative or Gram-positive bacteria included within Phyla phylogenetically diverse such as Proteobacteria, Firmicutes, Actinobacteria (reviewed in Peix et al. 2012, 2015; Velázquez et al. 2013) and the Cytophaga-Flavobacterium-Bacteroides (CFB) group

(De Meyer et al. 2015). Within Proteobacteria, endophytes were found mostly in alpha (Zakhia et al. 2006; Muresu et al. 2008; Deng et al. 2011), beta (Valverde et al. 2003; Li et al. 2008; Hoque et al. 2011), and gamma (Zakhia et al. 2006; Li et al. 2008; Ibañez et al. 2009; Deng et al. 2011; Hoque et al. 2011) subclasses. In Firmicutes, genera *Bacillus* and *Paenibacillus* encompass the majority of non-nodulating rhizobial endophytes (Zakhia et al. 2006; Li et al. 2008; Deng et al. 2011). Within Actinobacteria, bacteria belonging to the genera *Microbacterium*, *Mycobacterium*, *Agromyces*, *Ornithinicoccus*, *Nocardia*, *Streptomyces,* and *Micromonospora* were described as nodule endophytes (Zakhia et al. 2006; Trujillo et al. 2010; Deng et al. 2011). Considering all these reports, bacteria from *Agrobacterium*, followed by *Bacillus* and *Pseudomonas* are the most frequently genera obtained from inside nodules of a vast diversity of legumes.

In relation to the hosts, endophytic bacteria have been found to colonize nodules belonging to two of the three Fabaceae subfamilies (Papilionoideae and Mimosoideae) but, to our knowledge, there are no studies reporting nodule endophytic microorganisms on members of the basal Caesalpinioideae subfamily. This is probably related to the fact that nodulation is not so common within this basal legume group and also to the lack of deep studies on these plants. Expanding the studies of nodule endophytic bacteria to the nodulating members of this group of legumes will contribute to a better grasp of the bacterial diversity found within nodules.

Regarding the existence of specificity in the endophytic association, evidences suggest that there are no recognition mechanisms as strict as the ones involved in rhizobial symbiosis for endophytic colonization of the nodules. First, the great phylogenetic diversity of endophytic bacteria compared to the (relatively) narrow phylogenetic range of rhizobia. Second, some genera such as *Agrobacterium*, *Bacillus,* and *Pseudomonas* are able to colonize nodules of phylogenetically diverse legumes. Similarly, nodules from the same plant species can harbor a very diverse group of bacterial endophytes. For instance, bacteria from the phylogenetically distant genera *Bacillus*, *Agrobacterium,* and *Pantoea* were described as nodule endophytes of *Glycine max* (Velázquez et al. 2013). However, data seem to indicate that plants can select a specific subset of microorganisms to allow colonization of nodules. De Meyer et al. (2015) analyzed a large subset of nodule endophytic microorganisms from 30 species of indigenous legumes in Belgium and found that certain group of plants "prefers" some endophytes. Moreover, authors suggest a correlation between some rhizobial occupants of the nodules and certain groups of endophytic microorganisms. However, such concept is yet to be confirmed.

2.3.2 *Mode of Entry of Bacterial Endophytes to Legume Root and Nodule Tissues*

Bacterial genera most frequently isolated from inside nodules are also the most commonly found as root endophytes (including both legumes and non-legumes), suggesting that colonization of nodules does not rely on microbial specific traits

others than the ones required for root colonization. However, it is still not clear if there is any additional microbial trait particularly associated with nodule colonization, or a specific plant–microbe signaling for invasion of this specialized organ.

Sites for primary colonization and entry into the plant of non-symbiotic bacterial endophytes are undifferentiated tissues above the root tips and the points of emergence of lateral roots, as also described for rhizobia (Reinhold-Hurek and Hurek 1998). This first step in the tissue entry process of non-symbiotic endophytes also involves root adsorption and bacterial proliferation, forming biofilm structures at the surface of roots (Compant et al. 2010; Reinholdt-Hurek and Hurek 2011). Later, ways by which non-rhizobial endophytes can get access to the interior of legume roots have also been described in non-leguminous plants. In fact, they are able to use epidermal junction between root hair and adjacent epidermal cells, or disrupted endodermal cell layers resulting from the emergence of developing lateral roots, the mechanism known as "crack entry". Root hairs also represent a site for endophytic bacteria entry. *Pseudomonas* spp. the main entrance for endophytic colonization of olive are root hairs, regardless they have been previously colonized, but well-known root hair curling and infection thread induced by rhizobia in legumes were not observed (Prieto et al. 2011). In *Vigna radiata*, the invasion of infection threads by *Pseudomonas* and *Klebsiella* strains led to nodule colonization when co-inoculated with host-nodulating *Ensifer adhaerens*. The presence of the three strains: *E. adhaerens, P. fluorescens,* and *K. pneumoniae*, within the same root hair was demonstrated, and the inability of *P. fluorescens* and *K. pneumoniae* to colonize the interior of root hairs was attributed to their inability to secrete cellulase and pectinase (Pandya et al. 2013). In *Lotus japonicus*, infection threads initiated by *Mesorhizobium loti*, symbiont of *Lotus*, can guide endophytic bacteria toward nodule primordia. Inside these cells, competent strains multiply and colonize the nodule together with the symbiotic partner (Zgadzaj et al. 2015) (Fig. 2.1).

Nevertheless, how non-rhizobial rhizobacteria breach the rhizobial host specificity and enter root nodules remains unanswered. Even though symbiotic and non-symbiotic endophytes seem to use similar entry routes, to date, formation of nodules by endophytic bacteria other than rhizobia and *Frankia* has not been informed, with the exception of *Pseudomonas* spp. which induces nodules on *Robinia pseudoacacia* roots, probably after the acquisition in the soil of symbiotic genes from rhizobial species (Shiraishi et al. 2010).

Genetic diversity among nodule endophytes and their wide host-range suggest the absence of a sophisticated molecular recognition between the partners. However, it is becoming clear that plants are able to select their endophytic bacterial population by still not fully understood mechanisms. Possibly, the nodules endophytes use an ancestral form of colonization and accommodation, involving ancient traits. Studies focusing on the partners' genetic determinants allowing the endophytic colonization and accommodation inside the nodules could shed light on the evolution of the earlier steps of the beneficial interaction between plants and bacteria.

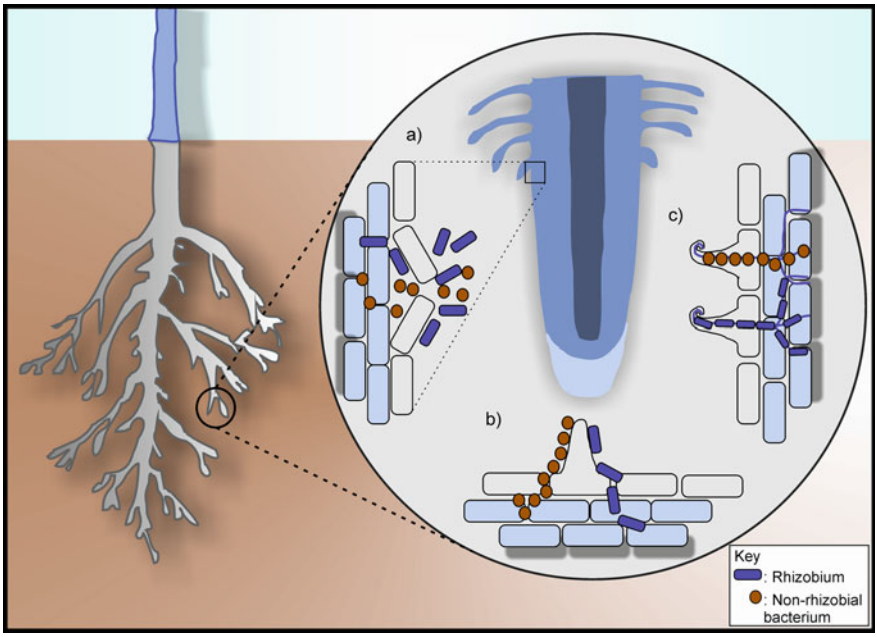

Fig. 2.1 Mode of entry to plant roots shared by rhizobial and non-rhizobial endophytes **a** through disrupted epidermal cell layers resulting from the emergence of developing lateral roots ("*crack entry*"), **b** root hairs colonization, without induction of morphological changes, and later invasion through intercellular spaces, **c** colonization of infection threads previously induced by rhizobial strains

2.3.3 How Plants May Benefit from Non-Symbiotic Nodule Endophytes?

Root nodule is an environmental niche induced by symbiotic bacteria. For a long time, it was believed that rhizobia or *Frankia* were the only nodule inhabitants in legumes and actinorhizal plants, respectively. Current data indicate that nodules may harbor a wide diversity of bacteria and that symbiotic and non-symbiotic endophytes coexist.

Recently, *Micromonospora saelicesensis* was identified as the most frequently bacterial species isolated from nodules of both leguminous and actinorhizal plants (Valdés et al. 2005; Trujillo et al. 2006, 2007, 2010; Garcia et al. 2010; Carro et al. 2012, 2013). However, the ecological role of bacterial endophytes others than rhizobia and *Frankia* inside the roots nodules, as well as their interaction with these nitrogen fixing bacteria, is unknown. In *Lotus japonicus,* it has been reported that colonization of nodules by endophytic bacteria is a selective process, host controlled, and that bacterial EPS are required for chronic infection of nodules. Therefore, it seems that the legume host invaded by infection threads formation

controls not only the symbiont access into nodules but also the endophytes (Zgadzaj et al. 2015). However, no information is available in legumes infected intercellularly without infection threads.

Plants have evolved sophisticated mechanisms to control microbial presence and infection. Therefore, only particular microbes are able to colonize the internal tissues with minimal or no host damage. Intracellular accommodation and multiplication of compatible symbionts are allowed only inside nodules.

Considering that some legumes may control the endophytes entry to nodule, it is possible to speculate that those bacteria located inside nodules are beneficial. In fact, reports indicate improved plant health, nodulation, and yield when co-inoculated with nodule endophytes, compared to inoculation with rhizobia alone (Sturz et al. 1997; Bai et al. 2002, 2003; Rajendran et al. 2008). It has also been shown that *Micromonospora* inoculation enhances alfalfa aerial growth, and an increase of nitrogen uptake by the plant is a general phenomenon in this interaction (Martínez-Hidalgo et al. 2014). In the same sense, co-inoculation of peanut with the bradyrhizobial symbiont and endophytic gammaproteobacteria belonging to *Enterobacter* increased number of nodules (Ibañez et al. 2009). Interestingly, these isolates were also capable to increase maize growth parameters when inoculated in a simulated peanut–maize rotation system (Ibañez et al. 2014). In *Vigna radiata*, nodule endophytic bacteria belonging to genera *Klebsiella, Agrobacterium, Dyadobacter, Chitinophaga, Paenibacillus,* and *Bacillus* were beneficial for plant growth (Pandya et al. 2015). In *Melilotus dentatus,* it was demonstrated that an *Agrobacterium* strain originally isolated from nodules of *Onobrychis viciifolia* could co-inhabit root nodules with the symbiotic *Sinorhizobium meliloti* strain, without affecting the growth and nodulation of plants (Wang et al. 2006).

It is known that legumes can recognize rhizobia performances in the nodules and impose sanctions that affect the symbiont fitness (Kiers et al. 2003). Therefore, a positive (or at least non-detrimental) effect of the nodule endophytes on the plant host can also drive the ecological fitness of these endophytes. However, inoculation with nodule endophytic bacteria may have a negative effect on growth and yield parameters. In the common bean, the nodule endophytic *Agrobacterium* strains might reduce the nodulation of *Rhizobium gallicum* (Mrabet et al. 2006). This effect seems to be host-specific, since they did not affect nodulation of *Sinorhizobium meliloti* with alfalfa (Wang et al. 2006).

Our knowledge of the interaction among symbiotic, non-symbiotic bacteria coexisting in nodules, and host plant is still scarce, and more studies are necessary to understand fully not only the role of this ecological process but also the molecular interaction between plants and non-symbiotic nodule endophytes.

2.4 Conclusions

As knowledge on plant–microorganism interaction expands, researchers have begun to consider that plants host not only different endophytic communities but also can recruit a subset of microorganisms, presumably for specific functions. Even plant specialized organs such as nodules are now considered susceptible to be colonized by different bacterial species. Many studies suggest that plants and their microbiome are in constant communication through the exchange of signals. However, it is just beginning to understand mechanisms and functions of these interactions. Most functional studies have been performed using experimental strategies commonly applied to the study of plant–individual microorganism interactions. Therefore, additional research around these concepts may help to determine the interactive functionalities that occur between plants and their microbiome and would provide a mean to further increases plant growth promoting potential, reaching maximum crop yields.

References

Agrios G (1997) Plant pathology, 4th edn. Academic, San Diego

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant pathogens: the European situation. Eur J Plant Pathol 114:329–341

Araujo W, Marcon J, Maccheroni W, Van Elsas J, Van Vuurde J, Azevedo J (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol 68:4906–4914

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48:230–238

Bai Y, Zhou X, Smith D (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci 43:1774–1781

Bais H, Park S, Weir T, Callaway R, Vivanco J (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Bais H, Weir T, Perry L, Gilroy S, Vivanco J (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Balachandar D Sandhiya G, Sugitha T, Kumar K (2006) Flavonoids and growth hormones influence endophytic colonization and in planta nitrogen fixation by a diazotrophic *Serratia* sp. in rice. World J Microbiol Biotechnol 22:707–712

Barka A, Belarbi E, Hachet C, Nowak J, Audran JC (2000) Enhancement of in vitro growth and resistance to gray mould of *Vitis vinifera* L. co-cultured with plant growth-promoting rhizobacteria. FEMS Microbiol Lett 186:91–95

Bent E, Chanway C (1998) The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. Can J Microbiol 44:980–988

Bhattacharjee R, Singh A, Mukhopadhyay S (2008) Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. Appl Microbiol Biotechnol 80:199–209

Biswas JC, Ladha JK, Dazzo FB (2000a) Rhizobia inoculation improves nutrient uptake and growth in lowland rice. Soil Sci Soc Am J 64:1644–1650

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG (2000b) Rhizobial inoculation influences seedling vigor and yield of rice. Agron J 92:880–886

Carro L, Spröer C, Alonso P, Trujillo ME (2012) Diversity of *Micromonospora* strains isolated from nitrogen fixing nodules and rhizosphere of *Pisum sativum* analyzed by multilocus sequence analysis. Syst Appl Microbiol 35:73–80

Carro L, Pujic P, Trujillo ME, Normand P (2013) *Micromonospora* is a normal inhabitant of actinorhizal nodules. J Biosci 38:685–693

Carvalho TLG, Ballesteros HGF, Thiebaut F, Ferreira PCG, Hamerly AS (2016) Nice to meet you: genetic, epigenetic and metabolic controls of plant perception of beneficial associative and endophytic diazotrophic bacteria in non-leguminous plants. Plant Mol Biol 90:561–574

Chaintreuil C, Giraud E, Prin Y, Lorquin JB, Gillis M, de Laudie P, Dreyfus B (2000) Photosynthetic bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. Appl Environ Microbiol 66:5437–5477

Chalk PM (2016) The strategic role of ^{15}N in quantifying the contribution of endophytic N2 fixation to the N nutrition of non-legumes. Symbiosis. doi:10.1007/s1399-016-0397-8

Chi F, Shen S-H, Cheng H-P, Jing Y-X, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271–7278

Cocking EC (2003) Endophytic colonization of plant roots by nitrogen-fixing bacteria. Plant Soil 252:169–175

Cohen A, Travaglia C, Bottini R, Piccoli P (2009) Participation of abscisic acid and gibberellins produced byendophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87:455–462

Compant S, Reiter B, Sessitsch A, Nowak J, Clement C, Barka E (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71:1685–1693

Compant S, Clement C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

De Meyer SE, Van Hoorde K, Vekeman B, Braeckman T, Willems A (2011) Genetic diversity of rhizobia associated with indigenous legumes in different regions of Flanders (Belgium). Soil Biol Biochem 43:2384–2396

De Meyer SE, De Beuf K, Vekeman B, Willems A (2015) A large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium). Soil Biol Biochem 83:1–11

Delaux PM, Radhakrishnan GV, Jayaraman D, Cheema J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, Rothfels CJ (2015) Algal ancestor of land plants was preadapted for symbiosis. Proc Natl Acad Sci USA 112:13390–13395

Deng ZS, Zhao LF, Kong ZY, Yang WQ, Lindström K, Wang ET, Wei GH (2011) Diversity of endophytic bacteria within nodules of the *Sphaerophysasalsula* in different regions of Loess Plateau in China. FEMS Microbiol Ecol 76:463–475

Dobbelaere S, Croonenborghs A, Thys A, Ptacek D, Vanderleyden J, Dutto P, Labandera-Gonzalez C, Caballero-Mellado J, Aguirre JF, Kapulnik Y, Brener S, Burdman S, Kadouri D, Sarig S, Okon Y (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879

Dörr J, Hurek T, Reinhold-Hurek B (1998) Type IV pili are involved in plant-microbe and fungus-microbe interactions. Mol Microbiol 30:7–17

Doty S (2011) Nitrogen-fixing endophytic bacteria for improved plant growth. In: Maheshwari DK (ed) Bacteria in agrobiology: Plant growth responses. Springer, Berlin, pp 183–199

Fabra A, Castro S, Taurian T, Angelini J, Ibañez F, Dardanelli M, Tonelli M, Bianucci E, Valetti L (2010) Interaction among *Arachis hypogaea* L. (peanut) andbeneficial soil microorganisms: how much is it known? Crit Rev Microbiol 36:179–194

Frommel M, Nowak J, Lazarovits G (1991) Growth enhancement and developmental modifications of in vitro grown potato (*Solanum tuberosum* spp. tuberosum) as affected by a nonfluorescent *Pseudomonas* sp. Plant Physiol 96:928–936

Gaiero J, Mc Call C, Thompson K, Day A, Best S, Dunfield K (2013) Inside the root: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750

Garcia LC, Martínez-Molina E, Trujillo ME (2010) *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. Int J Syst Evol Microbiol 60:331–337

Germida JJ, Siciliano SD, de Freitas JR, Seib AM (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26:43–50

Govindarajan M, Balandreau J, Muthukumarasamy R, Revathi G, Lakshminarasimhan C (2006) Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. Plant Soil 280:239–252

Gutiérrez-Zamora M, Martínez_Romero E. 2001) Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). J Biotechnol 91:117–126

Hallmann J, Quadt-Hallmann A, Mahaffee W, Kloepper J (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hardoim P, Van Overbeek L, Van Elsas J (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Hardoim PR, van Overbeek LS, 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

Hilali A, Prevost D, Broughton W, Antoun H (2001) Effets de l'inoculation avec des souches de *Rhizobium leguminosarum* biovar *trifolii* sur la croissance der bledansdeux sols der. Maroc Can J Microbiol 41:590–593

Hoque MS, Broadhurst LM, Thrall PH (2011) Genetic characterization of root-nodule bacteria associated with *Acacia salicina* and *A. stenophylla* (Mimosaceae) across south-eastern Australia. Int J Syst Evol Microbiol 61:299–309

Huang B, Lv C, Zhuang P, Zhang H, Fan L (2011) Endophytic colonization of *Bacillus subtilis* in the roots of *Robinia pseudoacacia*. L Plant Biol 13:925–931

Ibañez F, Angelini J, Taurian T, Tonelli ML, Fabra A (2009) Endophytic occupation of peanut root nodules by opportunistic Gammaproteobacteria. Syst Appl Microbiol 32:49–55

Ibañez F, Arroyo ME, Angelini J, Tonelli ML, Muñoz V, Ludueña L, Valetti L, Fabra A (2014) Non-rhizobial peanut nodule bacteria promote maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) growth in a simulated crop rotation system. App Soil Ecol 84:208–212

James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PPM, Olivares FL, Ladha JK (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant-Microbe Interact 15:894–906

Ji XL, Lu GB, Gai YP, Zheng CC, Mu ZM (2008) Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS Microbiol Ecol 65:565–573

Kandel S, Herschberger N, Kim S, Doty S (2015) Diazotrophic endophytes of poplar and willow for growth promotion of rice plants in nitrogen-limited conditions. Crop Sci 55:1765–1772

Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume–rhizobium mutualism. Nature 425:78–81

Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 24:895–904

Leplat J, Friberg H, Abid M, Steinberg C (2013) Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. Agron Sustain Dev 33:97–111

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246

Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X, Wan Y, Zeng G, Long F, Liu C, Liu Y (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. Appl Microbiol Biotechnol 93:1745–1753

Lupwayi N, Clayton G, Hanson K, Rice W, Bierderbeck V (2004) Endophytic rhizobia in barley, wheat, and canola roots. Can J Plant Sci 84:37–45

Marquez-Santacruz HA, Hernandez-Leon R, Orozco-Mosqueda MC, Velazquez-Sepulveda I, Santoyo G (2010) Diversity of bacterial endophytes in roots of Mexican husk tomato plants (*Physalis ixocarpa*) and their detection in the rhizosphere. Genet Mol Res 9:2372–2380

Martínez-Hidalgo P, Galindo-Villardón P, Trujillo M, Igual J, Martínez-Molina E. *Micromonospora* from nitrogen fixing nodules of alfalfa (*Medicago sativa* L.). A new promising Plant Probiotic Bacteria (2014) Scientific Reports 4:6389, doi:10.1038/srep06389

Meneses C, Rouws L, Simões-AraújoJ Vidal M, Baldani J (2011) Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus*. Mol Plant-Microbe Interact 24:1448–1458

Moënne-Loccoz Y, Mavingui P, Combes C, Steinberg C (2015) Microorganisms and biotic interactions. In: Bertrand JC et al (eds) Environmental microbiology: fundamentals and applications. Springer, The Netherlands, pp 395–444

Morris CE, Sands DC, Vanneste JL, Montarry J, Oakley B, Guilbaud C, Glaux C (2010). Inferring the evolutionary history of the plant pathogen *Pseudomonas syringae* from its biogeography in headwaters of rivers in North America, Europe, and New Zealand. mBio 1:e00107–10

Mrabet M, Mnasri B, Romdhane SB, Laguerre G, Aouani ME, Mhamdi R (2006) *Agrobacterium* strains isolated from root nodules of common bean specifically reduce nodulation by *Rhizobium gallicum*. FEMS Microbiol Ecol 56:304–309

Muresu R, Polone E, Sulas L, Baldan B, Tondello A, Delogu G, Cappuccinelli P, Alberghini S, Benhizia Y, Benhizia H, Benguedouar A (2008) Coexistence of predominantly nonculturable rhizobia with diverse, endophytic bacterial taxa within nodules of wild legumes. FEMS Microbiol Ecol 63:383–400

Pandya M, Kumar GN, Rajkumar S (2013) Invasion of rhizobial infection thread by non-rhizobia for colonization of *Vigna radiata* root nodules. FEMS Microbiol Lett 348:58–65

Pandya M, Rajput M, Rajkumar S (2015) Exploring plant growth promoting potential of non rhizobial root nodules endophytes of *Vigna radiate*. Microbiol 84:80–89

Peix A, Carro L, Cerda-Castillo E, Tejedor C, Ramírez-Bahena MH, Velázquez E (2012) New research on the genetic diversity of non-rhizobial endophytes inhabiting legume nodules. In: Amaya JAC, Jiménez, MMF (eds.) Genetic diversity: new research, Nova Science Publishers, pp 191–201

Peix A, Ramírez-Bahena MH, Velázquez E, Bedmar EJ (2015) Bacterial associations with legumes. Crit Rev Plant Sci 34:17–42

Peng SB, Biswas JC, Ladha JK, Yaneshwar PG, Chen Y (2002) Influence of rhizobial inoculation on photosynthesis and grain yield of rice. Agron J 94:925–929

Prayitno Stefaniak JJ, McIver J, Weinman J, Dazzo FB, Ladha JK, Baraquio W, Yanni YG, Rolfe BG (1999) Interactions of rice seedlings with nitrogen-fixing bacteria isolated from rice roots. Aust J Plant Physiol 26:521–535

Prieto P, Schilirò E, Maldonado-González M, Valderrama R, Barroso-Albarracín JB, Mercado-Blanco J (2011) Root hairs play a key role in the endophytic colonization of live roots by *Pseudomonas* spp. With biocontrol activity. Microb Ecol 62:435–445

Rajendran G, Sing F, Desai AJ, Archana G (2008) Enhanced growth and nodulation of pigeon pea by co-inoculation of *Bacillus* strains with *Rhizobium* spp. Bioresour Technol 99:4544–4550

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. Trends Microbiol 6:139–144

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14:435–443

Rinaudi L, Giordano W (2010) An integrated view of biofilm formation in rhizobia. FEMS Microbiol Lett 304:1–11

Rojas A, Olguin G, Lick B, Bashan Y (2001) Synergism between *Phyllobacterium* sp. (N_2 fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol Ecol 35:181–187

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837

Rudrappa T, Czymmek K, Paré P, Bais H (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556

Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda M, Glick B (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99

Savage D, Barbetti M, MacLeod W, Salam M, Renton M (2012) Seasonal and diurnal patterns of spore release can significantly affect the proportion of spores expected to undergo long-distance dispersal. Microb Ecol 63:578–585

Schulz B, Boyle C (2006) What are endophytes? In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial Root Endophytes. Berlin, Germany, Springer, pp 1–13

Shiraishi A, Matsushita N, Hougetsu T (2010) Nodulation in black locust by the Gammaproteobacteria *Pseudomonas* sp. and the Betaproteobacteria *Burkholderia* sp. Syst Appl Microbiol 33:269–274

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fert Soils 25(1):13–19

Tonelli ML, Furlán A, Taurian T, Castro S, Fabra A (2011) Peanut priming induced by biocontrol agents. Physiol Mol Plant Pathol 75:100–105

Trujillo ME, Kroppenstedt RM, Schumann P, Carro L, Martinez-Molina E (2006) *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriariamyrtifolia*. Int J Syst Evol Microbiol 56:2381–2385

Trujillo ME, Kroppenstedt RM, Fernandez-Molinero C, Schumann P, Martinez-Molina E (2007) *Micromonospora lupini* sp. nov. and*Micromonospora saelicesensis* sp. nov., isolated from root nodules of *Lupinus angustifolius*. Int J Syst Evol Microbiol 57:2799–2804

Trujillo ME, Alonso-Vega P, Rodríguez R, Carro L, Cerda E, Alonso P, Martínez-Molina E (2010) The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. ISME J 4:1265–1281

Truyens S, Weyens N, Cuypers A, Vangronsveld J (2014) Bacterial seed endophytes: genera, vertical transmission and interaction with plants. Environ Microbiol Rep 7:40–50

Valdés M, Perez NO, Estrada-de Los Santos P, Caballero-Mellado J, Pena-Cabriales JJ, Normand P, Hirsch AM (2005) Non-*Frankia* actinomycetes isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. Appl Environ Microbiol 71:460–466

Valverde A, Velázquez E, Gutiérrez C, Cervantes E, Ventosa A, Igual JM (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 53:1979–1983

Velázquez E, Martínez-Hidalgo P, Carro L, Alonso P, Peix A, Trujillo ME, Martínez-Molina E (2013) Nodular endophytes: an untapped diversity. In: González-López J (ed) Rodelas González MB. Ecology and Applications. CRC Press, Beneficial Plant-Microbial Interactions, pp 215–235

Villate L, Morin E, Demangeat G, Van Helden M, Esmenjaud D (2012) Control of *Xiphinema index* populations by fallow plants under greenhouse and field conditions. Phytopathology 102:627–634

Wang LL, Wang ET, Liu J, Li Y, Chen WX (2006) Endophytic occupation of root nodules and roots of *Melilotus dentatus* by *Agrobacterium tumefaciens*. Microbial Ecol 52:436–443

Wilson D (1995) Endophyte: The evolution of a term, and clarification of its use and definition. Oikos 73:274–276

Yanni Y, Rizk R, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn F, Stoltzfus R, Buckley D, Schmidt T, Mateos P, Ladha J, Dazzo F (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yanni YG, Rizk R, Abd El-Fattah F, Squartini A, Corich V, Giacomini A, de Bruijn F, Rademaker J, Maya-Flores J, Ostrom P, Vega-Hernandez M, Hollingsworth R, Martinez-Molina E, Mateos E, Velazquez E, Wopereis J, Triplett E, Umali-Garcia M, Anarna J, Rolfe B, Ladha J, Hill J, Mujoo R, Ng P, Dazzo F (2001) The beneficial plant

growth-promoting association of *Rhizobium leguminosarum* biovar *trifolii* with rice roots. Aust J Plant Physiol 62:845–870

Zakhia F, Jeder H, Willems A, Gillis M, Dreyfus B, de Lajudie P (2006) Diverse bacteria associated with root nodules of spontaneous legumes in Tunisia and first report for *nifH*-like gene within the genera *Microbacterium* and *Starkeya*. Microb Ecol 51:93–375

Zgadzaj R, James E, Kelly S, Kawaharada Y, de Jonge N, Jensen D, Madsen L, Radutoiu SA (2015) Legume genetic framework controls infection of nodules by symbiotic and endophytic bacteria. PLoS Genet 11:e1005280

Chapter 3
Quorum-Quenching Endophytes: A Novel Approach for Sustainable Development of Agroecosystem

Rajesh P Shastry and V Ravishankar Rai

Abstract Endophytes live within the plant, without causing apparent symptoms of infections. Plants and endophytes interactions are well known for symbiotic relationships, which substantially increases resistance against the plant pathogens as well as play a major role in growth promotion and nutrient uptake. Beneficial endophytes and plant pathogens use cell-to-cell communication to coordinate cell density known as quorum sensing (QS). Quorum sensing regulates most of the phenotypes which are beneficial in endophytes as well as expression of virulence in pathogens. In this chapter, endophytes and plants interactions were correlated interns of quorum sensing, and control strategies by quorum quenching were discussed based on QS-regulated phenotypes. Furthermore, the chapter also focuses on possible biotechnological application of quorum-quenching enzymes from endophytes to control QS-regulated virulence expression in plant pathogens.

Keywords Endophytes · Quorum quenching · Quorum sensing
Agroecosystem

3.1 Introduction

All plants are inhabited by a diverse microbial community, comprising of archaeal, bacterial, fungal and protistic taxa. Endophytic lifestyle is showing microorganisms play major roles in plant growth, fitness and diversification. Plant-microbe interactions and complexity depend on biotic and abiotic factors, including genotypes, environmental conditions and dynamic networks of interactions (Hardoim et al. 2015). Diverse endophytes have a few commonly found genera *Bacillus* sp.,

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_3

Enterobacter sp., *Phomopsis* sp., *Fusarium* sp., *Phyllostica* sp., *Cladosporium* sp. and so forth (Nair and Padmavathy 2014; Rajesh and Rai 2014a, b).

Growth stimulation by endophytes is a consequence of nitrogen fixation, control of phytopathogens by secondary metabolites at the root zone, production of phytohormones and volatile substances (Rosenblueth and Martínez-Romero 2006). Sixteen essential elements like C, H, N, O and P and 11 more are available to plant for their growth and development from atmosphere, soil, water and organic matter in chemical form. Endophytes play an important role in the uptake of these elements as nutrients to the plant (Nair and Padmavathy 2014). The phytohormones such as auxins, cytokinins and gibberellic acids responsible for promotion of plant growth also produced by many endophytes (Xin et al. 2009). The success of plant growth promotion by endophytic community depends on soil factors that influence survival, colonization and compatibility. The plant factors and microbial factors also play role in competition within the root and ability of the endophyte to survive (Gaiero et al. 2013). Furthermore, distribution within the plant depends on the availability of plant resources and abilities of endophyte colonization. Endophytes get entry through root cracks, lateral root emergence and below the root hair zone, establishing populations both inter- and intra-cellularly. After initial entry and colonization, these endophytes spread through vascular tissues and other part of plant systemically (Johnston-Monje and Raizada 2011).

Moreover, endophytes produce diverse bioactive compounds which showed the potentiality in biotechnological applications. Production of bioactive compounds depends directly on independent evolution of endophytes and promising potential of usefulness in safety as well as human health concerns (Pimentel et al. 2011). Secretion of a broad variety of secondary metabolites including benopyranones, alkaloids, flavonoids, chinones, phenolics, steroids, terpenoids, etc., are other metabolites originated from endophytes. This wide range of bioactive molecules known to have enormous applications in medicine and agrochemicals industry (Tan and Zou 2001; Pimentel et al. 2011). The endophytes also play an important role in balancing soil nutrients and make them available to each component of the ecosystem. Dead biomass is actively degraded by endophytes known to be saprophytes and make them available nutrients to the environment. Endophytes have the potential ability to break down most complex compounds into simpler utilizable form by the plants. This kind of applications has an important role in bioremediation of contaminated waste materials from the environment, possible by countless microbial diversity including endophytes (Müller et al. 2001).

Endophytes have the ability to produce different types of hydrolytic enzymes such as amylase, pectinase, cellulase, lipase, proteinase and laccase (Robl et al. 2013). These enzymes play major role in biodegradation and hydrolytic process in plant pathogen interaction against pathogen infection (Fouda et al. 2015). These enzymes are also required for the biodegradation of litter of the host plant (Gunatilaka 2006). Initially, endophytes colonize within the plant and increase litter decomposition through antagonistic interaction with the saprophytic microorganisms (Rodriguez et al. 2009). Endophytes disclosed various traits with potential capacity have multiple alternate lifestyles as saprophyte in the soil and as an

endophyte inside the root/root nodules. Interestingly, many plant pathogens and saprophytes are derived from the same lineages; relative prevalence of members belongs to endophytic communities (Rodriguez et al. 2009; Trujillo et al. 2015).

3.1.1 Endophytes and Host Plant Interaction

Endophytes interact with host plant, which ranges from antagonistic to mutualistic, and it is variable with respect to host plant as well as species of endophyte (Saikkonen et al. 1998). Traditionally, the plant endophytes considered to be mutualistic by reducing herbivores via production of mycotoxins. Most of the endophytes transmit horizontally with little or no effect on herbivore, but vertically transmitted endophytes of grasses increased resistant to herbivores (Faeth and Fagan et al. 2002). Many seeds carry a diverse species of endophytes; it can propagate vegetatively and transmit into the next generation without infection. But in the rhizosphere region is selective for competitive endophytes to colonize (Ryan et al. 2008).

Some of the endophytes induce plant host defence mechanism to counter attack the pathogen invasion, others to produce many antibiotics against invading pathogens as well as compete for hosting space and nutrient (Saikkonen et al. 1998). When *Chaetomium* and *Phoma* endophytes and their cell-free culture filtrate inoculated to wheat plants, it reduced the foliar disease severity caused by species of *Pyrenophara* and *Puccinia* (Istifadah and McGee et al. 2006). A fungal endophyte *Paraconiothyrium* sp., from *Taxus* sp., induces transcription of genes encoding a redundant taxol biosynthetic pathway in its host plant (Soliman et al. 2013).

3.2 Quorum Sensing

Quorum sensing (QS) is a phenomenon of coordination, in which bacteria sense the population density or growth by releasing specific signalling molecules, and these signalling molecules diffused in surrounding environment are recognized by respective organisms (Liu et al. 2012). Signalling molecules thus represent the bacterial population in such a way that, QS molecules produced until the threshold concentration is reached. The bacteria could express the virulence factor in its threshold population by the control of quorum-sensing mechanism. Thus, by interfering with quorum-sensing pathways, one could suppress the bacterial virulence expression without affecting the population density (Clatworthy et al. 2007). Population density regulation in Gram-negative bacteria is achieved by the synthesis of diffusible signalling molecules, which usually belong to *N*-acyl homoserine lactone (AHL) family which produced throughout the growth. More than a dozen AHL derivatives, which vary in length of acyl side chains have been identified and characterized. The threshold signalling molecules lead to activation

of specific QS-regulated functions like luminescence, production of extracellular enzymes, plasmid transfer, etc. (Boyer et al. 2008). The intra-specific communication is mediated by auto-inducer (AI) molecules in the case of gram-negative bacteria and inter-specific communication is mediated by boronated diester molecules (AI-2) in both Gram-positive and Gram-negative bacteria (Simões et al. 2010). The competitive nature of the bacterial community is the driving force for the development of cooperation among the cells, and adhesion is another requirement to form biofilms. This process involves the cell-to-cell signalling with the specific cell attachment and to form biofilms (dos Reis Ponce et al. 2012). The formation of biofilm exploits the colonial nature, which regulates adaptational regulation for environmental changes. The AHL quorum-sensing molecules have an important role in virulence factor production as well as biofilm formation to combine the bacterial infection and resistance (Khadar et al. 2011).

3.2.1 *Quorum Sensing in Plant-Associated Microorganisms and Pathogens*

Many Gram-negative bacteria associated with plant found to produce AHLs as QS signal molecules, including epiphytic, pathogenic, rhizosphere-inhabiting and nitrogen-fixing symbionts (Piper and Farrand 2000). Apart from AHLs, many plants and rhizosphere associated bacteria produce a number of molecules that affect AHL QS or act as own QS system (Fray 2002). Plant pathogen *Agrobacterium tumefaciens* uses AHL QS system for regulatory mechanism of opines which triggers the transfer of conjugal Ti plasmid between bacteria and plant (Piper and Farrand 2000). In case of octopine-type Ti plasmid regulation, a *lux*R homologue (*Tra*R) operates as octopine inducible operon includes the enzymes required for octopine catabolism. Receptor TraR responds for 3-oxo-C_8-HSL (3-oxo-octanyl homoserine lactone) and this triggers the induction of genes which are required for plasmid transfer (Piper and Farrand 2000). Different number of AHLs produced by *Rhizobium leguminosarum* using four different biosynthetic pathways, largest AHL contains an acyl side chain of 14 carbons. This long chain AHL induces the *rhiABC* operon to promote plasmid transfer; furthermore, mutant of *R. leguminosarum* for AHL receptor exhibited decreased nodulation (Cubo et al. 1992; Lithgow et al. 2000).

A soil borne bacterium, which colonizes on wheat rhizosphere, *Pseudomonas aureofaciens* has been used as a biocontrol agent against the take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*. The biocontrol activity is part of three phenazine antibiotic production, and C_6-HSL controls the expression of phenazine, synthesised by the *phzI* gene (Wood and Pierson 1996). In many biocontrol strains of *Pseudomonas*, AHLs are likely to have a major role in promoting production of active secondary metabolites (Whitehead et al. 2001). It is also confirmed that gross disruption of AHLs based cell–cell signalling in the rhizosphere region may adversely affect the colonization or process of growth promotion or biocontrol species (Zhang and Pierson 2001).

Several species of *Pseudomonas,* produce different molecule to AHLs such as cyclic dipeptides including biocontrol strains and they have the capacity to activate or antagonize the receptors normally used by the bacteria (Holden et al. 1999). The plant pathogen *Ralstonia solanacearum* causes wilt disease in many crops such as potato, tomato and banana. During infection, pathogen produces intercellular signal molecule 3-hydroxy-palmitic acid-methyl ester, volatile molecule acts on transcriptional activator PhcA, and then PhcA induces production of AHLs, extracellular polysaccharides and various other virulence factors (Denny 1999). The causative agent of black rot in crucifers is *Xanthomonas campestris* pv. *campestris* also produces diffusible intercellular signal molecules, which regulate production of extracellular polysaccharide production (He and Zhang 2008).

3.3 Quorum Quenching

Several prokaryotic and eukaryotic organisms were reported to interfere in QS of other organisms either by secreting enzymes that degrade the QS signals of different species by a phenomenon called quorum quenching (QQ) (Fig. 3.1) or by producing quorum-sensing inhibitors (QSI) that can block the communication. The production of signalling molecules depends on species and heterogeneous QS signal secretion by strains. Basically, AHL-mediated QS signals are degraded by three types of enzymes like oxidoreductase, acylase and Lactonase (Fig. 3.2). Furthermore, distinct pathways in degradation of signalling molecules also represent specificity of enzymes (Fekete et al. 2010).

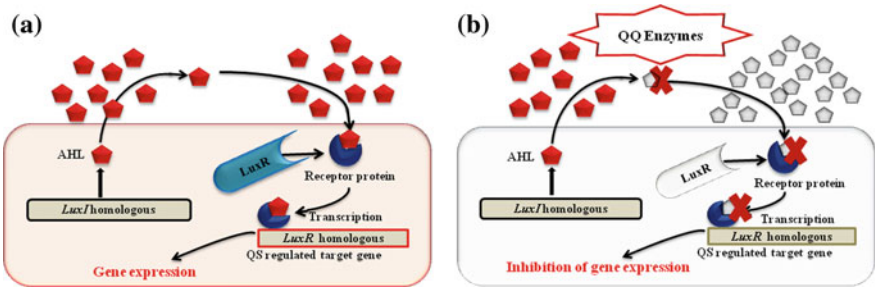

Fig. 3.1 Schematic representation of Quorum sensing and Quorum quenching in bacteria **a** expression of QS-regulated genes in bacteria by AHLs as signalling molecules, **b** possible action of quorum-quenching enzymes leads to inhibition of QS-controlled gene expression

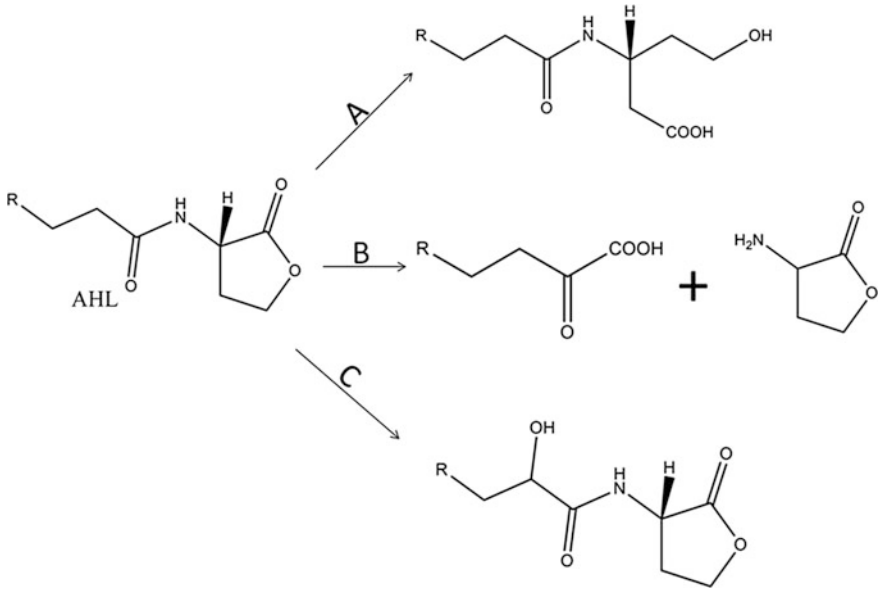

Fig. 3.2 Enzymatic degradation of AHL molecules by QQ enzymes. Hydrolytic cleavage of AHL by AHL-lactonase **A** and AHL-acylase, **B** breaks lactone ring of AHLs to form acyl homoserine and\removes the fatty acid side chain from AHLs (HSL-Homoserine lactone) respectively. **C** Reduction of 3-oxo-substituted AHLs by AHL-oxidoreductases

3.3.1 Quorum-Quenching Inhibitors

Molecules which are capable of mimic or interfere with the QS signals are known as quorum-sensing inhibitors (QSI). Halogenated funanone affects the architecture of *P. aeruginosa* biofilm and process enhances the bacterial detachment, leading to a loss of bacterial biomass from the substratum (Hentzer et al. 2002). On the other hand, Butyrolactones (2(3H)-furanones) from *Streptomyces* sp. (Kinoshita et al. 1997), intermediate of the butanolide (2(5H)-furanones) biosynthetic pathway in *Streptomyces antibioticus* and *Hortonia* sp., effectively reduced the QS in *Chromobacterium violaceum* CV026 (Martinelli et al. 2004).

The better selection of QSI would help in possible success in drug discovery against infectious diseases. Therefore, QSI proposed to have many criteria such as molecule should be small with efficient ability to reduce QS-regulated gene expression (Hentzer and Givskov 2003). QSI should be highly specific without any adverse effects on the bacteria or to the host and chemically stable, resistant for metabolic degradation by host metabolism system (Kalia 2013). More importantly, these are not likely to become resistant by the bacteria and compound not likely to adversely affect the population of bacteria directly (Rasmussen et al. 2005).

Fungal endophytes such as *Fusarium graminearum* and *Lasidiplodia* sp., isolated from *Ventilago madraspatana* Gaertn., significantly inhibited the production of violacein more than 60% in biosensor strain *Chromobacterium violaceum* CV026 (Rajesh and Rai 2013). Interestingly, some of marine endophytes belong to the genera of *Sarocladium, Fusarium, Epicoccum* and *Khuskia* found to inhibit bacterial quorum sensing (Martín-Rodríguez et al. 2014). Similarly, endophytic fungus *Penicillium restrictum* from stem of milk thistle (*Silybum marianum*) known to produce polyhydroxyanthraquinones inhibited QS in a clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) (Figueroa et al. 2014).

3.3.2 Quorum-Quenching Enzymes

Quorum sensing in bacteria is represented by the production of signalling molecules and they widely control the broad range of activities, including virulence factor. AHL-mediated quorum sensing is found in most Gram-negative bacterial species belonging to the genera *Agrobacterium, Aeromonas, Citrobacter, Burkholderia, Ralstonia, Chromobacterium, Enterobacter, Erwinia, Nitrosomonas, Pseudomonas, Rhodobacter, Rhizobium, Serratia, Vibrio* and *Yersinia* (Eberl 1999). The major biosynthesis of AHL is encoded by enzyme acyl homoserine lactone synthase (*Lux*I) which uses S-adenosyl methionine and an acyl chain carrier protein to form AHL molecule. As the threshold of QS molecules reaches, the AHL binds to a receptor protein (*Lux*R) in the bacterial cytoplasm which activates the *lux* operon to regulate the gene expression (Hartmann and Schikora 2012) and this regulatory mechanism allows bacteria to coordinate swarming, biofilm formation, stress resistance, production of toxins and secondary metabolites (Steidle et al. 2002).

Production and degradation of signalling molecules are an evidence for microbial interaction under growth stimulated conditions. This was observed in culture medium and in pork extract as a food stimulated medium for *Bacillus cereus* and *Yersinia enterocolitica* (Medina-Martínez et al. 2007). Therefore, the QS regulatory mechanism involved a battle target for the control of pathogenic bacteria with respect to the same species, In fact, QS is also observed in species specifically as well as in interspecies signalling mechanisms. In many polymicrobial communities, the cell-to-cell communication occurs in interspecies and signalling molecules are of the same or related signals of communities. Interspecies signalling alters the virulence and persistence of pathogens and also affects the development of beneficial microbial communities (Ryan et al. 2008). The bacteria also respond to the secreted molecules from closely related bacteria having phylogenetic relation to the interacting bacteria (Shank et al. 2011). The enzymatic degradation of the AHL has been reported due to the presence of different types of genes with respective organisms. In fact, metabolism of AHL molecules utilized as nitrogen source by *Variovorax paradoxus* (Leadbetter and Greenberg 2000).

3.3.2.1 Sources of Lactonase

The lactonases preferentially act on the lactone ring of AHL signalling molecule, produced by the Gram-negative bacteria. The firmicutes such as *Bacillus* sp, the Gram-positive bacteria are reported as the major AHL molecule degrades from diverse sources. Similarly, *B. cereus* and *B. mycoides* activity has not been reported in *aiiA* gene for AHL-lactonase activity but such *B. fusiformis* and *B. sphaericus* strains (Dong et al. 2002). *Bacillus sonorensis* isolated from the fermentation brine of Chinese soy sauce has the ability to degrade AHL, but devoid of the *aiiA* homologue suggesting the presence of different AHL-degrading gene (Yin et al. 2012). There are many *Bacillus* species which have lactonase producing capacity similar to that *Bacillus marcorestinctum* (Han et al. 2010) and *B. licheniformis* (Mani et al. 2012). The genetic diversity among the strains reflects the activity of AHL-lactonase as evidenced by the presence of *aiiA* gene. In case of *Bacillus* sp., the genetic diversity with respect to lactonase gene predominates and it varies with the strains of the same species (Huma et al. 2011).

The lactonase produced by *Arthrobacter* sp. IBN110 by utilizing 3-oxo-C_6-HSL as sole carbon source. When this bacterium was co-cultured with *Erwinia carotovora*, amount of AHL and pectin lyase activity reduced significantly. The catalysis of AHL molecules is encoded by *AhlD* gene with hydrolysis capacity on the lactone ring of C_8-HSL. Furthermore, AHL-degrading activity was detected in *Klebsiella pneumoniae* and *Bacillus stearothermophilus* with *ahlK*, an *ahlD* homologue gene encodes the AHL-degrading enzymes respectively (Park et al. 2003). There are actively different lactonases with the selection of the same substrate (AHL molecules) but variation in structural constituent of enzyme. *Rhodococcus erythropolis* encoded by *qsdA* (for quorum-sensing signal degradation) as a major gene and is related to phosphotriesterases and constitutes a new type of lactonase (Uroz and Heinonsalo 2008). Multi-substrate utilizing capacity is reported in *Chryseobacterium* spp. which degrade C_{10}-HSL as well as 3-oxo-substituted AHLs and is purely based on strain specific (Rashid et al. 2011). These types of enzymes assist in quorum quenching of multi-species, which are producers of more than one signalling molecule. The broad spectrum inactivation of AHL family was reported in *Comamonas* sp.; it was able to degrade AHL with acyl side chains ranging from 4 to 6 C to form HSL instead of *N*-acyl homoserine, therefore, it is considered as amidohydrolase. This amidohydrolase has the ability to suppress pathogenicity and antibiotic production in *Pectobacterium* under the control of quorum sensing (Uroz et al. 2007). The *aiiM* gene, which is the part of *Microbacterium testaceum*, a Gram-negative, leaf surface inhabiting bacterium of potato highly homologous to α/β hydrolase family from *Actinobacteria* and expressed with plant pathogen *Pectobacterium carotovorum* subsp. *carotovorum;* it effectively attenuates the soft rot symptoms of potato (Wang et al. 2010).

A phosphotriesterase-like lactonase (PLL) produced from *Geobacillus kaustophilus* has thermostable quorum-quenching lactonase activity, which hydrolyzes AHLs (Chow et al. 2010). *Rhizobium* sp., demonstrates autoinducer I hydrolase which functions as AHL— lactonase enzyme able to inhibit the formation of

biofilm based on quorum-sensing processes in *P. aeruginosa*, *A. tumefaciens* and *C. violaceum* (Krysciak et al. 2011). The rhizobacteria belongs to *Bacillus, Streptomyces*, *Arthrobacter*, *Pseudomonas* and *Mesorhizobium* showed AHL-degrading ability against *Pectobacterium carotovorum* strain are able to reduce the tissue maceration on potato tubers (Mahmoudi et al. 2011).

Recently, *aiiA* homologous gene from endophytic bacteria *Bacillus firmus* PT18 and *Enterobacter asburiae* PT39 exhibited potent quorum-sensing molecule hydrolysis against short and long chain AHLs. These bacteria were isolated as endophytes from *Pterocarpus santalinus* Linn., and the protein tentatively predicted as AHL-lactonase (Rajesh and Rai 2014a, b). Furthermore, endophytes of *Ventilago madraspatana* significantly degraded AHL molecules more than 99%, collectively identified as *E. asburiae VT65*, *E. aerogenes VT66* and *E. ludwigii VT70*. Molecular sequence analysis revealed that QQ enzyme belongs to the family of AHL-lactonase along with the presence of two zinc binding sites, "HXHXDH" motif as well as tyrosine residue at the position of 194 (Rajesh and Rai 2014a, b).

3.3.2.2 Microbial Sources of Acylase

Bacterial acylase is produced to degrade AHL molecules into fatty acids and homoserine lactone by cleaving amide bond, so it is also called as amidohydrolase. The specificity of substrate or degradation of AHL depends on the type of acylase secreted or otherwise length of the carbon chain in AHL molecule (Lin et al. 2003). The existence of quorum-quenching and quorum-sensing system reveals the controlled regulation of cell-to-cell communication. Enzymatic degradation of these diffusible signals by amidohydrolases abolishes AHL regulated virulence; which may be utilized to suppress the quorum-sensing machinery of pathogens (Beeson et al. 2011). On the other hand, *Pseudomonas aeruginosa* MW3A, an isolate of sea water, is capable to utilize 3-oxo-C_8-HSL as the sole source of carbon. The degradation prefers the presence of substituted molecule of AHL rather than the unsubstituted groups at C_3 position of acyl side chain. The gene responsible for the degradation property is *quiP* and *pvdQ* homologue gene sequences with that of acylases (Huang et al. 2003; Wong et al. 2012). *Ralstonia* sp., was isolated from biofilm of mixed bacterial species, revealed to inactivate AHLs signalling molecules by hydrolysis of the amide bond encoded with *aiiD* gene. It shared most similar amino acid poly peptides with aculeacin A acylase (AAC) from *Actinoplanes utahensis*, cephalosporin acylases and similar other N-terminal (Ntn) hydrolases (Lin et al. 2003). Similar acylase from different microbial source also found active against most of AHLs such as Aac from *Shewanella* sp. MIB015 (Morohoshi et al. 2008) and AiiC from *Anabaena* sp. PCC7120 (Romero et al. 2008) belonging to Ntn hydrolase family, AiiO from *Ochrobactrum* sp. A44 belongs to α/β-Hydrolase family (Czajkowski et al. 2011).

3.3.2.3 Sources of Oxidoreductase

These enzymes catalyse oxidoreduction reactions, commonly called as dehydrogenases or oxidases. The cell extract and whole cell assay of *Rhodococcus erythropolis* strain W2 have shown oxidoreductase activity to reduce compounds such as *N*-(3-oxo-6-phenylhexanoyl) homoserine lactone and 3-oxododecanamide as well as capable of reducing both D- and L-isomers of *N*-(3-oxododecanoyl)-l-homoserine lactone (Uroz et al. 2005). Similarly, AHL-oxidoreductase reported as CYP102A1 a Cytochrome P450 Monooxygenase from *B. megaterium* capable of high efficient oxidation of AHLs signalling molecules. The observed oxidation primarily takes place at the ω-1, ω-2, and ω-3 carbons of the acyl chain and also on fatty acids, but AHLs judged to be better substrates in comparison to that of fatty acids (Chowdhary et al. 2007).

3.4 Control of QS Mediated Plant Disease by Quorum Quenching

Most of the plant pathogens to establish the disease severity by QS-regulated expression of virulence factors strongly depend against the plant protection mechanism. The earliest application of QQ strategy against microbial infection to protection of plant disease was conducted on Chinese cabbage. The phytopathogen *Erwinia carotovora* could cause decay in Chinese cabbage, the virulence expression was regulated by QS. The decay phenotype of Chinese cabbage is significantly controlled by expression of *aiiA* gene encode for AHL-lactonase in transformed phytopathogen *E. carotovora* (Dong et al. 2000). Furthermore, expression of *aiiA* gene in tobacco leaves and on potato tuber showed as feasible approach for prevention of bacterial infection (Dong et al. 2001).

Similarly, Co-inoculation of *P. chlororaphis* biocontrol strain expressing *aiiA* lactonase with plant pathogen *Fusarium oxysporum* impaired severity of disease in tomato plants (Molina et al. 2003). Expression of *aiiA* in transformed *E. amylovora* abolished induction of AHL biosensors, tolerance to hydrogen peroxide, impaired extracellular polysaccharide production and reduced virulence on apple leaves (Molina et al. 2005). *Bacillus cereus* U92 remarkably inactivated all synthetic AHLs up to 80%, successfully reduced the frequency of Ti plasmid conjugal transfer in *A. tumefaciens* by about 99% and this strain acted as a biocontrol agent by attenuating *Pectobacterium* soft rot on potato tubers (up to 60%) as well as efficient in alleviating QS-regulated crown gall incidence on tomato roots (up to 90%) (Zamani et al. 2013). Expression of AHL-acylase encoded by *Pvd*Q gene from *P. aeruginosa* exhibited significant effect on virulence in *Burkholderia cenocepacia* in larvae of the great wax moth *Galleria mellonella*. Furthermore, exogenous addition of *Pvd*Q showed a dramatic decrease in QS molecules and inhibited QS-regulated phenotypes (Koch et al. 2014).

Most important strategy used by microbes in terms of the QS regulation is competitive behaviour for survival and beneficial support for plant growth. AHL-lactonase is necessary for rhizosphere colonization of microbes, its survival in the soil and also for preventing microbial diseases. A mutant strain with defective gene encode AHL-lactonase was unable to successfully colonize in the rhizosphere region and its viability was significantly decreased (Park et al. 2008). The co-culture on sliced potato tubers with QQ bacterium *Bacillus marcorestinctum* from soil strongly quenches the AHL QS signal and *Pectobacterium carotovorum* effectively attenuated QS mediated soft rot symptoms on potato tuber (Han et al. 2010). Furthermore, expression of *aiiA* gene from *Bacillus* sp. DMS133 under the constitutive *lac* promoter in *P. carotovorum* drastically reduced the tissue maceration activity on potato tuber (Mahmoudi et al. 2011). AHL-degrading enzyme AidH from *Ochrobactrum* sp. strain T63 belonging to the α/β-hydrolyase family hydrolyses the ester bond of the homoserine lactone ring of AHLs significantly reduces the pathogenicity of *P. carotovorum* and biofilm formation by *P. fluorescens* 2P24 (Mei et al. 2010).

The second AHL-lactonase identified was AttM from *A. tumefaciens* (Zhang et al. 2002), that regulates the horizontal transfer and vegetative replication of oncogenic Ti plasmids with the help of cell-to-cell communication (Lang and Faure 2014). Naturally occurring molecules such as salicylic acid and nonprotein amino acid GABA (gamma-Aminobutyric acid) have been shown to induce *attM* expression (Chevrot et al. 2006) and over-expression of these metabolites increased the plant resistant against *A. tumefaciens* (Yuan et al. 2007). Furthermore, interference with *A. tumefaciens* QS by the expression of *attM* gene significantly induced the plant immune response (Haudecoeur et al. 2009).

3.4.1 Endophytes in Control of Virulence in Plant Pathogen *Pectobacterium Carotovorum*

Here we follow to test the efficacy of QQ enzyme from endophytic bacterium isolated from root sample of medicinal plant, *Coscinium fenestratum* Gaertn. Endophytic bacterium *Enterobacter* sp. CS25 identified with *aiiA* homologous gene encode for AHL-lactonase. Production of AHL molecules by *P. carotovorum* was confirmed by co-culturing on LB agar with *C. violaceum* CV026 biosensor. Violacein production by biosensor strain confirmed the AHLs production (Fig. 3.3a). The pathogenicity assay was performed as described previously (Chankhamhaengdecha et al. 2013; Rajesh and Rai 2016) with some modifications. Briefly, potato tubers of same dimension (4 ± 0.5 cm diameters) were surface sterilized and washed with sterile water followed by 90 µl of 12 h culture of *P. carotovorum* (1.5×10^8 cfu/ml), mixed with 10 µl of partially purified AHL-lactonase. Then, the reaction mixture containing enzyme and test organism was incubated at 37 °C for 1 h and inoculated into potato tuber using pipette tips

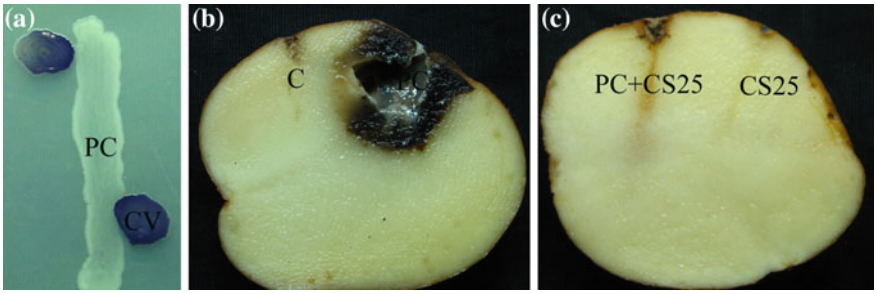

Fig. 3.3 Bioassay test of *P. carotovorum* using *C. violaceum* CV026 biosensor (CV) and *in vitro* analysis of inhibition of soft rot disease (tissue maceration activity). **a** detection method to confirm the production of AHL molecules as QS molecules by *P. carotovorum*. **b** *In vitro* assay for inhibition of tissue maceration activity and **c** by treatment with AHL-lactonase in potato tubers (C is sterile water, PC is *P. carotovorum* alone, CS25 is AHL-lactonase from *Enterobacter* sp. CS25 alone, CS25 + PC is *P. carotovorum* was treated with AHL-lactonase). The decrease in tissue maceration in potato tubers upon treatment with AHL-lactonase (CS25 + PC) indicates that control of virulence regulated by QS compared to control (PC)

(20–22 mm depth punch). The untreated *P. carotovorum* culture was used as control. Each potato was inoculated with treated and untreated cultures (control) in different punch holes. Potato punch holes filled with AHL-lactonase solution and sterile water served as controls. Inoculated tubers were wrapped in aluminium foil and incubated at 30 °C for 5 days.

The inoculum of *P. carotovorum* alone strongly showed tissue maceration in potato tuber but no infection was seen either in AHL-lactonase preparation alone or in sterile control. But, inoculum of *P. carotovorum* mixed with 10 µl of AHL-lactonase reduced tissue maceration substantially (Fig. 3.3b and c). Most of the *P. carotovorum* use 3-oxo-C_6-HSL as the major signalling molecules, whereas some group of bacteria uses 3-oxo-C_8-HSL as QS signal (Jafra et al. 2006). The AHL-lactonase can effectively degrade AHLs produced by most of the Gram-negative bacteria including *P. carotovorum* for QS-regulated virulence factor expression. It has been demonstrated that expression of *aiiA* from *Bacillus* sp. 240B1 in *E. carotovorum* significantly decreases the production of extracellular pectinase and reduces bacterial pathogenicity (Dong et al. 2002). HSL-acylase from endophytic *Streptomyces* LPC029 degraded long chain HSL and attenuated soft rot disease caused by *P. carotovorum* (Chankhamhaengdecha et al. 2013).

3.5 Conclusion

Endophytes and plants are known to have beneficial interactions including improvement of plant immunity and nutrient uptake. The partially purified AHL-lactonase from *Enterobacter* sp. CS25 can degrade AHLs molecules by

cleaving the lactone ring and which impart direct application in the control of QS-regulated virulence in Plant pathogen *P. carotovorum*. Therefore, the enzyme could be used as biocontrol agent in plant diseases caused by plant pathogens, in which virulence is regulated by quorum sensing.

References

Beeson WT, Iavarone AT, Hausmann CD, Cate JHD, Marletta MA (2011) Extracellular Aldonolactonase from *Myceliophthora thermophile*. Appl Environ Microbiol 77(2):650–656

Boyer M, Bally R, Perrotto S, Chaintreuil C, Wisniewski-Dyé F (2008) A quorum-quenching approach to identify quorum-sensing-regulated functions in *Azospirillum lipoferum*. Res Microbiol 159(9–10):699–708

Chankhamhaengdecha S, Hongvijit S, Srichaisupakit A, Charnchai P, Panbangred W (2013) Endophytic actinomycetes: a novel source of potential acyl homoserine lactone degrading enzymes. BioMed Res Int vol. 2013

Chevrot R, Rosen R, Haudecoeur E, Cirou A, Shelp BJ, Ron E, Faure D (2006) GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*. Proc Natl Acad Sci USA 103(19):7460–7464

Chow JY, Xue B, Lee KH, Tung A, Wu L, Robinson RC, Yew WS (2010) Directed evolution of a thermostable quorum-quenching lactonase from the amidohydrolase superfamily. J Biol Chem 285(52):40911–40920

Chowdhary PK, Keshavan N, Nguyen HQ, Peterson JA, González JE, Haines DC (2007) *Bacillus megaterium* CYP102A1 oxidation of acyl homoserine lactones and acyl homoserines. Biochemistry (Mosc.) 46(50):14429–14437

Clatworthy AE, Pierson E, Hung DT (2007) Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3(9):541–548

Cubo MT, Economou A, Murphy G, Johnston AW, Downie JA (1992) Molecular characterization and regulation of the rhizosphere-expressed genes rhiABCR that can influence nodulation by *Rhizobium leguminosarum* biovar. *viciae*. J Bacteriol 174(12):4026–4035

Czajkowski R, Krzyżanowska D, Karczewska J, Atkinson S, Przysowa J, Lojkowska E, Williams P, Jafra S (2011) Inactivation of AHLs by *Ochrobactrum* sp. A44 depends on the activity of a novel class of AHL acylase. Environ Microbiol Rep 3(1):59–68

Denny TP (1999) Autoregulator-dependent control of extracellular polysaccharide production in phytopathogenic bacteria. Eur J Plant Pathol 105(5):417–430

Dong YH, Xu JL, Li XZ, Zhang LH (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci 97(7):3526–3531

Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH (2001) Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 411(6839):813–817

Dong YH, Gusti AR, Zhang Q, Xu JL, Zhang LH (2002) Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. Appl Environ Microbiol 68(4):1754–1759

dos Reis Ponce A, Martins ML, de Araujo EF, Mantovani HC, Vanetti MCD (2012) AiiA quorum-sensing quenching controls proteolytic activity and biofilm formation by *Enterobacter cloacae*. Curr Microbiol 65(6):758–763

Eberl L (1999) N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. Syst Appl Microbiol 22(4):493–506

Faeth SH, Fagan WF (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. Integr Comp Biol 42(2):360–368

Fekete A, Kuttler C, Rothballer M, Hense BA, Fischer D, Buddrus-Schiemann K, Lucio M, Müller J, Schmitt-Kopplin P, Hartmann A (2010) Dynamic regulation of N-acyl-homoserine lactone production and degradation in *Pseudomonas putida* IsoF. FEMS Microbiol Ecol 72(1):22–34

Figueroa M, Jarmusch AK, Raja HA, El-Elimat T, Kavanaugh JS, Horswill AR, Cooks RG, Cech NB, Oberlies NH (2014) Polyhydroxyanthraquinones as quorum sensing inhibitors from the guttates of *Penicillium restrictum* and their analysis by desorption electrospray ionization mass spectrometry. J Nat Prod 77(6):1351–1358

Fouda AH, Hassan SED, Eid AM, Ewais EED (2015) Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Ann Agric Sci 60 (1):95–104

Fray RG (2002) Altering plant–microbe interaction through artificially manipulating bacterial quorum sensing. Ann Bot 89(3):245–253

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: Bacterial root endophytes and plant growth promotion. Am J Bot 100(9):1738–1750

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):509–526

Han Y, Chen F, Li N, Zhu B, Li X (2010) *Bacillus marcorestinctum* sp. nov., a novel soil acylhomoserine lactone quorum-sensing signal quenching bacterium. Int J Mol Sci 11(2):507–520

Hardoim PR, van Overbeek LS, 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(3):293–320

Hartmann A, Schikora A (2012) Quorum sensing of bacteria and trans-kingdom interactions of N-acyl homoserine lactones with eukaryotes. J Chem Ecol 38(6):704–713

Haudecoeur E, Planamente S, Cirou A, Tannières M, Shelp BJ, Moréra S, Faure D (2009) Proline antagonizes GABA-induced quenching of quorum-sensing in *Agrobacterium tumefaciens*. Proc Natl Acad Sci 106(34):14587–14592

He YW, Zhang LH (2008) Quorum sensing and virulence regulation in *Xanthomonas campestris*. FEMS Microbiol Rev 32(5):842–857

Hentzer M, Givskov M (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J Clin Invest 112(9):1300–1307

Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L, Molin S, Høiby N, Kjelleberg S, Givskov M (2002) Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology 148(1):87–102

Holden MT, Ram Chhabra S, de Nys R, Stead P, Bainton NJ, Hill PJ, Manefield M, Kumar N, Labatte M, England D, Rice S, Givskov M, Salmond GP, Stewart GS, Bycroft BW, Kjelleberg S, Williams P (1999) Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. Mol Microbiol 33(6):1254–1266

Huang JJ, Han JI, Zhang LH, Leadbetter JR (2003) Utilization of acyl-homoserine lactone quorum signals for growth by a soil *Pseudomonad* and *Pseudomonas aeruginosa* PAO1. Appl Environ Microbiol 69(10):5941–5949

Huma N, Shankar P, Kushwah J, Bhushan A, Joshi J, Mukherjee J, Raju J, Purohit HJ, Kalia VC (2011) Diversity and polymorphism in AHL-lactonase gene (aiiA) of *Bacillus*. J Microbiol Biotechnol 21(10):1001–1011

Istifadah N, McGee PA (2006) Endophytic *Chaetomium globosum* reduces development of tan spot in wheat caused by *Pyrenophora tritici-repentis*. Australia Plant Pathol 35(4):411–418

Jafra S, Jalink H, Van Der Schoor R, Van Der Wolf JM (2006) *Pectobacterium carotovorum* subsp. *carotovorum* strains show diversity in production of and response to N-acyl homoserine lactones. J Phytopathol 154(11–12):729–739

Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in zea across boundaries of evolution, ethnography and ecology. Plos One 6(6):6 e20396

Kalia VC (2013) Quorum sensing inhibitors: An overview. Biotechnol Adv 31(2):224–245
Khadar SM, Shunmugiah KP, Arumugam VR (2011) Inhibition of quorum-sensing-dependent phenotypic expression in *Serratia marcescens* by marine sediment *Bacillus* spp. SS4. Ann Microbiol 62(1):443–447
Kinoshita H, Ipposhi H, Okamoto S, Nakano H, Nihira H, Yamada Y (1997) Butyrolactone autoregulator receptor protein (BarA) as a transcriptional regulator in *Streptomyces virginiae*. J Bacteriol 179(22):6986–6993
Koch G, Nadal-Jimenez P, Reis CR, Muntendam R, Bokhove M, Melillo E, Dijkstra BW, Cool RH, Quax WJ (2014) Reducing virulence of the human pathogen *Burkholderia* by altering the substrate specificity of the quorum-quenching acylase PvdQ. Proc Natl Acad Sci USA 111(4):1568–1573
Krysciak D, Schmeisser C, Preuss S, Riethausen J, Quitschau M, Grond S, Streit WR (2011) Involvement of multiple loci in quorum quenching of autoinducer I molecules in the nitrogen-fixing symbiont *Rhizobium* (*Sinorhizobium*) sp. strain NGR234. Appl Environ Microbiol 77(15):5089–5099
Lang J, Faure D (2014) Functions and regulation of quorum-sensing in *Agrobacterium tumefaciens*. Plant Biot Interact 5(14)2014
Leadbetter JR, Greenberg EP (2000) Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. J Bacteriol 182(24):6921–6926
Lin YH, Xu JL, Hu J, Wang LH, Ong SL, Leadbetter JR, Zhang LH (2003) Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. Mol Microbiol 47(3):849–860
Lithgow JK, Wilkinson A, Hardman A, Rodelas B, Wisniewski-Dyé F, Williams P, Downie JA (2000) The regulatory locus *cinRI* in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. Mol Microbiol 37(1):81–97
Liu P, Gao Y, Huang W, Shao Z, Shi J, Liu Z (2012) A novel bioassay for high-throughput screening microorganisms with N-acyl homoserine lactone degrading activity. Appl Biochem Biotechnol 167(1):73–80
Mahmoudi E, Hasanzadeh N, Tabatabaei BES, Venturi V (2011) Virulence attenuation of *Pectobacterium carotovorum* using N-acyl-homoserine lactone degrading bacteria isolated from potato rhizosphere. Plant Pathol J 2011
Mani A, Hameed S, Ramalingam S, Narayanan N (2012) Assessment of quorum quenching activity of *Bacillus* species against *Pseudomonas aeruginosa* MTCC 2297. Global J Pharmacol 6:118–125
Martinelli D, Grossmann G, Séquin U, Brandl H, Bachofen R (2004) Effects of natural and chemically synthesized furanones on quorum sensing in *Chromobacterium violaceum*. BMC Microbiol 4(1):1–10
Martín-Rodríguez AJ, Reyes F, Martín J, Pérez-Yépez J, León-Barrios J, Couttolenc J, Espinoza C, Trigos Á, Martín VS, Norte M, Fernández JJ (2014) Inhibition of bacterial quorum sensing by extracts from aquatic fungi: first report from marine endophytes. Mar Drugs 12(11):5503–5526
Medina-Martínez MS, Uyttendaele M, Rajkovic A, Nadal P, Debevere J (2007) Degradation of N-acyl-l-homoserine lactones by *Bacillus cereus* in culture media and pork extract. Appl Environ Microbiol 73(7):2329–2332
Mei GY, Yan XX, Turak A, Luo ZQ, Zhang LQ (2010) AidH, an alpha/beta-hydrolase fold family member from an *Ochrobactrum* sp. strain, is a novel N-acylhomoserine lactonase. Appl Environ Microbiol 76(15):4933–4942
Molina L, Constantinescu F, Michel L, Reimmann C, Duffy B, Défago G (2003) Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. FEMS Microbiol Ecol 45(1):71–81
Molina L, Rezzonico F, Défago G, Duffy B (2005) Autoinduction in *Erwinia amylovora*: evidence of an acyl-homoserine lactone signal in the fire blight pathogen. J Bacteriol 187(9):3206–3213

Morohoshi T, Nakazawa S, Ebata A, Kato N, Ikeda T (2008) Identification and characterization of N-acylhomoserine lactone-acylase from the fish intestinal *Shewanella* sp. strain MIB015. Biosci Biotechnol Biochem 72(7):1887–1893

Müller MM, Valjakka R, Suokko A, Hantula J (2001) Diversity of endophytic fungi of single norway spruce needles and their role as pioneer decomposers. Mol Ecol 10(7):1801–1810

Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. Sci World J e250693

Park SY, Lee SJ, Oh TK, Oh JW, Koo BT, Yum DY, Lee JK (2003) AhlD, an N-acylhomoserine lactonase in *Arthrobacter* sp., and predicted homologues in other bacteria. Microbiol Read Engl 149(6):1541–1550

Park SJ, Park SY, Ryu CM, Park SH, Lee JK (2008) The role of AiiA, a quorum-quenching enzyme from *Bacillus thuringiensis*, on the rhizosphere competence. J Microbiol Biotechnol 18 (9):1518–1521

Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM, Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process, the use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Int Biotechnol Res Int e576286

Piper KR, Farrand SK (2000) Quorum sensing but not autoinduction of Ti plasmid conjugal transfer requires control by the opine regulon and the antiactivator *TraM*. J Bacteriol 182 (4):1080–1088

Rajesh PS, Rai VR (2013) Hydrolytic enzymes and quorum sensing inhibitors from endophytic fungi of *Ventilago madraspatana* Gaertn. Biocatal Agric Biotechnol 2(2):120–124

Rajesh PS, Rai VR (2014a) Quorum quenching activity in cell-free lysate of endophytic bacteria isolated from *Pterocarpus santalinus* Linn., and its effect on quorum sensing regulated biofilm in *Pseudomonas aeruginosa* PAO1. Microbiol Res 169(7–8):561–569

Rajesh PS, Rai VR (2014b) Molecular identification of *aiiA* homologous gene from endophytic *Enterobacter* species and in silico analysis of putative tertiary structure of AHL-lactonase. Biochem Biophys Res Commun 443(1):290–295

Rajesh PS, Rai VR (2016) Inhibition of QS-regulated virulence factors in *Pseudomonas aeruginosa* PAO1 and *Pectobacterium carotovorum* by AHL-lactonase of endophytic bacterium Bacillus cereus VT96. Biocatal Agric Biotechnol 7:154–163

Rashid R, Morohoshi T, Someya N, Ikeda T (2011) Degradation of N-acylhomoserine lactone quorum sensing signaling molecules by potato root surface-associated *Chryseobacterium* strains. Microbes Environ JSME 26(2):144–148

Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO, Hentzer M, Eberl L, Hoiby N, Givskov M (2005) Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. Microbiol Read Engl 151(5):1325–1340

Robl D, Delabona P daS, Mergel CM, Rojas JD, Costa P dos S, Pimentel IC, Vicente VA, da Cruz Pradella JG, Padilla G (2013) The capability of endophytic fungi for production of hemicellulases and related enzymes. BMC Biotechnol 13:94

Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Romero M, Diggle SP, Heeb S, Cámara M, Otero A (2008) Quorum quenching activity in *Anabaena* sp. PCC 7120: identification of AiiC, a novel AHL-acylase. FEMS Microbiol Lett 280(1):73–80

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interac 19(8):827–837

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278(1):1–9

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal Endophytes: a continuum of interactions with host plants. Ann Rev Ecol Syst 29(1):319–343

Shank EA, Klepac-Ceraj V, Collado-Torres L, Powers GE, Losick R, Kolter R (2011) Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. Proc Natl Acad Sci 108(48):e1236–e1243

Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT-Food Sci Technol 43(4):573–583

Soliman SS, Trobacher CP, Tsao R, Greenwood JS, Raizada MN (2013) A fungal endophyte induces transcription of genes encoding a redundant fungicide pathway in its host plant. BMC Plant Biol 13:93

Steidle A, Allesen-Holm A, Riedel A, Berg A, Givskov A, Molin A, Eberl L (2002) Identification and characterization of an N-acylhomoserine lactone-dependent quorum-sensing system in *Pseudomonas putida* strain IsoF. Appl Environ Microbiol 68(12):6371–6382

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18 (4):448–459

Trujillo ME, Riesco R, Benito P, Carro L (2015) Endophytic actinobacteria and the interaction of micromonospora and nitrogen fixing plants. Extreme Microbiol 1341

Uroz S, Heinonsalo J (2008) Degradation of N-acyl homoserine lactone quorum sensing signal molecules by forest root-associated fungi. FEMS Microbiol Ecol 65(2):271–278

Uroz S, Chhabra SR, Cámara M, Williams P, Oger P, Dessaux Y (2005) N-Acylhomoserine lactone quorum-sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. Microbiol Read Engl 151 (10):3313–3322

Uroz S, Oger P, Chhabra SR, Cámara M, Williams P, Dessaux Y (2007) N-acyl homoserine lactones are degraded via an amidolytic activity in *Comamonas* sp. strain D1. Arch Microbiol 187(3):249–256

Wang WZ, Morohoshi T, Ikenoya M, Someya N, Ikeda T (2010) AiiM, a novel class of N-acylhomoserine lactonase from the leaf-associated bacterium *Microbacterium testaceum*. Appl Environ Microbiol 76(8):2524–2530

Whitehead NA, Barnard AML, Slater H, Simpson NJL, Salmond GPC (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25(4):365–404

Wong CS, Yin WF, Choo YM, Sam CK, Koh CL, Chan KG (2012) Coexistence of quorum-quenching and quorum-sensing in tropical marine *Pseudomonas aeruginosa* strain MW3A. World J Microbiol Biotechnol 28(2):453–461

Wood DW (1996) Pierson LS (1996) The *phzI* gene of *Pseudomonas aureofaciens* 30-84 is responsible for the production of a diffusible signal required for phenazine antibiotic production. Gene 168(1):49–53

Xin G, Zhang G, Kang JW, Staley JT, Doty SL (2009) A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol Fertil Soil 45(6):669–674

Yin WF, Tung HJ, Sam CK, Koh CL, Chan KG (2012) Quorum quenching *Bacillus sonorensis* isolated from soya sauce fermentation brine. Sensors 12(4):4065–4073

Yuan ZC, Edlind MP, Liu P, Saenkham P, Banta LM, Wise AA, Ronzone E, Binns AN, Kerr K, Nester EW (2007) The plant signal salicylic acid shuts down expression of the vir regulon and activates quormone-quenching genes in *Agrobacterium*. Proc Natl Acad Sci USA 104 (28):11790–11795

Zamani M, Behboudi K, Ahmadzadeh M (2013) Quorum quenching by *Bacillus cereus* U92: a double-edged sword in biological control of plant diseases. Biocontrol Sci Technol 23(5):555–573

Zhang Z, Pierson LS (2001) A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in *Pseudomonas aureofaciens*. Appl Environ Microbiol 67 (9):4305–4315

Zhang HB, Wang LH, Zhang LH (2002) Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. Proc Natl Acad Sci 99(7):4638–4643

Chapter 4
Harnessing Fungal Endophytes for Plant and Human Health

Deepanwita Deka, Kumananda Tayung and Dhruva Kumar Jha

Abstract Endophytic fungi reside inside the healthy tissues of plants. Expansion of the world population has increased the health problems in human and plant and drug resistance in pathogens. Endophytic fungi have the ability to benefit plant growth, metabolism and defense against pathogens, herbivores, insects, etc. They can produce various potential commercially valued secondary metabolites. This has generated worldwide interest among the researchers to study and exploit them for applications in pharmacy and agriculture. Extensive research has led to the discovery of endophytic fungi which provides a great source of medicine for therapeutic applications in human and plant protection under adverse conditions. Secondary metabolites isolated from endophytes possess antimicrobial, antioxidant, cytotoxic activities. It is believed that screening for antimicrobial compounds from endophytes is a promising way to overcome the increasing threat of drug resistant strains of human and plant pathogen. In this review, many important, well-studied areas regarding endophytic fungi and their potential secondary metabolites are presented. Metabolomics and metagenomics of fungal endophytes have also been described. This source of noble compound (secondary metabolites) would bring the endophytic fungi to light to be utilized in the field of pharmacy and agriculture. Metagenomics of endophytes is very important now a day to study the diversity of the endophytic fungi in its environment because all endophytes are not culturable from the host.

Keywords Endophytic fungi · Secondary metabolite · Antimicrobial Metabolomics

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Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_4

4.1 Introduction

Microorganisms are metabolically highly diverse and are regarded as the store house of biomolecules important for human as well as plant health. Since time immemorial, microorganisms like fungi and bacteria have been isolated from different sources. The appearance of multi drug resistant fungal and bacterial strains (MDR) (Daleyi 2002), like *Pseudomonas*, *Streptococcus,* and *Staphylococcus* (Chitnis et al. 2000), has necessitated the discovery of a special group of microorganisms called endophytes which inhabit the internal plant tissues. Endophytic microorganisms are defined as microorganisms colonizing healthy plant tissues without causing overt symptoms or apparent injuries to the host (Bills 1996). Conceptually, the term "endophyte" has undergone various transformations from time to time, and there still is considerable disagreement as what constitute an endophyte. They form inconspicuous infections within tissues of healthy plants for a part or throughout their life cycle (Limsuwan et al. 2009). Endophytes are ubiquitous and have been found in all the species of plants studied to date. Almost all the plant species ($\sim$400,000) harbor one or more endophytic microorganisms (Tan and Zou 2001). Endophytes, therefore, represent an enormous, relatively unexplored source of microbial diversity (Strobel and Daisy 2003). The actual relationship between the host and the microorganisms, however, is not properly understood.

A wide range of relationships with the hosts including symbiotic, mutualistic, commensalistic, and trophobiotic have been hypothesized. It is said that most endophytes appear to originate from the rhizosphere or phyllosphere. There are many signal transduction mechanisms which trigger the rhizosphere or phyllosphere fungus to become endophytic. Ryan et al. (2008) advocated that flavanoids, isoflavanoids, and phenolic signaling molecules excreted by the plant roots attract the rhizosphere fungus to colonize inside the plant tissues. Different workers described different signaling mechanisms which help in maintaining the association of endophyte with its host. Signaling mechanism between cool season grasses and fungi of the family Clavicipitaceae has been studied by Eaton et al. (2011). It is observed that a stress-activated MAP kinase signal pathway is responsible for the stability of the mutualistic relationship between endophytic fungus *Epichloe festucae* and *Lolium perenne.* This mutualistic relationship gets converted to parasitic one when the compounds of Nox complex (*Nox*A, *Nox*R, and *Rac*A) or the stress-activated MAP kinase (SakA) are disrupted (Eaton et al. 2011). The host lacking the functional Nox complex or the stress-activated MAP kinase with the fungus shows dwarf phenotype and premature senescence. This indicates the effect of Nox complex or MAP kinase signal pathway on the fungus for their mutualistic relationship with the host. The molecular basis of the mechanism which regulates these physiological changes was studied (Eaton et al. 2011). Transcriptomes of both the host and symbiont in SakA wild type and the mutant were studied by the high throughput mRNA sequencing which helped in understanding of the inside mechanism of Nox complex or MAP kinase signal pathway. In the mutant

association, fungal hydrolases and transporters are observed to increase drastically which switches the nature of the fungus from restricted symbiotic form to proliferative pathogenic form. Similarly, in the plant, the expression of the gene responsible for the pathogen defense is changed and the transcriptome of host revealed this change. Along with the gene transposon activation, hormone biosynthesis and response were also changed. This example vividly describes the role of different molecules in signaling mechanism between the endophytic fungi and the host for their association (Eaton et al. 2011).

Almost all groups of microorganisms, i.e., fungi, bacteria, and actinomycetes, have been found to be associated with plants as endophyte. They stimulate the production of secondary metabolites having wide range of biological activities either by the host or by itself. Some of the endophytic microorganisms can produce the secondary metabolites similar to the one produced by the plant, thus making them a promising source of novel biomolecules which might help in the sustainable utilization and conservation of some of the economically important rare and endangered plants. For example, the plant *Taxus brevifolia* is a commercially important plant as the leaves and bark produce taxol, an anticancerous secondary metabolite. The fungus *Taxomyces andreanae,* an endophyte isolated from *T. brevifolia,* found to produce taxol (Stierle et al. 1993) consequently attracting the attention of microbiologists toward endophytes. Each plant is a repository of one or more fungal endophytes, and one endophytic species may possess several to a few hundred strains (Strobel and Daisy 2003; Huang et al. 2007). In recent years, the biosynthetic potential of endophytic fungi has gained more significance owing to isolation of fungal endophytes capable of synthesizing molecules as plants do. It is thus imperative to study the complex relationship of endophytes with coexisting endophytes, host plants, insect pests, and other specific herbivores, which regulate the ability of endophytes to produce compounds similar to their hosts (Kusari et al. 2013).

There are enough evidences to prove that endophytes produce numerous bioactive compounds which help them to protect the host plants from insects which are carrier of many plant pathogens (Webber 1981; Claydon et al. 1985; Azevedo et al. 2000; Arnold and Lewis 2005). However, there is insufficient information regarding the role of endophytes in producing compounds in plants against insects and the corresponding effect of the insects on the metabolic processes of these endophytes inside the host plants. As we know, plants do not possess immune system as animals do to fight against disease-causing organisms. Kusari et al. (2013) described the paradigm 'attack-defense-counter defense strategies' linking insects with plants and endophytes that produce either similar or the same compounds which might be useful in defending the hosts from insects (Fig. 4.1). They hypothesized three different mechanisms for defense in plant system. The direct mechanism comprises the permanently available constitutive defense, the permanently available induced defense, in which the constitutive defense gets upregulated after the attack is recognized, and the activated defense in which toxic metabolites are produced instantly from the non-toxic plant metabolites in response to an attack (Kusari et al. 2008). A plant undergoes any of these defense reactions, as soon as it

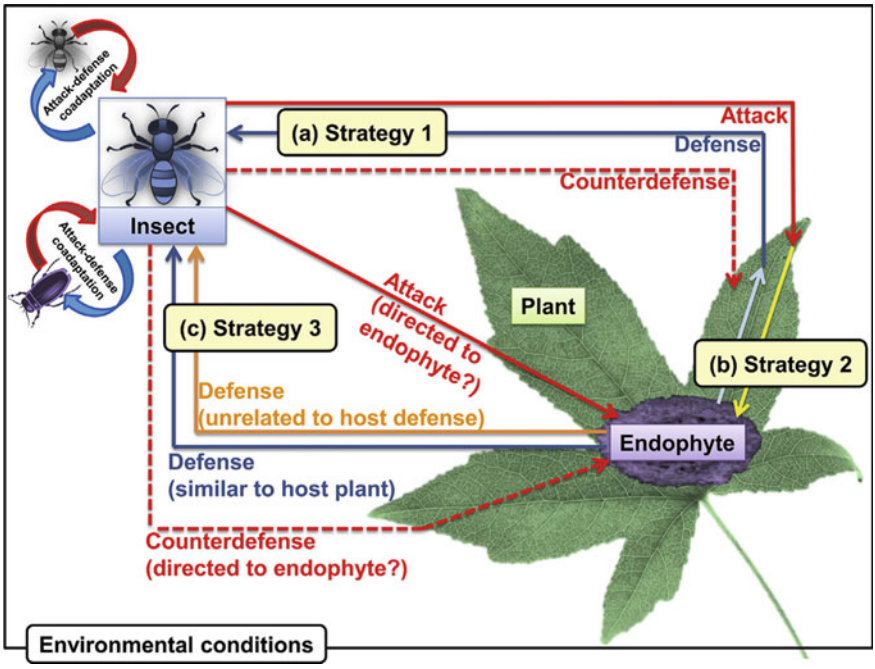

Fig. 4.1 Different attack on defense-counter defence strategies linking insects with plants and endophytes producing the same compounds (**a**–**c**). Three different strategies (Adapted from Kusari et al. 2013)

is attacked by an insect (Fig. 4.2). For example, when a number of insect pests attack *Hypericum* sp., a photodynamic compound, hypericin (7) is produced by the plant as a mode of chemical defense describing the constitutive defense (Guillet et al. 2000). Different researchers proposed that hypericin (7) is synthesized in specialized glandular structures called 'dark glands' (Cellarova et al. 1994; Onelli et al. 2002) of above-ground plant tissues (Briskin et al. 2000). Repeated attack by insect pests increases the production of hypericin (7) in the plant tissues (Sirvent et al. 2003). The endophytic fungus, *Thielavia subthermophila,* isolated from the stem of *H. perforatum* which produced hypericin (7) and emodin (8) (Kusari et al. 2008, 2009) in vitro. Thus, it can be inferred that this endophytic fungus might help in triggering the chemical defense of its host. Kusari et al. (2013) considered the plant–endophyte signaling mechanism as a second line of defense by plants which activate the endophyte to produce the same (or similar) active compounds (Fig. 4.1). Endophytic *Fusarium solani* introduces camptothecin but requires strictosidine synthase, a key plant enzyme to complete the biosynthesis of camptothecin (2) (Kusari et al. 2011). There are many evidences proving that the host plants induce their native endophytes to synthesize of biomolecules. This can be illustrated by the observation of Young et al. (2006), who observed that expression of gene, in vitro, responsible for lolitrem production in endophytic fungus,

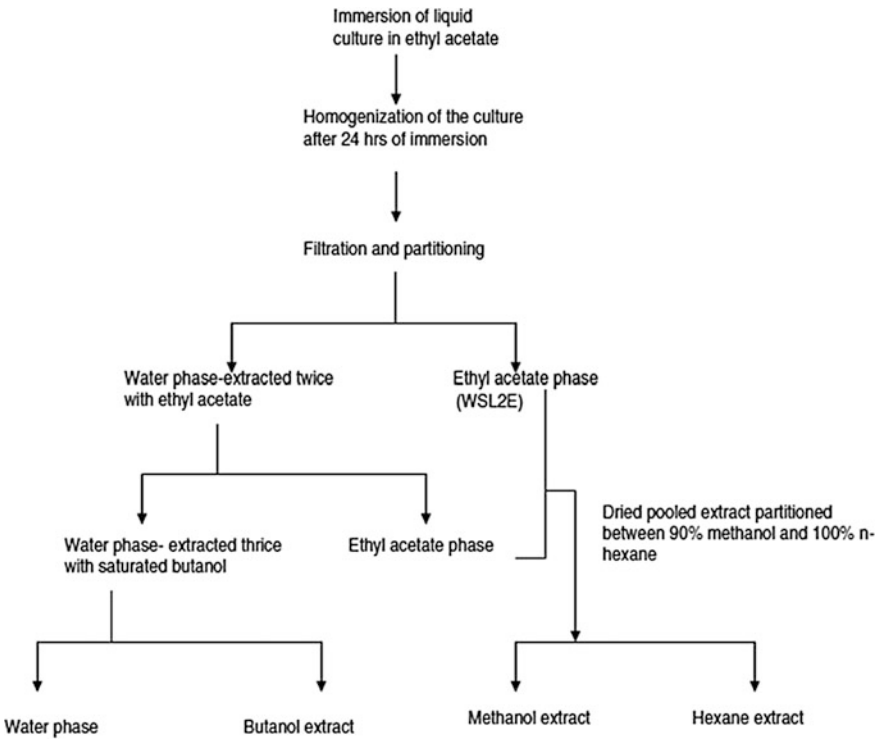

Fig. 4.2 Extraction procedure for obtaining crude fungal extract of *C. globosum* EF 18 (WSL2) (Adapted from Kumar et al. 2013)

Neotyphodium lolli, was very low or undetectable compound to when expressed in vitro in the host plant perennial rye grass. This compound causes neurological mycotoxicosis in herbivores.

There exists another mechanism inside the plant body in which coexisting endophytes interact with each other and provide a 'mutualistic trigger' for plant–endophyte interaction to produce chemical responses. It is evident that endophytes interact with other associated inter- or intraspecies of endophytes within the plant body (Kusari et al. 2013). It is not always possible to isolate and identify each and every endophyte and subsequent structural elucidation of the compounds they produce under laboratory conditions. The endophyte–endophyte interactions, therefore, are unavoidable. This can be understood by the observations made by Schroeckh et al. (2009), who found that the fungus *Aspergillus nidulans* produced orsellinic acid (12), lecanoric acid (13) (lichen metabolite), and some cathepsin K inhibitors only during its intimate interaction with *Streptomyces rapamycinicus*. Thus, further studies on endophyte–endophyte communication would not only help the discovery of unknown compounds, but also the sustainable production of plant compounds by endophytes. Kusari et al. (2013) described a general approach to

evaluate the effect of coexisting endophytes on each other in triggering the production of host plant compounds. Kusari et al. (2013) depicted a generalized layout of co-culture systems using a large number of endophytes. This provides the knowledge of the ability of the fungi to produce a particular metabolite or their structural analogs, individually or synergistically with other microbes and the host plant. As the different microbes exist and interact with each other within the same host plant, suitable co-culture systems could be developed using the endophytic fungi, endophytic bacteria, and fungi–bacteria together (Schroeckh et al. 2009). The co-culture of different microbes can lead to the production of desired secondary metabolite by influencing and altering the production mechanism.

The study of molecular and signaling mechanisms of interaction of plant pathogen, plant rhizobial microbes, and plant endophytes has been studied by different scientists. Molecular and ecological model plant species, such as *Arabidopsis thaliana* and wild Solanaceous plants, are being used successfully to study signaling mechanisms of such interactions. Many molecules are capable which response to changes in the extracellular and intracellular environment of the plant during microbial interactions. According to Vinagre et al. (2006), a group of molecules called 'receptor-like kinases' (RLKs) are involved in such mechanisms of perception and transduction of extracellular signals into the cell. For example, *Arabidopsis has* more than 400 RLKs out of which the group of 'Leucine- Rich-Repeat' RLKs (LRR-RLKs) was responsible for plant growth, development, and defense of plants against phytopathogens. More than 100 genes were estimated in *Arabidopsis* for calcium-binding proteins and channels, which got activated in response to specific type of interaction (Ranf et al. 2011). The recognition of endophytic microbe, for example *Piriformospora indica* (Vadassery et al. 2009), involved a rapid infusion of signaling proteins. The pathway of 'Oxidative Signal Inducible 1' (OXI1) kinase (member of AGC protein kinase family) has been found to be involved in endophytic *P. indica–Arabidopsis* interaction (Camehl et al. 2011). This pathway is regulated by H_2O_2 and PDK1 (3-phosphoinositide-dependent protein kinase 1). This pathway ultimately results in the production of phytohormones like ethylene (ET) (Guo and Ecker 2004), jasmonic acid (JA) and salicylic acid (SA) (Pieterse et al. 2009), and auxins (Long et al. 2008) which consequently influences plant growth and development.

Endophytic fungi, a most common type of endophytic organisms, are repository of noble secondary metabolites having potential therapeutic applications (Tejesvi et al. 2005). The nobility of the metabolites have been attributed to the specific biotope and/or host (Schulz et al. 2002) from where the endophytes are isolated. These compounds have antimicrobial, antioxidant, anticancer, and cytotoxic properties. Bioprospecting of endophytic fungi has led to tremendous possibilities to explore and utilize their potential. There are several strategies which might be employed in order to explore potent endophytes with desirable traits from unexplored sites. Random sampling of different plants from any population to isolate the associated endophytes, or a detailed study of an ecosystem in order to determine its features with regard to its natural population of plant species, their relationship with the environment, soil composition, and biogeochemical cycles, may lead to the discovery of potent endophytes. The evolutionary relatedness among groups of

plants at a particular sampling site can also be evaluated in relation to species, genus, and populations, through morphological data matrices and molecular sequencing, followed by isolation of endophytes from the desired plants. Traditional medicinal plants are also studied for endophytic associations, especially for those which are capable of producing one or more bioactive secondary metabolites present in the host plants. Finally, all the valuable data obtained using the different bioprospecting schemes can be pooled together and applied for further investigations.

In this review, we have tried to highlight endophytic fungal diversity, their metabolic pathways, communication, and relationship of endophytic fungi with their host plants, bioprospection of endophytic fungal secondary metabolites in relation to plant and human health. This might help not only in discovering and sustainable production of desirable natural products but also in discovering unexplored metabolites which would be renewable and easily obtainable without destroying the plants (Liu et al. 2001).

4.2 Diversity of Fungal Endophytes

Almost all groups of microorganism, viz. fungi, bacteria, and actinomycetes colonize plants and remain as endophyte within the plants. All plants investigated so far are found to harbor one or many fungal endophytes. This highly diverse group of fungi significantly affects plant communities by improving their fitness by conferring abiotic and biotic stress tolerance, increasing biomass, etc.

The endophytic fungi from the red listed, critically endangered medicinal plant, *Coscinium fenestratum* was investigated for the first time by Goveas et al. (2011). Goveas et al. (2011) identified a total of 41 endophytic fungi belonging to sixteen different taxa from 195 samples of healthy leaves and stems using classical methods. The overall colonization rate of endophytic fungi in both the leaf and the stem was found to be 21.02%. Stem had low percentage frequency of colonization than that of the leaf segments. Among the endophytic flora, *Phomopsis jacquiniana* was found to be dominant with a colonization frequency of 4.6%. Fifty-three endophytes were isolated from stems and roots of *Dendrobium devonianum* and *D. thyrsiflorum* (orchids) which exhibited strong impacts on their hosts (Xing et al. 2011). *Fusarium* sp. colonizing both *Dendrobium* sp. showed host specificity of the endophyte. Diversity of fungal endophytes colonizing *Panax quinquefolium* (American ginseng) was studied by Xing et al. (2010). Xing and his coworkers isolated 134 fungi from *P. quinquefolium of* different age groups. The infection frequencies of these fungi, however, varied with age and tissues of the host which they colonized. A total of 81 Thai medicinal plant species were examined growing in four geographical regions for the presence of endophytic fungi (Wiyakrutta et al. 2004).

Endemic medicinal plants of Tirumala hills of Seshachalam range falling under the Eastern Ghats of India were investigated for endophytes to be utilized as a

possible source of bioactive secondary metabolites (Dandu et al. 2013). Six hundred and ten (610) segments from four different plants, viz. *Boswellia ovalifoliolata, Pterocarpus santalinus, Shorea thumbuggaia,* and *Syzygium alternifolium*, were investigated for the presence of endophytic fungi. A total of 14 fungal species, viz. *Fusarium oxysporum, Colletotrichum falcatum, Pestalotiopsis* sp, *Aspergillus fumigatus, A. flavipes,* Sterile mycelia, *Penicillium senticosum, Gliocladium roseum, Phomopsis jacquiniana, P. archeri, Nigrospora sphaerica, Leptosphaeria* sp, and *Alternaria alternata,* were isolated and identified based on their morphological and spore characteristics. Among all the isolates, *Colletotrichum falcatum* was found to be the core-group fungus with colonization frequency of 12.5%. Sterile mycelia were common to the entire host, and few were host specific. The frequency of colonization of endophytes in case of stem was low than that of the leaf segments.

Endophytes were isolated for the first time from symptomless leaves, stem, fruits, and roots of four ethnomedicinal angiospermic plants, viz. *Digitalis lanata* (wooly foxglove), *D. purpurea* (purple foxglove), *Plantago ovata* (psyllium/isabgol), and *Dioscorea bulbifera* (air potato) (Ahmed et al. 2012). A total of one hundred and thirty-two isolates of microbial endophytes were isolated from these plants.

The genetic diversity of fungal endophytes in root, bark, and twigs of four medicinally important plants, *Azadirachta indica*, *Holarrhena antidysenterica*, *Terminalia arjuna,* and *T. chebula,* was studied by Tejesvi et al. (2007). Tejesvi et al. (2007) on the basis of RAPD analysis grouped thirty isolates of *Pestalotiopsis* and two isolates of *Bartalinia robillardoides* into four groups (group I contained 12 isolates, group II contained 3 isolates of *P. virgatula*, group III contained 10 isolates including *P. microspora*, *B. robillardoides*, *P. theae,* and *Pestalotiopsis* sp., and group IV contained five isolates of *P. microspora,* and finally one *Pestalotiopsis* sp. did not fall into any group).

The formulation containing different parts of three herbs, namely *Echinacea purpurea*, *E. pallida*, and *E. angustifolia,* is commercially available in Europe and USA. *Echinacea* genus is one of the top ten selling medicinal herbs in Europe and USA. The diversity of microbial community associated with healthy *E. purpurea* clones and their ability to produce defense compounds were studied (Rosa et al. 2012). Thirty-nine fungal endophytes were recovered and identified through the molecular methods in 15 distinct phylotypes, which were closely related to species of the genera, viz. *Ceratobasidium*, *Cladosporium, Colletotrichum*, *Fusarium*, *Glomerella*, and *Mycoleptodiscus*.

One mycelia sterilia and five fertile taxa (*Alternaria, Fusarium, Epicoccum, Phoma,* and *Cladosporium*) were isolated from twigs of *Buddleja asiatica* at two sites within Kathmandu city, Nepal (Chetri et al. 2013). *Epicoccum, Phoma,* and *Cladosporium* were found to be site specific. Colonization frequency, isolation frequency, and diversity of fungi were higher in site II than that of site I.

Traditional medicinal plants are a rich and reliable source of novel endophytic fungi. Different species of *Colletotrichum, Phoma, Phomopsis*, genus of xylariales, and sterile mycelia were the main isolates out of 1160 endophytic fungi isolated

from 29 Chinese medicinal plants (Huang et al. 2008). Some phenolic compounds were extracted from endophytic fungi of the same plant.

One hundred and thirty endophytic fungi were isolated from 12 Chinese traditional medicinal plants collected by Li et al. (2005a, b), at Yuanmou County and Dawei Mountain, Yunnan province, southwest China some of which were found to be promising source of novel bioactive compounds. The fungus *Bartalinia robillardoides* (strain AMB-9) was isolated as an endophyte from *Aegle marmelos* which is an important medicinal plant (Gangadevi and Muthumary 2008).

A total of 292 morphologically distinct endophytic fungi isolated from 29 traditional Chinese medicinal plants showing the immense diversity of endophytic fungi of Chinese traditional medicinal plants (Huang et al. 2007). Microbial communities and their host plants have integrated functions for successful survival in the nature. For example, fungal endophytes of *Schedonorus phoenix* (syn. *Lolium arundinaceum*), particularly *Neotyphodium coenophialum*, have been reported to reduce colonization of other plants by arbuscular mycorrhizal (AM) fungi by producing different allelochemicals (Antunes et al. 2008).

The diversity and frequency of endophytic fungi associated with young and old leaves of fungal endophytes of the endemic plant *Cordemoya integrifolia* occurring inside and outside the Maccabhe Conservation Management Area (CMA) were investigated by Toofanee and Dulymamode 2002). *Pestalotiopsis* sp. and *Penicillium* sp. were the dominant among all 26 fertile fungal taxa and one sterile morphospecies. Old leaves, veins of leaves, and petioles were colonized more by endophytes than relatively younger leaves and inter vein tissues. Thus, differences were observed between the endophytic communities isolated from different tissues and tissues of different ages.

Five endophytic fungi were isolated from the roots of *Capsicum annuum*, *Cucumis sativus,* and *Glycine max* and were screened on dwarf mutant rice (*Waito-C*) and normal rice (Dongjin-byeo) (Khan et al. 2012).

The diversity of endophytic fungi present in the leaves of *S. saponaria* L. was evaluated by Garcia et al. (2012). The bark, roots, and fruits of this plant are traditionally used in tonics, blood depurative, and cough medicine. They observed the colonization of host plants by endophytic fungi, using light and scanning electron microscopy. Species of *Cochliobolus*, *Alternaria*, *Curvularia*, *Phomopsis*, *Diaporthe,* and *Phoma* were isolated and identified.

The leaves and branches of five different species of *Garcinia* plants, *G. atroviridis, G. dulcis, G. mangostana, G. nigrolineata,* and *G. scortechinii,* were also found to be inhabited by a total of 376 endophytic fungi, in southern Thailand (Phongpaichit et al. 2006).

Withania somnifera (L.) Dunal is a medicinal plant with high endophyte biodiversity. The biodiversity of endophytic fungi residing in *W. somnifera* and their potential novel compounds of medicinal importance were evaluated by Khan et al. (2010). Thirty-three fungal strains of 24 species were isolated from a total of 643 segments (202 leaf, 391 stem, and 50 root samples) from 20 different plants; four belonged to the class Ascomycetes and 20 to class Deuteromycetes. *Aspergillus niger, A. terreus,* and *A. alternata* were exception by showing organ specificity.

A total of 10 different species, viz. *Aspergillus brevipes, Aspergillus spp, Aureobasidium spp. Curvularia lunata, Fusarium moniliforme, Colletotrichum acutatum, Colletotrichum gloeosporioides, Phyllosticta hymanaeae,* and sterile mycelia, were isolated from 210 segments of *Rauvolfia serpentina* during winter and summer seasons in Kerala (Meenatchi et al. 2016). Among all, Hyphomycetes was found to be dominant. The diversity of endophytic fungi was found to be the highest in leaves followed by bark and stem. The colonization frequency and diversity of endophytes were observed higher during winter season than summer season.

From the roots, stems, leaves, and fruit of the medicinal plant *Brucea javanica,* a total of 4 genera of endophytic fungi were isolated and identified (Amin et al. 2015). *Trichoderma* sp. was isolated from roots and stems, *Fusarium* sp. and *Penicillium* sp. from fruits, and *Aspergillus* sp. from leaves of the plant. The presence of endophytic fungi isolated from the holoparasitic plant *Balanophora japonica* (Balanophoraceae) collected from Kochi and Shikoku in western Japan revealed the ecological diversity of endophytes (Ikeda et al. 2016). A total of 23 fungal strains from inflorescences and tubers of three *B. japonica* plants growing on the host plant *Symplocos lancifolia* (Symplocaceae) dominant endophytes were *Trichoderma-Hypocrea*, *Penicillium,* and *Phialemonium.*

4.2.1 Endophytic Fungal Metabolomics

Metabolomics implies the unique chemical fingerprints of metabolites which are the end product of cellular processes occurring in a biological cell, tissue, organ, or organism. Metabolome includes the collection of all the metabolites that a biological cell, tissue, organ, or organism have. The chemical fingerprinting of metabolite is also known as metabolite profiling.

In order to study fungal endophytes and their metabolomics, one must have to isolate the proper endophyte. Surface sterilization of plant samples using different chemicals is the first and foremost step to isolate endophyte. The process of surface sterilization may be time-consuming and varies tissue to tissue and potentially limiting the number of samples processed. To overcome these limitations, a novel method was developed by Greenfield et al. (2015) to surface sterilize the plant samples in bulk simultaneously and discretely. A set of 24 perforated Falcon™ tubes, each containing a sample, were used. The samples were transferred successively through the series of containers holding the sterilizers. Through this method, samples of roots, stems, and leaves or entire seedlings can be sterilized. It was emphasized that this method could increase the throughput by a factor of 24 relative to conventional surface sterilization methods (Greenfield et al. 2015).

4.2.2 Extraction of Metabolites

Extraction of the secondary metabolites involves culture of isolates in broth media, and after a required or optimum incubation period, the culture is filtered to remove the mycelia. To separate the metabolite from the broth, the filtrate is then treated with different organic solvents like ethyl acetate, methanol *n*-hexane, and dichloromethane. Solvent extraction method usually is used for the extraction of metabolites. After separation of metabolites, the solvents are removed using rotary evaporator at required temperature depending on the boiling point of the compound and the solvents. The resulting residue is the crude extract which is subjected to various activity tests. The crude extracts are dissolved in Dimethyl sulphoxide (DMSO) in order to stabilize the compounds which are stored at 4 °C or used for further studies (Meneses et al. 2009; Bhagobaty and Joshi 2011; Muharni et al. 2012; Desai et al. 2012; Pavithra et al. 2012; Desale and Bodhankar 2013).

Mass culture of the fungus was done in 11.7 liters of wickerham medium [Malt extract (3 g/l); Yeast extract (3 g/l); Peptone (5 g/l); Glucose (Qualigens)−10 g/l; pH-7.2–7.4] at 24 °C for 3–4 weeks for extraction of the fungal crude extract as shown in Fig: 4.2 (Kumar et al. 2013). This protocol is proposed by Wicklow et al. (1998).

4.2.3 Metabolic Profiling

Metabolomics require special approaches for sample preparation, purification, and analysis using different techniques. Nowadays, different techniques are available to characterize the secondary metabolite extracted from fungus. Different researchers have used different methods for metabolite profiling. For purification and identification of compounds, widely used authentic techniques are CC, FC, FT-IR, TLC, GC-MS, LC-MS, LC-MS/MS, LC-UV (DAD), different types of HPLC, gas–liquid chromatography, NMR, LC/TOF-MS, etc. (Amna et al. 2006; Senyuva et al. 2008; Bhagobaty and Joshi 2011; Devi and Singh 2013; Senthilkumar et al. 2014; Devi and Prabakaran 2014).

A schematic diagram of procedure for separation and purification of endophytic *Chaetomium globosum* extract is presented in Fig. 4.3 which was proposed by Kumar et al. (2013). They subjected the extract into vacuum liquid chromatography (VLC) and eluted with dichloromethane: methanol in different concentrations. The concentrations of dichloromethane: methanol were 100% DCM, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 30:50, 25:75, and 100% MeOH. These obtained fractions were dried in rotary vacuum evaporator and analyzed using TLC, HPLC, LC-MS. Based on TLC pattern, fractions were grouped into 5 groups—WSL2E VI: I–V which were analyzed by HPLC and LC-MS. Depending on the HPLC and LC-MS profiles of the fractions, one fraction (no. WSL2E V: I) was further purified using Sephadex LH 20 column with dichloromethane and methanol at 50:50

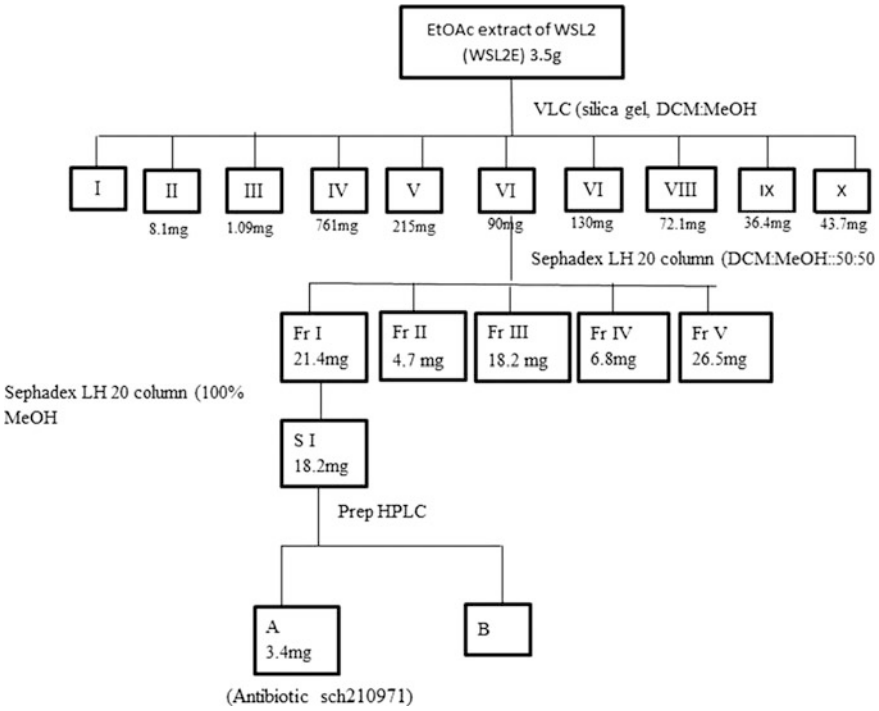

Fig. 4.3 Procedure of separation/purification of ethyle acetate extracts of *C. globosum* isolate EF18 WSL2E (Adapted from Kumar et al. 2013)

concentration, 100% methanol followed by preparative HPLC which has been shown in Fig. 4.3. The gradient of flow of the solvent in preparative HPLC was as follows: 0–5 min 50% methanol and 5–35 min increase from 50 to 100% of methanol and from 35 to 40 100% methanol. By applying these techniques, Kumar et al. (2013) obtained one major compound (compound 'A' in Fig. 4.3) from the endophytic fungal extract which was isolated from the plant *Withania somnifera*.

Chromatography helps in separation of the compounds. A compound isolated from endophytic fungus *Chrysosporium tropicum* when purified using flash chromatography effectively controlled mosquito (Verma and Prakash 2010). Two compounds were extracted from *Phomopsis cassiae* which was isolated from *Cassia spectabilis* using TLC and flash chromatography (Silva et al. 2005), which were identified as ethyl 2,4-dihydroxy-5,6-dimethylbenzoate, and phomopsilactone using FT-IR, MS, and NMR. These compounds showed strong in vitro antifungal activity against the phytopatogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum*, besides being cytotoxicity against human cervical tumor cell line (HeLa).

With the help of some spectroscopic methods, viz. UV, IR, HR-ESIMS, and extensive 1D-and 2D-NMR techniques, a new cytochalasin named as phomocytochalasin along with cytochalasin H, cytochalasin N, RKS-1778, dankasterone B, cyclo (L-Ile-L-Leu) isolated from *Phomopsis theicola* BCRC 09F0213 was identified (Hsiao et al. 2016). This endophytic fungus was isolated from the leaves of *Litsea hypophaea* Hayata (an endemic Formosan plant).

Endophytic fungal metabolites using agar plug paper chromatography, TLC, and LC-MS analysis which was carried out in a Waters' ultra-performance liquid chromatography (UPLC) coupled with Waters' Q-ToF Premier Mass Spectrometer (Bhagobaty and Joshi 2011). The isolates were RS07PF, RS07OS, RS07OC, RS07CC, and RS07SK which produced aurantioclavine, austdiol, oleic acid, jasmonic acid-ethyle ester, diaportin acid, and walleminone. Except RS07PF, the isolates also produced abscisic acid, and except RS07CC, others produced aflatoxin I, aflatoxin B2, aflatoxin G2, aflatoxin G2, and aflatoxin M.

The GC-MS/MS analysis of the extracts of an endophytic fungus *Phomopsis* sp. of *Tectona grandis* showed eleven major compounds (Senthilkumar et al. 2014). Compounds, namely 1,2-dioxy-3,5-octetraisopropyldisiloxane, 3-diyl-3-beta riborotroxy, dodecanoic acid ethylester, phthalic acid, and octyl 2-pentyl ester, were obtained from the culture filtrate of endophytic fungus Phomopsis sp. isolated from *Tectona grandis* (Senthilkumar et al. 2014) using GC-MS/MS. These compounds showed insecticidal properties. 1,2-dioxy-3,5-octetraisopropyldisiloxane, and 3-diyl-3-beta riborotroxy were recorded as the major compounds having the best insecticidal activity. Endophytic fungi *Aspergillus flavus* and *Nigrospora sphaerica* from that plant could produce some phytochemicals, viz. duroquinone, adamantine derivative, dodecanoic acid, tetradecanoic acid, pentadecanoic acid, and myristic acid which were identified by GC-MS analysis by Senthilkumar et al. (2014).

The volatile secondary metabolites present in ethyl acetate extract of endophytic *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* were determined by using GC-MS analysis (Devi and Singh 2013). GC-MS analysis revealed the presence of phenol-2,4-bis-(1,1-dimethylethyl), 1-hexadecene, 1-hexadecanol, hexadecanoic acid, octadecanoic acid methyl ester, and 1-nonadecene as major compounds in the extract. FT-IR and GC-MS analysis of ethyl acetate extract of endophytic *Penicillium* sp. of *Centella asiatica* showed the presence of benzeneethanol 4-hydroxy, 2-tert-butyl-4-isopropyl-1,5-methylphenol, benzoic acid-4-hydroxyl-propyl ester, p-hydroxyphenylacetamide, N-[2-methyl-1-prenylpropyl] formamide, cyclo-(L-leucyl-L-propyl), 3-(3-azidopropyl)-1H-indene, and dihydroergotamine (Devi and Prabakaran 2014).

The secondary metabolite of *Aspergillus flavus* was studied by LC/TOF-MS and HPLC (Senyuva et al. 2008). The metabolites were identified as aflatoxin B1, aflatoxin B2, aflatoxin B3, and aflatoxin G. Structural elucidation of the extract of *Entrophospora infrequens* isolated from the inner bark of *Nothapodytes foetida* was analyzed by MS-MS and XRD (Amna et al. 2006).

The ethyl acetate extracts of secondary metabolites were derived from endophytic *Aspergillus fumigatus* using TLC under UV light, NMR (^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC and H-H COSY). This fungus was isolated from the

tissues of the fruits of *Garcinia griffithii* and was found to produce identified 4,6-dihydroxy, 3,8α-dimethyl-1-oxo-5-(3′-oxobutan-2′-yl)-1,4,4a,5,6,8α-hexahydron aphthalen-2-yl-1,2-dimethyl-5-(2-methylprop-1-enyl) cyclopentane-carboxylate after structural elucidation (Elfita and Indah 2011).

A total of three different compounds from the crude secondary metabolite of endophytic *Colletotrichum* sp. were isolated from *Artemisia annua* using IR, MS, and ^{1}H and ^{13}C NMR (Lu et al. 2000). The compounds were identified as (a) 6-isoprenylindole-3-carboxylic acid, (b) 3β,5α-hydroxy-6β-acetoxy-ergo sta-7,22-diene, and (c) 3β,5α,-dihydroxy-6β–phenylacetyloxy-ergosta-7,22-diene which showed antimicrobial activity. They also identified IAA produced by the endophytic fungus co-TLC and –HPLC. The molecular formula of the new compound (a) was analyzed to be $C_{14}H_{15}O_2N$ by its spectral data (EIMS, DEPT, ^{1}H and ^{13}C NMR). The 1H and 13C NMR spectra of compound (b) were closely similar to 3β,5α,6β-trihydroxy-ergosta-7,22-diene suggesting that compound (b) was presumably a derivative of 3β,5α,6β-trihydroxy-ergosta-7,22-diene. The ^{1}H and ^{13}C NMR spectra of compound (c) were similar in some parts to those of compound (b) indicating that it was also a derivative of 3β,5a,6b-trihydroxy-ergosta-7,22-diene. EI mass spectrum, 13C NMR H-6 and H-7 demonstrated that the phenylacetyl group of compound (c) was anchored on C–6, and therefore, the structure of the new sterol 3 was determined as 3β,5α-dihydroxy-6β-phenylacetyloxy-ergosta-7,22-diene.

From the twigs of the *J. communis* L. Horstmann plant, isolated a novel endophytic fungus, which was identified as *Aspergillus fumigates* (Kusari et al. 2009). This fungus specifically and consistently produced one anticancerous compound deoxypodophyllotoxin which displayed antimicrobial activity against pathogenic bacteria. This compound was identified and quantified by high-resolution LC-MS, LC-MS^2, and LC-MS^3.

4.3 Mechanisms of Metabolite Production

Secondary metabolites are produced as result of accumulation of several intermediate products in culture media or in the cells during primary metabolism. The pathway of secondary metabolite production is anabolic which depends on the growth conditions and composition of the medium (Khan 2007).

Three pathways have been identified for production of secondary metabolites by endophytic fungi.

(a) Mevalonic acid pathway
(b) Polyketide pathway
(c) Shikimic acid pathway.

4.3.1 Mevalonic Acid Pathway

According to Garraway et al. (Garraway and Evans 1984), acetyl-CoA is the most common precursor for this pathway. However, it is also found that in some fungi leucine can be an alternative possible precursor for secondary metabolite production. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which then reacts with a third acetyl-CoA to form hydroxymethylglutaryl-CoA (HMG-CoA) and then mevalonic acid. In the alternative pathway, where leucine is the precursor, leucine is deaminated, carboxylated, and converted to HMG-CoA. Mevalonic acid is then phosphorylated, carboxylated, and converted to isopentenylpyrophosphate (IPP) which is the first molecule in the pathway containing the isoprene (hemiterpene) carbon skeleton. Terpenoids are produced from isomer of IPP, dimethylallyl pyrophosphate, the "chain-initiating unit." Mevalonate is the key intermediate in terpenoid biosynthesis. Mevalonate is converted to isopentenyl pyrophosphate and dimethylallyl pyrophosphate, from which geraniol, farnesol, geranylgeraniol, and squalene are formed. These compounds undergo a variety of cyclization reactions to form, respectively, mono-, sesquidi-, and triterpenes. Two molecules of farnesyl-pyrophosphate condensed to form 30 carbon triterpenes squalene. This triterpenes squalene reacts with molecular oxygen and cyclizes to form the steroid lanosterol. Lanosterol is thus can be classified as a sterol which serves as the precursor for all fungal sterols, such as ergosterols, cholesterol, and fucosterol. These compounds may be modified by alkylation using S-adenosylmethionine, demethylation, dehydrogenation, and reduction. It can be said that there is a network of interlinking biosynthetic pathways varying in activity depending on the organisms and the stage of the life cycle. The 20-carbon diterpene derivative geranylgeranyl-pyrophosphate is the precursor for a number of biologically important secondary metabolites. Two molecules of geranylgeranyl-pyrophosphate condense in a tail-to-tail manner to form 40-carbon tetraterpenoids which is the carotenoids. The immediate product of this reaction is the carotenoid phytoene. Phytoene is dehydrogenated in several steps to form lycopene, which is then cyclized to β- or γ-carotene. β- or γ-carotene as well as oxygen-containing carotenoids called xanthophylls are the common pigments in many fungi.

4.3.2 Polyketide Pathway

Many fungi prefer polyketide pathway to produce secondary metabolites than any other pathways (Turner 1976). Condensation of one molecule of acetyl-CoA with at least three molecules of malonyl-CoA produces polyketides. Condensation of acetyl units, three molecules of carbon-di- oxide is released. By a type of aldol condensation the resulting tri-β-ketomethylene (tri ketide) chain cyclizes to form a variety of aromatic compounds including orsellinic acid, dihydroxy-dimethylbenzoic acid, 6-methylsalicylic acid, and acetylphloroglucinol (Fig. 4.4). Then, three aromatic

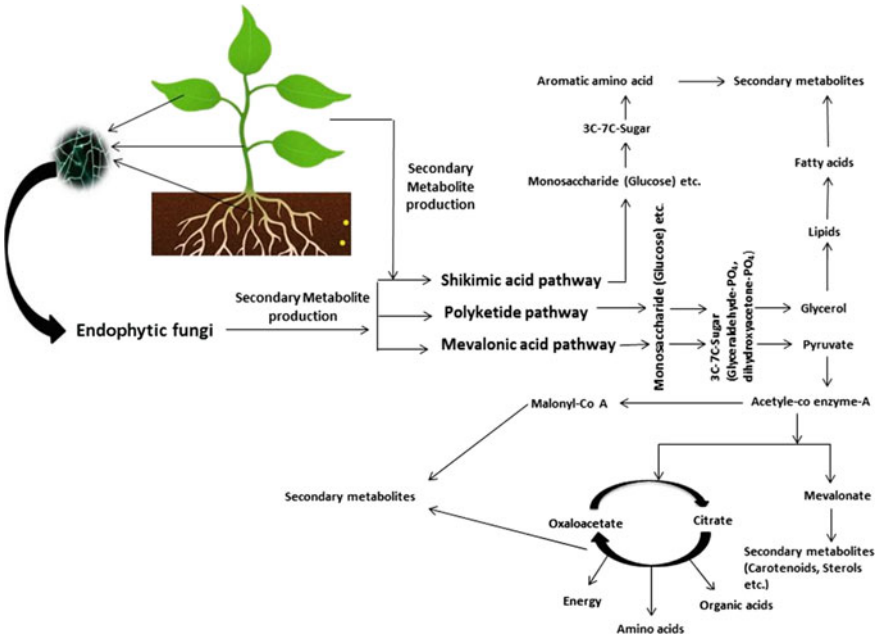

Fig. 4.4 Hypothesis describing the different mechanisms responsible for production of secondary metabolites by endophytic fungi and the host plant (Fig. 4.4)

compounds can be modified further by reduction, hydroxylation, oxidation, decarboxylation, and methylation, and a tremendous variety of compounds can be generated (Zhang et al. 2004). Furthermore, polyketide produced can interact with metabolites from other biosynthetic pathways and generates new metabolites.

4.3.3 Shikimate-Chorismate Pathway

This pathway is common in fungi, bacteria, and plants also. A wide variety of aromatic compounds are produced by this pathway (Garraway and Evans 1984). Condensation of phosphoenolpyruvate and erythrose-4-phosphate initiates this pathway to form a cyclized product dehydroquinic acid. Phosphoenolpyruvate and erythrose-4-phosphate both are glycolytic intermediates. This product is converted first to shikimic acid and then to chorismic acid by multienzyme complex system. From chorismate some aromatic amino acids like phenylalanine, tyrosine, and tryptophane, the aromatic moiety of ubiquinone, and p-aminobenzoic acid moiety of folic acid are synthesized. These aromatic amino acids in turn serve as precursors for the synthesis of more complex compounds. Phenylalanine serves as the precursor for synthesis of cinnamic acid and its derivatives. Many important products are produced through this pathway such as amino acids phenylalanine, tyrosine, and

tryptophane, cinnamic acid derivatives such as coumarin and methyl-cis-ferulate, the antibiotics penicillin and cephalosporin; different ergot alkaloids like ergosterine and lysergic acid (Fig. 4.4).

Upregulated metabolites are shown in bold solid boxes, downregulated metabolites in dashed boxes, and unchanged metabolites in fine solid boxes. Shown are only metabolites/quality parameters that were quantified in the analyses, except for acetyl-CoA (dotted circle). Arrows and lines do not represent direct biochemical relationships, but rather indicate possible connections between those metabolites. Metabolites produced exclusively by the endophytic fungus and connections to them are highlighted in gray. Amino acids synthesized from the same precursor were grouped: P3G AA—L-serine, L-cysteine (not quantified), L-glycine derived from 3-phosphoglycerate; a-KG AA—L-glutamate, L-glutamine, L-proline, L-arginine derived from a-ketoglutarate; OA AA—L-aspartate, L-asparagine, L-methionine, L-threonine, L-lysine, L-isoleucine derived from oxaloacetate; Pyr AA—L-alanine, L-valine, L-leucine derived from pyruvate; PEP? E4P AA—L-phenylalanine, L-tyrosine, L-tryptophan derived from erythrose 4-phosphate and phosphoenolpyruvate (Strayer et al. 2000). Fatty acids were grouped into saturated fatty acids (Sat FA; only C17:0 and C18:0 were downregulated) and unsaturated fatty acids (Unsat FA). Plant quality parameters were analyzed by near-infrared spectroscopy: NDF neutral detergent fiber; ADF acid detergent fiber; ME metabolizable energy; OMD organic matter digestibility. (Adapted from Rasmussen et al. 2009).

Rasmussen et al. (2009) described a hypothesis of the network of endophytic fungal metabolism and plant metabolism in ryegrass blades infected with endophytic *Neotyphodium lolii* strain Lp19 (CS), which is shown in Fig. 4.5. In the schematic diagram, they only showed those metabolites and plant quality parameters which were actually measured in their studies (except for acetyl-CoA, which was not analyzed, but which was the central compound in most processes). They did not detect many of the metabolites by analytical methods, like phosphorylated sugars and CoA esters which were known to be important intermediates of metabolic pathways, as well as the metabolites usually present in very low concentrations, like phytohormones. Figure 4.5 does not represent a direct biosynthetic relationship between the metabolites, but rather it shows a simplified scheme of possible metabolic network connections of the fungi and the plant.

4.4 Endophytic Fungi and Plant Health

Association between fungal endophytes and host plants is considered as unique and unavoidable. Many researchers believe that asymptomatic, systemic fungi that colonize the healthy leaves, stems, roots, reproductive organs of the host significantly affect the physiology, ecology, and reproductive biology (Bonnet et al. 2000; Clay and Schardl 2002; Clay et al. 2005; Malinowski and Belesky 2006; Knop et al. 2007; Alfaro and Bayman 2011) of the host plants. There are sufficient evidences to prove that endophytic fungi provide protection to their hosts from insects, pests,

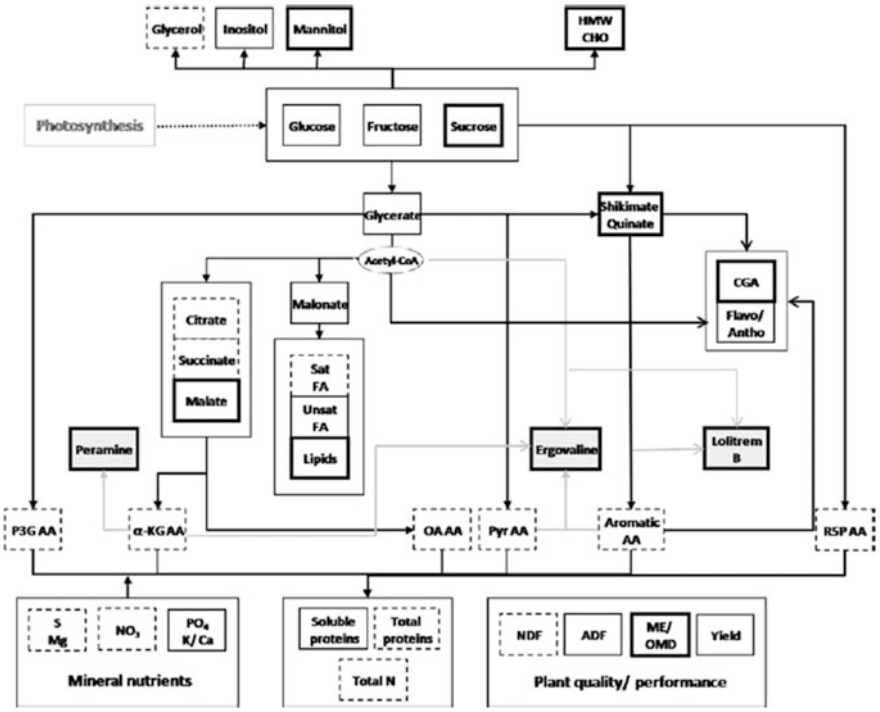

Fig. 4.5 Hypothetical schematic representations of metabolic endophyte effects and possible metabolic network connections in *L. perenne* mature blades infected with *N. lolii* Lp19 (CS) strain (Fig. 4.5)

herbivores, abiotic stresses, etc., and in turn they receive nutrition, shelter and propagation opportunities inside the host body (Thrower and Lewis 1973; Clay and Schardl 2002).

It has been established that there exist a complex relationship between endophytes and their host plants. Researchers are of the opinion that endophytes directly or indirectly promote plant growth by producing active secondary metabolites, which may also inhibit the growth and/or activity of pests and insects. As many of these secondary metabolites have been observed to inhibit number of microorganisms, they might be used for controlling different plant diseases (Gurney and Mantle 1993; Fisher et al. 1994).

A number of bioactive substances are produced by endophytes that provide protection and vitality to the plant. In the field of medicine, agriculture and industry endophyte is a new, unexplored, and potential source of novel natural products which has been evidenced by many scientists. Therefore, the use of endophytes is becoming a promising way to overcome the increasing threat of multi drug resistant strains (MDRS) of human as well as plant pathogens.

Endophytic fungi protect host plants from different natural enemies. Endophytes silently infect the host and move from the ovule to the seeds resulting into

substantial metabolic cost develops throughout the aerial parts of the host plant (Carrol 1988). Carrol (1988) proposed that endophytes which are "inducible mutualists" are not involved with host seed and disseminate independently through air or water, rather they infect and grow rapidly and produce toxins against herbivores when herbivores damage host tissues and provide new sites for infection. There are many examples of fungal endophytes which protect the plants in different environmental prohibitions. For example, *Piriformospora indica*, a member of the order Sebacinales, helps plant to overcome abiotic stresses (Yuan et al. 2010).

Colonization of hosts by endophytes lead to the production of bioactive metabolites and development of induced resistance in hosts as enhanced by over expression of stress related enzymes are responsible for direct or indirect protection and growth promotion of the hosts. Herbivorous mammals get poisoned and suffer from several types of diseases after taking the host plant by the mycotoxins produced by the endophytes (Carrol 1988; Roberts and Andre 2004). In *Festuca arundinaceae* grass, the endophyte produces a number of compounds like alkaloids, lysergic acid amides, and ergopeptines which are the cause of fescue toxicosis in mammalian herbivores. In fescue toxicosis, the animal suffers from vasoconstriction, increase in body temperature, increased respiration, suppressive immune system serious reproductive problems, etc. (Roberts and Andre 2004). Through the production of different bioactive compounds, endophytic fungi defend their host plants against a wide range of insects also (Spiering et al. 2005). For example, the fungus *Phomopsis oblonga* produced some metabolites which directly controls the beetle *Physocnemum brevilineu* or induced the elm tree plant to protect itself against the beetle (Webber 1981). This beetle is the vector for spreading elm Dutch disease-causing pathogen *Ceratocystis ulmi* (Gaynor and Hunt 1983).

The endophytic fungi of coffee plants, viz. *Beauveria bassiana* and *Clonostachys rosea*, can control the coffee berry borer which is the most destructive pest of coffee throughout the world. These two endophytes were found to be active against pest (Vega et al. 2008).

Beauveria bassiana was used to seeds of tomato and cotton and was found to be colonized as endophyte in tomato and cotton seedlings. This endophyte protected the plants against plant pathogenic *Rhizoctonia solani* and *Pythium myriotylum* caused damping off of seedlings and root rot of older plants (Ownley et al. 2008). *B. bassiana* also induced systemic resistance in cotton plant against bacterial blight-causing pathogen *Xanthomonas axonopodis* pv. *malvacearum*. Hyphae of *B. bassiana* were observed coiling around hyphae of *Pythium myriotylum* when parasitism assays were done by Ownley et al. (2008).

Endophytic fungi produce antimycotic, nematicidal, insecticidal compounds to protect the host plants. They also improve the growth and yield of crops under various environmental stressed conditions (Gond et al. 2010). Endophytes are rich source of antimicrobial metabolites which helps the plant in active defense mechanisms against pathogens. These mechanisms of plants mainly involved oxidative burst, hypersensitive responses, accumulation of phytoalexins, different kinds of enzymes, proteins, alkaloids, phenols, etc. (Khan, 2007). Protection of the host plant by endophytic fungi against pathogens, herbivores, abiotic stress, etc. results

in the increase in the primary production by the plant. These fungi may also help the plant to produce or to capture the limiting resources which are required for primary production (Mandyam and Jumpponen 2005).

Endophytic *Fusarium* and *Curvularia* species were isolated from *Leymus mollis*, collected from several coastal beach habitats in the San Juan Island Archipelago, WA (Rodriguez et al. 2008). Then these endophytes were applied to sterilized seeds of Tomato (*Solanum lycopersicum*), dunegrass (*L. mollis*), panic grass (*Dichanthelium lanuginosum*), and rice (*Oryza sativa* subspecies *japonica*, var. *dongjin*). These plants were examined for water consumption, salt, drought, and heat resistance. These plants showed significant salt, drought, and heat resistance compared to the control plants.

The antifungal potential of fungal endophytes associated with *Schima wallichii* and their potential to produce bioactive compounds according to detection of the conserved ketosynthase domain (KS) of polyketide synthase (PKS) gene were evaluated (Rodriguez et al. 2008). Out of 15 morphologically different endophytic fungal genera, *Alternaria*, *Phomopsis*, *Colletotrichum*, *Chaetomium,* and *Penicillium* were found to be most frequently colonized genera. The strains were screened for their biocontrol ability against *Macrophomina phaseolina*, *Aspergillus flavus,* and seven phytopathogens of the genus *Fusarium. Penicillium simplicissimum* (KJ826510) and *Talaromyces verruculosus* (KJ826513), respectively, showed highest degree of antagonisms against tested pathogens indicating that they are the good source of biocontrol agents which could be used against phytopathogens.

The affect of endophytic *Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5 on sunflower (*Helianthus annuus* L.) growth and disease resistance were studied (Waqas et al. 2015). The capability of these endophytes for regulation of hormone signaling networks involved in plant defense against the stem rot caused by *Sclerotium rolfsii* were also studied. The shoot length, shoot diameter, shoots fresh/dry weight, transpiration, stomatal conductance; photosynthesis and chlorophyll content of the plant were found to increase after the fungal treatment. The endophytes could relieve the biotic stress in the diseased plant and lowered the level of endogenous salicylic acid and jasmonic acid contents which were significantly higher in control diseased plants. These resulted in the reduced stem rot in *H. annuus.* This result revealed the usefulness of endophytic fungi in plant health control in a sustainable and eco-friendly manner by reducing excessive fungicide use in agriculture.

The fungal endophyte *Cryptosporiopsis* sp. Norway spruce root could inhibit the well-known genera of phytopathogens, viz. *Heterobasidion parviporum*, *Phytophtora pini,* and *Botrytis cinerea,* and also could protect Norway spruce seedlings against *H. parviporum* infection (Terhonen et al. 2016). The endophyte *Phialocephala sphareoides* was able to inhibit all the tested phytopathogens promoting the root shoot growth of Norway spruce seedlings.

These are a few examples of role of endophytic fungi in plant health. Thus, one can say that endophytic fungi are store house of different metabolic compounds which tremendously take part in plant health protection and growth promotion (Table 4.1).

Table 4.1 Endophytic fungi and their activity in relation to plant health

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Antagonistic against	References
Banana	*Fusarium oxysporum*	Not identified	Nematode (*Radopholus similis)*	Athman et al. (2006)
Banana, Tomato	*Trichodermaatroviride*	Not identified	*Pratylenchus goodeyi* *Meloidogyne incognita* (Root rot causing Nematode)	Xia et al. (2011) Tucci et al. (2011)
Elm tree	*Phomopsis oblonga*	Not identified	*Physocnemum brevilineum* which spreads the pathogen *Ceratocystis ulmi*	Webber (1981)
Fagus	*Xylaria sp*	Not identified	Beetle larvae	Claydon et al. (1985)
Hypericum perforatum	*Thielavia subthermophila*	Hyperici, Emodin	Insect pests	Kusari et al. (2008, 2009)
Nothapodytis nimmoniana	*Entrophospora infrequens*	Camptothcin	Analogous to antineoplstic agent causing DNA damage	Puri et al. (2005)
Festuca arundinacea Nothapodytes nimmoniana	*Neurospora crassa Fusarium solani*	Camptothecin	Analogous to antineoplstic agent causing DNA damage	Kusari et al. (2009, 2012); Sweta et al. (2010)
Festuca arundinacea	*Neotyphodium coenophialum*	Alkaloids, lysergic acid amides, ergopeptines	a. Cattle "Fescue toxicosis" b. Biotic and abiotic stress tolerance	Bacon et al. (1977); Read and Camp (1987); Roberts et al. (2005); Rehman et al. (2008)
Bontia daphnoides	*Nodulisporium* sp.	Nodulisporic acid	Blowfly larvae	Bills et al. (2002); Schardl et al. (2004)
Azadirachta indica	*Geotrichum sp.*	Epimeric 1,3-oxazinane derivatives	Nematode, viz., *Bursaphelenchus xylophilus*, *Panagrellus redivivus*	Li et al. (2007); Jalgaonwala et al. (2011)

(continued)

Table 4.1 (continued)

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Antagonistic against	References
Arachis hypogaea (peanut)	*Aspergillus caelatus*	Flavonoids (eriodicyol, medicapin and quercetin-3-glucoside) Stilbenes (stilbenoid phytoalexins)	The growth or reproduction of plant pathogenic bacteria, fungi, viral invaders as well as protozoans *Aspergillus flavus*, *A. parasiticus*	Edwards (1997); Kim et al. (2008); Sobolev et al. (2008)
Lolium perenne	*Acremonium lolli*	Lolitrem B	Reduce insect attack in infected host plants.	Prestige and Gallagher (1988)
Echinopogum ovatus	*Neotyphodium* sp.	N-formilonine and a paxiline analog	*Listronotus bonariensis* and other insects	Miles et al. (1998)
Woody plants	*Phyllosticta sp.*	Heptelidic acid,	Insects	Calhoun et al. (1992); Bills et al. (1992)
Woody plants	*Hormonema dematioides*	Rugulosine	Insects	Calhoun et al. (1992); Bills et al. (1992)
Theobroma cacao	*Colletotrichum* sp.	Not identified	Black pod pathogen *Phytophthora* sp.	Arnold (2003); Herre et al. (2007)
Barley	*Piriformospora indica*	Not identified	Powdery mildew-causing pathogen *Blumeria graminis* f. sp. *Hordei*	Waller et al. (2005)
Barley	*Piriformospora indica*	Not identified	Salt stress	Waller et al. (2005)
Dicanthelium lanuginosum	*Curvularia sp.*	Not identified	Thermo tolerance (Cell-wall melanin pigment of the endophyte can dissipate heat along the hyphae)	Redman et al. (2002)
Festuca arundinacea	*Acremonium coenophialum*	Not identified	Increases primary productivity and thus increasing biomass by producing or induces to produce phytohormones, cytokines, and other growth-promoting substances	Clay (1986)
Lolium perenne	*Acremonium loliai*	Not identified	Increases primary productivity and thus increasing biomass by producing or induce to produce	Clay (1986)

(continued)

Table 4.1 (continued)

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Antagonistic against	References
			phytohormones, cytokines and other growth-promoting substances	
Festuca obtuse	Not identified	Not identified	Increases primary productivity and thus increasing biomass by enhancing the ability to produce or capture the limiting resources, controls herbivores	Bier (1995)
Poa sylnestris	Not identified	Not identified	Increases primary productivity and thus increasing biomass enhancing the ability to produce or capture the limiting resources, controls herbivores	Bier (1995)
Theobroma cacao	*Botryosphaeria* sp.	Not identified	Black pod disease-causing pathogen *Phytophthora* sp.	Herre et al. (2007)
Theobroma cacao	Not identified	Not identified	Witches broom pathogen	Rubini et al. (2005)
Theobroma cacao	Not identified	Not identified	Rust pathogen	Shiomi et al. (2006)
Tectona grandis L.	*Aspergillus flavus*	Duroquinone, Adamantine derivative, Dodecanoic acid,	*Hyblea purea, Ailanthus defoliators,* and *Eligma narcissus*	Senthilkumar et al. (2014)
T. grandis	*Nigrospora sphaerica*	Tetradecanoic acid, pentadecanoic acid, and Myristic acid	*H. purea, A. fabriciella, and E. narcissus*	Senthilkumar et al. (2014)

4.5 Endophytic Fungi and Human Health

Endophytic fungi are a precious source of antimicrobial, antioxidant, and anti-cancerous compounds. They are the less explored treasure house of wide range of bioactive molecules which can be used directly or transformed for controlling different human diseases. Diverse array of endophytic fungi from different hosts is useful to check microbial diseases summarized in Table 4.2.

Phoma isolated from *Dendrobium devonianum* and *D. thyrsiflorum,* showed strong inhibitory activity against different human pathogens namely *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus* (Xing et al. 2011). *Epicoccum nigrum* isolated from *D. thyriflorum* exhibited antibacterial activity stronger than ampicillin sodium salt (antibiotic) used. *Fusarium* isolated from the *Dendrobium* species showed antagonistic activity against bacterial as well as fungal pathogens. This study revealed that *Dendrobium* sp. is a store house of fungi producing potential antibacterial and/or antifungal compounds. Wiyakrutta et al. (2004) reported that many isolates isolated from 81 Thai medicinal plant species inhibited *Mycobacterium tuberculosis* when tested using microplate Alamar blue assay. Some of these isolates were also active against human oral epidermoid carcinoma cells, while some showed cytotoxicity against breast cancer cells.

Aegle marmelos, widely used medicinal plant, harbored taxol producing fungi (Gangadevi and Muthumary 2008). Taxol is an important and costly anticancer drug widely used in the clinics. Endophytic fungus *Bartalinia robillardoides* (strain AMB-9) produced 187.6 l g/l of taxol which exhibited in vitro cytotoxic activity against BT 220, H116, Int 407, HL 251 and HLK 210 human cancer cells when tested by Apoptotic assay. This result suggests that the fungus can be genetically improved to increase the production of taxol.

Strains of *Pestalotiopsis* and *Bartalinia robillardoides* isolated from the medicinal plant *Terminalia arjuna* (Gangadevi and Muthumary 2008) exhibited antifungal activity. The ethyl acetate extracts of *Pestalotiopsis* showed greater antifungal activity than those isolated from other medicinal plants against six test organisms viz., *Alternaria carthami*, *Fusarium oxysporum*, *F. verticilloides, Macrophomina phaseolina*, *Phoma sorghina,* and *Sclerotinia sclerotiorum*.

The combinations of different plant parts of three herbs *Echinacea purpurea*, *E. pallida*, and *E. angustifolia* is commercially available formulations in Europe and USA. This genus is one of the top ten selling medicinal herbs in Europe and USA. The diversity of microbial community associated with healthy *E. purpurea* clones and their ability to produce defense compounds were evaluated (Rosa et al. 2012). Thirty-nine fungal endophytes were recovered and identified through the molecular methods in 15 distinct phylotypes, which were closely related to species of the genera, viz., *Ceratobasidium*, *Cladosporium Colletotrichum*, *Fusarium*, *Glomerella*, and *Mycoleptodiscus*. These endophytic fungi produced compounds against phytopathogenic fungi, insects, etc. A total of 16 crude extracts showed

Table 4.2 Endophytic fungi of different hosts and their antimicrobial products

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Activity/Antagonistic against	References
Lannea corammendalica	*Colletotrichum gloeosporioides*	9-octadecenamide, hexadecenamide, diethyl pythalate, 2-methyl-3-methyl-3-hexene, 3-ethyl-2,4-dimethyl-pentane	*Staphylococcus aureus*	Premjanu and Jaynthy (2015)
Centella asiatica	*Penicillium* sp.	Benzeneethanol 4-hydroxy, 2-tert-Butyl-4-Isopropyl-1-5 methylphenol, Benzoic acid 4- hydroxy propyl ester, p-hydroxyphenylacetamide, N-[2-Methyl-1-prenylpropyl] formamide, Cyclo(L-leucyl-L-propyl), 3-(3-azidopropyl)-1H-indene, and Dihydroergotamine	Cytotoxic activity against HeLa, A431, High antioxidant activity, Against human breast cancer (MCF7)	Devi and Prabakaran (2014)
Adathoda beddomei	*Syncephalastrum* sp.	Acarbose	Antimicrobial and antidiabetic activity	Prabavathy and Nachiyar (2013)
Phlogacanthus thyrsiflorus	*Colletotrichum gloeosporioides*	2,4-bis (1,1-dimethylethyl), 1-Hexadecene, 1-Hexadecanol, 1-Nonadecene	Antioxidant	Devi and Singh (2013)
Vismia latifolia	*Lewia infectoria*	Pyrrocidine C	*Candida albicans* Uterine cervical carcinoma, melanoma, human lung fibroblasts	Casella et al. (2013)
Taxus baccata	*Fusarium solani*	1-tetradecene, 8-octadecanone, 8-pentadecanone, octylcyclohexane and 10-nonadecanone.	Active against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Shigella flexneri, Candida albicans, Candida tropicalis*	Tayung et al. (2011)
Mangrove plant	*Phomopsis* sp. ZSU H76	Phomopsin A, B, and C together with two compounds Cytosporone B and C	*Candida albicans* and *Fusarium oxysporum*	Bhimba et al. (2011)

(continued)

Table 4.2 (continued)

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Activity/Antagonistic against	References
Mangrove plant	*Halorosellinia* sp.	Anthracenedione derivatives	Growth of KB and KBv200 cells (Cancer)	Bhimba et al. (2011)
Mangrove plant	*Guignardia* sp.	Anthracenedione derivatives	Growth of KB and KBv200 cells (Cancer)	Bhimba et al. (2011)
Rosa damacaena (rose)	*Aspergillus niger*	2-phenylethanol	Microbial infections	Massod et al. (2010)
Salvia officinalis	*Chaetomium* sp.	Chochliodinol, Isocochliodinol	Cytotoxic activity	Debbab et al. (2009)
Ginkgo biloba	*Chaetomium globosum*	Gliotoxin	Phytopathogenic fungi	Li et al. (2011)
Hypericum perforatum	*Thielavia subthermophila*	Hypericin, emodin	Human acute monocytic leukemia cell line	Kusari et al. (2009)
Excoecaria agallocha	*Phomopsis* sp.	Aliphatic compounds	*Candida albicans* and *Fusarium oxysporum*	Huang et al. (2008)
Acrostichum aureurm	*Penicillium* sp.	Peptides	*Staphylococcus aureus, Candida albicans*	Cui et al. (2008)
Polygonum senegalense	*Alternaria* sp.	Alternariol and its monomethyl ethers alternariol, Alternariol 5-O-methyl ether, Altenusin, 2,5-dimethyl-7-hydroxychromone, Tenuazonic acid, and Altertoxin	Cytotoxic activity	Aly et al. (2008)
Nerium oleander L.	*Chaetomium* sp.	Not identified	Antioxidant	Huang et al. (2007)
Nerium oleander L.	*Hyphomycete* sp., Mycelia sterila	Phenolic acid (chlorogenic acid and di-O-caffeoylquinic acid) and rutin	*Candia krusei, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Bacillus cereus*, and *Salmonella annatum*	Huang et al. (2007)

(continued)

Table 4.2 (continued)

Host(s)	Endophytic fungi	Bioactive metabolite(s)	Activity/Antagonistic against	References
Podophyllum peltatum	*Phialocephala fortinii*	Lignan podophyllotoxin	Cancer	Eyberger et al. (2006)
Taxus chinensis var. *mairei*	BT2	Taxol, Taxane baccatin III	Breast cancer, lung cancer, and refractory ovarian cancer	Guo et al. (2006)
Terminalia morobensis	*Pestalotiopsis microspore*	Pestacin, isopestacin	Antioxidant	Strobel (2002) Harper et al. (2003)
Taxus mairei	*Tubercularia* sp	Taxol	Breast cancer, lung cancer, and refractory ovarian cancer	Wang et al. (2000)
Tripterigeum wilfordii	*Cryptosporiopsis* cf. *quercina*	Cryptocin	*Pyricularia oryzae*	Li et al. (2000)
Tripterigeum wilfordii	*Cryptosporiopsis* cf. *quercina*	Cryptocandin	Microbes	Strobel et al. (1999)
Taxus wallichi, Wollemia nobilis	*Pestalotiopsis microspore*	Taxol	Breast cancer, lung cancer, and refractory ovarian cancer	Strobel (2002), Strobel et al. 1997, Strobel et al. 1999)
Taxus brevifolia	*Taxomyces andreanae*	Taxol	Breast cancer, lung cancer, and refractory ovarian cancer	Stierle et al. (1993); Strobel et al. (1996)
Torreya taxifolia	*Pestalotiopsis microspore*	Torreyanic acid	Breast cancer, lung cancer, and refractory ovarian cancer	Lee et al. (1996)

antifungal properties; while just the extract of *M. indicus* exhibited larvicidal activity against *A. aegypti*.

Phomopsis sp. isolated from *Erythrina crista-galli* (ceibo or coral tree) is used in Argentinean ethnopharmacology as anti-inflammatory medication, narcotic, disinfectant, and for the treatment of wounds (Webera et al. 2005). Besides several new metabolites, a number of known compounds were detected from the metabolite of the fungus, viz., mellein, nectriapyrone, 4-hydroxymellein, scytalone, tyrosol, lavatol, mevinic acid, and mevalonolactone which were biologically active.

The endophytic fungus *Fusarium oxysporum* NFX06 isolated from leaf of *Nothapodytes foetida* of Agumbe forest, Karnataka, showed the good activity against all the four test pathogenic strains, viz. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 69548) (Musavi and Balakrishnan 2014). The secondary metabolite of this endophyte was extracted using microwave for the first time.

The nutrient uptake and cell growth kinetics of an endophytic fungus *Fusarium oxysporum* NFX 06 isolated from *Nothapodytes foetida* producing metabolites with antimicrobial and anticancerous property, was studied (Fathima et al. 2013).

Endophytic fungi isolated from 12 Chinese traditional medicinal plants, were studied for antitumour and antifungal activities by MTT assay on human gastric tumor cell line BGC-823 and the growth inhibition of phytopathogenic test fungi (Li et al. 2005a, b). The fermentation broth from 9.2% of the isolates exhibited antitumor activities, while 30% exhibited antifungal activities. Some of these isolates exhibited broad-spectrum antifungal activities. This indicates that the endophytic fungi of Chinese traditional medicinal plants are a promising source of novel bioactive compounds having applications on pharmaceutical industries.

Plants and their endophytes are important resources for extraction of different medicinal natural products. The isolates showed good antioxidant activity which was significantly correlated with their total phenolic contents. Thus, phenolics were found to be the major antioxidant constituents of the endophytes (Huang et al. 2007). This investigation reveals that the metabolites produced by the endophytic fungi can be a potential source of novel natural antioxidants for human benefits.

The diversity and frequency of endophytic fungi associated with young and old leaves of fungal endophytes of the endemic plant *Cordemoya integrifolia* occurring inside and outside the Maccabhe Conservation Management Area (CMA) were investigated by Toofanee et al. (Toofanee and Dulymamode 2002).

Pestalotiopsis sp. and *Penicillium* sp. were the dominant among all 26 fertile fungal taxa and one sterile morphospecies. Old leaves, veins of leaves, and petioles were colonized more by endophytes than relatively younger leaves and inter vein tissues. Thus, differences were observed between the endophytic communities isolated from different tissues and tissues of different ages.

Five endophytic fungi were isolated from the roots of *Capsicum annuum*, *Cucumis sativus,* and *Glycine max* by Khan et al. (2012). The culture filtrates (CF) of isolates were screened on dwarf mutant rice (*Waito-C*) and normal rice (Dongjin-byeo). The endophyte *Paraconiothyrium* sp. which was identified by

sequencing the ITS rDNA region and phylogenetic analysis, significantly inhibited the growth of *Waito-C* and Dongjin-byeo. The ethyl acetate fraction of this fungus suppressed the germination of *Lactuca sativa* and *Echinochloa crus-galli* seeds. The compound responsible for inhibition was characterized through NMR and GC/MS techniques, as the phytotoxic compound ascotoxin. This compound was isolated for the first time from *Paraconiothyrium* sp.

Endophytes can co-evolve with its host plants and possess species-specific interactions. They protect the plant from insect attacks, herbivore attacks, and diseases by producing different substances of biotechnological interest.

The antimicrobial activity of endophytic fungi isolated from leaves and branches of five different species of *Garcinia* plants, *G. atroviridis, G. dulcis, G. mangostana, G. nigrolineata,* and *G. scortechinii*, in southern Thailand, was screened (Phongpaichit et al. 2006). Seventy isolates (18.6%) showed antimicrobial activity against at least one pathogenic microorganism, such as *Staphylococcus aureus*, *Candida albicans,* and *Cryptococcus neoformans*. *Phomopsis* sp. and *Botryosphaeria* sp. showed the strongest antibacterial activity against *S. aureus*. *Botryosphaeria* sp. also showed strong antifungal activity against *M. gypseum*. These results indicate that some of the endophytic fungi from *Garcinia* plants are a potential source of antimicrobial compound.

The endophytic fungi isolated from some ethnomedicinal plants stimulate the production of secondary metabolites with a diverse range of biological activities that can be exploited for human health and welfare (Ahmed et al. 2012). Some of the endophytes could produce the same secondary metabolites as that of the plant making them a promising source of novel compounds. For example, *Dioscorea bulbifera* belonging to the dioscoreaceae family produces steroidal and iridoid group of secondary metabolites. These groups of compounds were also found to be produced by some of the fungal isolates in greater amount which have enormous applications in the medicinal/pharmaceutical areas.

Phomopsis sp. GJJM07, an endophytic fungi isolated from *Mesua ferrea* was tested for its potent antimicrobial activity against some test pathogens, gram positive bacteria viz., *Bacillus subtilis, Micrococcus luteus*; gram negative bacteria viz., *Escherichia coli, Klebsiella pneumoniae* and yeast, *Candida albicans* (Jayanthia et al. 2011). The inhibition was highest against the test pathogen *B. subtilis* (18 ± 0.13 mm). This fungus was also examined for the in vitro antioxidant activity by DPPH radical scavenging assay which was significant.

In vitro antioxidant property of culture filtrate of *Phyllosticta* sp. isolated from *Guazuma tomentosa* was tested (Srinivasan et al. 2010). It showed good antioxidant property for which total phenol and flavonoid were found to be responsible. Thus, *Phyllosticta* sp. is a potential source of natural antioxidant.

A total of 27 species belonging to 18 endophytic fungal genera were isolated from a medicinal plant, *Salvadora oleoides*, an endangered species, from Haryana, India (Dhankhar et al. 2012). Crude extracts of the isolates were screened for antioxidant activities by six potential assays, out of which extracts of four fungal endophytes viz., *Aspergillus* sp. JPY2, *Aspergillus* sp. JPY1, *Penicillium chrysogenum and Phoma* sp. showed positive activity. The acetonic extract of *Phoma*

sp. showed super oxide radical scavenging activity with a higher value than the rest three and showed moderate reducing power and ferrous ion chelating activity. The phytochemical screening of these four fungal extracts of acetonic, methanolic and water, revealed the presence of alkaloids, flavanoids, saponins, carbohydrates, tannins, sterols, and terpenoids.

Rhodiola crenulata, *R. angusta*, and *R. sachalinensis* are rare and endangered alpine medicinal plants in Arctic and mountainous regions of Asia and Europe, from which 347 endophytic fungi were, isolated (Cui et al. 2015). Five isolates out of 114 active isolates showed DPPH radical scavenging rates more than 90%. These endophytes showed much more antioxidant activity than that of the host plant. Salidrosides and p-tyrosol were found to be the compounds for antioxidant activity which were also produced by the host plant *Rhodiola*. These results suggested that *Rhodiola* source of antioxidants could be exploited for versatile endophytic fungi for novel antioxidant compounds.

There is a report of seaweed endophytic fungi possessing cytotoxic, antifungal, and antibacterial activities. A total of 45 endophytic fungal strains were isolated from *Bostrychia tenella* (seaweed) out of which *Penicillium decaturense* and *P. waksmanii* showed positive results in different assays. A known antitumor and antibiotic compound cytochalasin D was isolated from Xylaria sp. *Acremonium implicatum*, *Trichoderma atroviride* and *Nigrospora oryzae* were also isolated as marine seaweed endophytes which showed good antimicrobial activity.

Endophytic *Cladosporium* sp. and *Curvularia* sp. isolated from needle of *Cupressus torulosa* showed antagonistic activity against human pathogen *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium,* and *Macrophomina phaesolina* (Bisht et al. 2016).

Three new arylbenzofurans, 7-methoxy-2-(4-methoxyphenyl)-3-methyl-5-(3-prenyl)-benzofuran (1), 2-(4-methoxyphenyl)-3-methyl-5-(3-prenyl)-benzofuran-7-ol (2) and 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-methyl-5-(3-prenyl)benzofuran-7-ol (3), along with four known arylbenzofurans (4–7) were isolated using 1D- and 2D-NMR techniques from the fermentation products of an endophytic *Phomopsis* sp. (Dua et al. 2016). Among all, compound 3 exhibited anti-TMV activity with inhibition rate of 35.2%. The other compounds also showed potential anti-TMV activity with inhibition rates in the range of 18.6–25.7%, respectively.

Endophytic fungi isolated from *Menthe viridis* collected from Khamariya, Jabalpur Madhya Pradesh (India), were screened for in vitro antibacterial activity against six pathogenic bacteria, i.e., *Bacillus subtilis*, *Streptococcus pyogenes Escherichia coli,* Klebsiella *pneumoniae*, *Salmonella typhimurium,* and *Enterococcus* sp. (Kumar et al. 2016). *Aspergillus fumigatus, Aspergillus niger, Fusarium solani, Aspergillus repens, Alterneria alternata, Alternaria* sp., *Phoma hedericola* and *Fusarium oxysporum* were isolated and *Fusarium oxysporum* found to produce effective antibacterial compounds.

The recognition that many new species of endophytic fungi have yet to be found which is of fundamental importance to plant pathologists, agronomists, environmentalists, microbiologist, etc. for the improvement of plant as well as human health with sustainable use of the plants.

4.6 Conclusion and Future Prospects

Endophytes are rich sources of novel secondary metabolites with a wide variety of biological activity. The fungal extracts revealed their potential as a source of bio-control agents, antioxidant, anti-inflammatory, antimicrobial compounds which could be used in the development of compounds against a wide spectrum of plant and human diseases.

This chapter mainly deals with the research progress on endophytic fungi for plant and human benefits and the plant–endophyte interactions. However, the relations between endophytic fungi and their host plants, effect of endophytes on plant metabolite production and vice versa, action mechanisms of the endophytic fungal metabolites, methods for efficiently promoting production of these bioactive compounds as well as their potential applications in different field will get much importance in near future.

The production of bioactive compounds by endophytic fungi, especially those exclusive to their host plants, is significant from the molecular and biochemical perspective as well as the ecological and economical viewpoint. The production of beneficial plant secondary metabolites by endophytes leads to the expectations and utilization of them as alternative and sustainable sources of these compounds in place of the plants. However, the extraction and utilization of desirable compounds produced by endophytic fungi still remains untouchable in commercial fields (Kusari et al. 2011). According to Kusari et al. (2012), one of the major obstacles preventing the biotechnological application of endophytes is the perplexing problem of reduction of secondary metabolite production on repeated sub culturing under axenic monoculture conditions. As the endophytes reside within the plants and are constantly communicating and interacting with their hosts, it is compelling that plants would have a substantial influence on the metabolic processes of the endophytes and in turn the endophytes also influence the plant metabolomics. Moreover, the endophytes give us tremendous bioactive metabolites in in vitro conditions. Nowadays, the whole genome sequencing strategies have shown that the number of genes encoding the biosynthetic enzymes in endophytes is much greater than the known secondary metabolites produced by various bacteria and fungi (Winter et al. 2011). The endophytic fungi always remain in versatile interactions with the host plant as well as other endophytes, and even slight variation in the in vitro cultivation conditions can impact the kind and range of endophyte isolated and secondary metabolites they produce (Scherlach and Hertweck 2009). This tremendous source of bioactive metabolite can take us to a much enthralling world if further researches are done to systematically understand the endophyte–endophyte and endophyte-host interspecies crosstalk which is desirable for sustainable production of compounds using endophytes (Kusari et al. 2011). It is beneficial for us to better understand and take advantage of less explored plant endophytic fungi to ensure a continuous and sustained gain of bioactive pro-drugs against the present and emerging diseases.

Different types of signal molecules are the language of communication between host plants and the endophytes. These molecules and the pathways where and how these molecules work will help us in manipulating the pathways for the synthesis and discovery of many known and unknown beneficial natural compounds from plants and endophytes. Recent emerging technologies in the field of 'omics' such as proteomics, metabolomics, metagenomics, transcriptomics and secretomics and also the high throughput and next-generation sequencing (NGS) technologies, and bioinformatics can be taken as privilege for us to further fortify and visualize a complete picture of the complex plant–endophyte, endophyte–endophyte interactions proficiently for agricultural and environmental benefits. These may further provide the ample understanding of the endophytic evolution, molecular interactions and signal transduction, synthesis of the desired compound by regulating the responsible gene, etc. There is another technique known as the conventional suppression subtractive hybridization (SSH) technique through which endophyte-endophyte differential gene expression can be enumerated (Diatchenko et al. 1996). Recently, several NGS technologies have been developed in order to make the studies easier. Moreover, the metagenomic approaches or other culture-independent techniques now and in near future will help researchers to reveal more information on endophytes and their metabolomics and interaction with other microbes and the host plants.

Thus, the studies on the endophytic diversity, their metabolites and also the endophyte–endophyte and plant–endophyte interaction using different available and promising tools will help not only in the identification and discovery of new compounds but also in sustainable production of desirable bioactive compounds in near future. The traditional knowledge on endophyte when combined with the modern tools and technique, this would show a promising pathway for metabolic engineering in order to get novel secondary metabolite.

References

Ahmed M, Hussain M, Dhar MK, Kaul S (2012) Isolation of microbial endophytes from some ethnomedicinal plants of Jammu and Kashmir. J Nat Prod Plant Resour 2(2):215–220

Alfaro AP, Bayman P (2011) Hidden fungi, emergent properties: endophytes and microbiomes. Ann Rev Phytopath 49:291–315

Aly AH, Edrada ER, Indriani ID, Wray WE, Totzke F, Zirrgiebel U, Schachtele C, Kubbutat MHG, Lin WH (2008) Cytotoxic metabolites from the fungal endophyte *Alternaria* sp. and their subsequent detection in its host plant *Polygonum senegalense*. J Nat Prod 71:972–980

Amin N, Fitrianti M, Rahim D (2015) Isolation and characterization of endophytic fungi from medicinal plant, buah Makassar (Makassar fruit: *Brucea javanica*). J Chem Pharma Res 7 (1):757–762

Amna T, Puri SC, Verma V, Sharma JP, Khajuria RK, Musarrat J, Spiteller M, Qazi GN (2006) Bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of an anticancer alkaloid camptothecin. Can J Microbiol 52:189–196

Antunes PM, Miller J, Carvalho LM, Klironomos JN, Newman JA (2008) Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants. Funct Ecol 22:912–918

Arnold AE (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Nat Acad Sci 100(26):15649–15654

Arnold AE, Lewis LC (2005) Ecology and evolution of fungal endophytes and their roles against insects. In: Vega FE, Blackwell M (eds) Insect-fungal association: ecology and evolution. Oxford University Press, New York, pp 74–90

Athman SY, Dubois T, Coyne D, Gold CS, Labuschagne N, Viljoen A (2006) Effect of endophytic *Fusarium oxysporum* on host preference of *Radopholus similis* to tissue culture banana plants. J Nematology 38(4):455–460

Azevedo JL, Jr WM, Pereira JO, Araujo WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Elec J Biotechnol 3(1):40–65

Bacon CW, Porter JK, Robbins JD, Luttrell ES (1977) *Epichloe typhina* from toxic tall fescue grasses. Appl Environ Microbiol 34(5):576–581

Bhagobaty RK, Joshi SR (2011) Metabolite profiling of endophytic fungal isolates of five ethno-pharmacologically important plants of Meghalaya, India. J Metabol Syst Biol 2(2):20–31

Bhimba BV, Franco DAAD, Jose GM, Mathew JM, Joel EL (2011) Characterization of cytotoxic compound from mangrove derived fungi *Irpex hydnoides* VB4. Asian Pacific J Trop Biomed 1(3):223–226

Bier J (1995) Thesis: Relationship between transmission mode and mutualism in the grass-endohyte. Indiana University, Bloomington

Bills GF (1996) Isolation and Analysis of Endophytic fungal communities from woody plants. In: Redin S, Carris LM (eds) Systematics, ecology and evolution of endophytic fungi in grasses and woody plants. APS Press, St. Paul, pp 31–65

Bills GF, Giacobbe RA, Lee SH, Pelaez F, Tkacz JS (1992) Tremorgenic mycotoxins paspalitrem A and C from a tropical *Phomopsis*. Mycologic Res 96:977–983

Bills G, Dombrowski A, Pelaez F, Polishook J, An Z (2002) In Watling R, Frankland JC, Ainsworth AM, Issac S, Robinson CH (eds) Tropical mycology: micromycetes, vol 2. CABI Publishing, New York, pp 165–194

Bisht DDR, Sharma D, Agrawal PK (2016) Antagonistic and antibacterial activity of endophytic fungi isolated from needle of *Cupressus torulosa*. Asian J Pharma Clin Res 9(3):1–7

Bonnet M, Camares O, Veisseire P (2000) Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll-a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cv Apollo). J Exp Bot 51(346):945–953

Briskin DP, Leroy A, Gawienowski M (2000) Influence of nitrogen on the production of hypericins by St. John's wort. Plant Physiol Biochem 38:413–420

Calhoun LA, Finddrlay JA, Miller JD, Whitney NJ (1992) Metabolites toxic to spruce budworm from balsam fir needle endophytes. Mycolog Res 96:281–286

Camehl I, Drzewiecki C, Vadassery J, Shahollari B, Sherameti I, Forzani C, Munnik T, Hirt H, Oelmuller R (2011) The OXI1 kinase pathway mediates *Piriformospora indica*-induced growth promotion in *Arabidopsis*. PLoS Pathog 7(5):e1002051

Carrol G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69(1):2–9

Casella TM, Eparvier V, Mandavid H, Bendelac A, Odonne G, Dayan L, Duplais C, Espindola LS, Stien D (2013) Antimicrobial and cytotoxic secondary metabolites from tropical leaf endophytes: isolation of antibacterial agent pyrrocidin C from *Lewia infectoria* SNB-GTC2402. Phytochemistry 96:370–377

Cellarova E, Daxnerova Z, Kimakova K, Haluskova J (1994) The variability of hypericin content in the regenerants of *Hypericum perforatum*. Acta Biotechnol 14:267–274

Chetri BK, Maharjan S, Budhathoki U (2013) Endophytic fungi associated with twigs of *Buddleja asiatica* lour. Kathmandu Univ J Sci Eng Technol 9:90–95

Chitnis V, Patil S, Kant R (2000) Hospital effluent: a source of multiple drug-resistant bacteria. Curr Sci 79:83–89

Clay K (1986) Microbiology of the phylosphere. In: Fokkenna NJ, Heuvel VDJ (eds) Grass endophyte. Cambridge University Press, Cambridge, pp 188–204
Clay K, Schardl C (2002) Evolution origin and ecological consequences of endophyte symbiosis with grasses. Am Naturalist 160:99–127
Clay K, Holah J, Rudgers JA (2005) Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. Proc Natl Acad Sci USA 102:12465–12470
Claydon N, Grove JF, Pople M (1985) Elm bark beetle boring and feeding deterrents from *Phomopsis oblonga*. Phytochemistry 24:937–943
Cui HB, Mei WL, Miao CD, Lin HP, Hong K, Dai HF (2008) Antibacterial constituents from the endophytic fungus *Penicillium* sp. 0935030 of mangrove plant *Acrostchum aureurm*. Chemical J Chin Univ 33:407–410
Cui JL, Guo TT, Ren ZX, Zhang NS, Wang ML (2015) Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of *Rhodiola crenulata*, *R. angusta*, and *R. sachalinensis*. PlosOne 10(3):e0118204
Daleyi CMD (2002) Transmission of multidrug-resistant tuberculosis limited by man or nature. Am J Resp Crit Care Med 165:742–743
Dandu A, Tartte V, Duggina P, Netala VR, Kalla CM, Nagam V, Desaraju SB (2013) Isolation and characterization of endophytic fungi from endemic medicinal plants of tirumala hills. Int J Life Sci Biotechnol Pharma Res 2(3):367–373
Debbab A, Aly AH, Ru AEE, Muller WEG, Mosaddak M, Hakiki A, Ebel R, Proksch P (2009) Bioactive secondary metabolites from the endophytic fungus *Chaetomium* sp. isolated from *Salvia officinalis* growing in Moroco. Biotechnol Agronom Soc Environ 13(2):229–234
Desai S, Metrani R, Vantamuri S, Ginigeri V, Phadke K, Hungund B (2012) Phytochemical analysis, antimicrobial and antitumour screening of endophytes of *Tinospora cordifolia*. Int J Pharma Bio Sci 3(4):533–540
Desale MG, Bodhankar MG (2013) Antimicrobial activity of endophytic fungi isolated from *Vitex negundo* Linn. Int J Curr Microbiol Appl Sci 2(12):389–395
Devi NN, Prabakaran JJ (2014) Bioactive metabolites from an endophytic fungus *Penicillium* sp. isolated from *Centella asiatica.* Curr Res Environ Appl Mycol 4(1):34–43
Devi NN, Singh MS (2013) GC-MS analysis of metabolites from endophytic fungus *Colletotrichum gloeosporioides* isolated from *Phlogacanthus thyrsiflorus* Nees. Int J Pharma Sci Rev Res 23(2):292–295
Dhankhar S, Kumar S, Dhankhar S, Yadav JP (2012) Antioxidant activity of fungal endophytes isolated from *Salvadora oleoidesdecne*. Int J Pharma Pharma Sciences 4(2):975–1491
Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Nat Acad Sci USA 93:6025–6030
Dua G, Wang ZC, Hub WY, Yan KL, Wang XL, Yang HM, Yang HY, Gao YH, Liu Q, Hu QF (2016) Three new 3-methyl-2-arylbenzofurans from the fermentation products of an endophytic fungus *Phomopsis* sp. and their anti-TMV activity. Phytochem Lett 1144:1–4
Eatona CJ, Coxa MP, Scott B (2011) What triggers grass endophytes to switch from mutualism to pathogenism? Plant Sci 180:190–195
Edwards EF (1997) New fatty acid-based signals, a lesson from the plant world. Science 5314:912–913
Elfita Muharni, Indah T (2011) Secondary metabolite of *Aspergillus fumigatus*, endophytic fungi of the medicinal plant *Garcinia griffithii*. Makara Sains 15(2):124–128
Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolate from *Podophyllum peltatum* produces podophyllotoxin. J Nat Prod 69(8):1121–1124
Fathima BS, Abhinandan D, Kumar BS, Mohan BR (2013) Mathematical modelling of an endophytic fungus *Fusarium oxysporum* NFX06 isolated from *Nothapodytes foetida*. Int J Chem Chemic Eng 3(2):123–130
Fischer PJ, Petrini O, Scott LHM (1994) The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). New Phytopathol 122(2):299–305

Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi, isolated from a medicinal plant, *Aegle marmelos* Correa ex Roxb. World J Microbiol Biotechnol 24:717–724

Garcia A, Rhoden SA, Filho CJR, Nakamura CV, Pamphile JA (2012) Diversity of foliar endophytic fungi from the medicinal plant *Sapindus saponaria* L. and their localization by scanning electron microscopy. Biol Res 45:139–148

Garraway MO, Evans RC (1984) In: John (ed.) Fungal nutrition and physiology secondary metabolites. Wiley & Sons, New York, pp 336–366

Gaynor DL, Hunt WF (1983) The relationship between nitrogen supply, endophytic fungus and Argentine stem weevil resistance in ryegrass. Proc New Zealand Grassland Asso 44:257–263

Gond SK, Verma VC, Mishra A, Kharwar RN (2010) Management of fungal plant pathogens. In Arya A, Perello A (eds) Role of fungal endophytes in plant protection, vol 632. CABI Publishing, pp 183–196

Goveas SW, Madtha R, Nivas SK, D'Souza L (2011) Isolation of endophytic fungi from *Coscinium fenestratum*-a red listed endangered medicinal plant. Eur Asian J Bio Sci 5:48–53

Greenfield M, Parejaa R, Ortiza V, Gomez-Jimeneza MI, Vegab FE, Parsa S (2015) A novel method to scale up fungal endophyte isolations. Bio Sci Technol 25(10):1208–1212

Guillet G, Podeszfinski C, Regnault RC, Arnason JT, Philogene BJR (2000) Behavioral and biochemical adaptations of generalist and specialist herbivorous insects feeding on *Hypericum perforatum* (Guttiferae). Environ Entomol 29:135–139

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol 7 (1):40–49

Guo BH, Wang YC, Zhou XW, Hu K, Tan F, Miao ZQ, Tang KX (2006) An endophytic Taxol-producing fungus BT2 isolated from *Taxus chinensis* var. *mairei*. African. J Biotechnol 5(10):875–887

Gurney KA, Mantle PG (1993) Biosynthesis of 1-n-methylalbonoursin by an endophytic *Streptomyces* sp. Isolated from perennial ryegrass. J Nat Prod 56(7):1194–1198

Harper JK, Arif AM, Ford EJ, Strobel GA, Porko JA, Tomer DP, Oneill KL, Heider EJ, Grant DM (2003) Pestacin: a 1,3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. Tetrahedron 59(14):2471–2476

Herre EA, Mejia LC, Kyllo DA, Rojas E, Maynard Z, Butler A, Bael SAV (2007) Ecological implications of antipathogen effects of tropical fungal endophytes and mycorhizae. Ecology 88 (3):550–558

Hsiao Y, Chang HS, Liu TW, Hsieh SY, Yuan GF, Cheng MJ, Chen IS (2016) Secondary metabolites and bioactivity of the endophytic fungus *Phomopsis theicola* from Taiwanese endemic plant. Rec Nat Prod 10(2):189–194

Huang WY, Cai YZ, Xing J, Corke H, Sun M (2007) A potential antioxidant resource: endophytic fungi from medicinal plants. Econ Bot 61(1):14–30

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Diver 33:61–75

Ikeda H, Fukuda T, Yokoyama J (2016) Endophytic fungi associated with a holoparasitic plant, *Balanophora japonica* (Balanophoraceae). Am J Plant Sci 7:152–158

Jalgaonwala RE, Mohite BV, Mahajan RT (2011) A review: Natural products from plant associated endophytic fungi 1:21–32

Jayanthia G, Kamalraja S, Karthikeyanb K, Muthumarya J (2011) Antimicrobial and antioxidant activity of the endophytic fungus *Phomopsis* sp. GJJM07 isolated from *Mesua ferrea*. Int J Curr Sci 1:85–90

Khan R (2007) Isolation, identification and cultivation of endophytic fungi from medicinal plants for the production and characterization of bioactive fungal metabolites. PhD thesis

Khan R, Shahzad S, Choudhary MI, Khan SA, Ahmad A (2010) Communities of endophytic fungi in medicinal plant *Withania somnifera*. Pak J Bot 42(2):1281–1287

Khan AL, Hamayun M, Hussain J, Kang SM, Lee IJ (2012) The newly isolated endophytic fungus *Paraconiothyrium* sp. LK1 Produces Ascotoxin. Molecules 17:1103–1112

Kim H, Chen F, Wu C, Wang X, Chung HY, Jin Z (2008) Evaluation of antioxidant activity of Australian tea tree (*Melabuca alternifolia*) oil and its components. J Agric Food Chem 52:2849–2854

Knop M, Pacyna S, Voloshchuk N, Kjant S, Mullenborn C, Steiner U, Kirchmair M, Scherer HW, Schulz M (2007) Zea Mays: Benzoxalinone Detoxification under sulfur deficiency conditions-A complex allelopathic alliance including endophytic *Fusarium verticilloides*. J Chem Ecol 33(2):225–237

Kumar S, Kaushik N, Proksch P (2013) Identification of antifungal principle in the solvent extract of an endophytic fungus *Chaetomium globosum* from *Withania somnifera*. SpringerPlus 2:37

Kumar S, Upadhyay R, Agarwal RP, Sandhu SS (2016) Antibacterial activity of some isolated endophytic fungi from *Menthe viridis*. Int J Appl Biol Pharma Technol 7(1):239–248

Kusari S, Lamshoft M, Zuhl KS, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces Hypericin. J Nat Prod 71(2):159–162

Kusari S, Lamshoft M, Zuhlke S, Spiteller M (2009) *Aspergillus fumigates* Fresenius, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J Appl Microbiol 107(3):1019–1030

Kusari S, Kosuth J, Cellarova E, Spiteller M (2011) Survival-strategies of endophytic *Fusarium solani* against indigenous camptothecin biosynthesis. Fungal Ecol 4:219–223

Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798

Kusari S, Pandey SP, Spiteller M (2013) Untapped mutualistic paradigms linking host plant and endophytic fungal production of similar bioactive secondary metabolites. Phytochemistry 91:81–87

Lee JC, Strobel GA, Lobkovsky E, Clardy J (1996) Torreyanic acid: a selective cytotoxic quinine dimmer from the endophytic fungus *Pestalotiopsis microspora*. J Org Chem 61(10):3232–3233

Li JY, Strobel G, Harper J, Lobkovsky E, Clardy J (2000) Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. Org Lett 2:767–770

Li H, Qing C, Zhang Y, Zhao Z (2005a) Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World J Microbiol Biotechnol 21:1515–1519

Li Y, Song YC, Liu JY, Ma YM, Tan RX (2005b) Anti-*Helicobacter pylori* substance from endophytic cultures. World J Microbiol Biotechnol 21:553–558

Li WC, Zhou J, Guo SY, Guo LD (2007) Endophytic fungi associated with lichens in Baihua mountain of Beihing, China. Fungal Divers 25:69–80

Li HQ, Li XJ, Wang YL, Zhang Q, Zhang AL, Gao JM, Zhang XC (2011) Antifungal metabolites from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*. Biochem Syst Ecol 39 (4):876–879

Limsuwan S, Trip EN, Kouwenc TRHM, Piersmac S, Hiranrat A, Mahabusarakam W (2009) Rhodomyrtone: a new candidate as natural antibacterial drug from *Rhodomyrtus tomentosa*. Phytomedicine 16:645–651

Liu CH, Zou XW, Lu H, Tan RX (2001) Antifungal activity of *Artemisia annua* endophyte cultures against phytopathogenic fungi. J Biotechnol 88:277–282

Long HH, Schmidt DD, Baldwin IT (2008) Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. PLoS ONE 3:e2702

Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. Plant Sci 151:67–73

Malinowski DP, Belesky DP (2006) Ecological importance of *Neotyphodium* sp. grass endophyte in agroecosystems. Grassland Sci 52(1):23–28

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonizing dark septate endophytic fungi. Stud Mycol 53:173–189

Massod AW, Kaul S, Dhar MK, Dhar KL (2010) GC-MS analysis reveals production of 2-phenylethanol from *Aspergillus niger* endophytic in rose. J Basic Microbiol 50(1):110–114

Meenatchi A, Velmurugan R, Bagyalakshmi Rajendran A (2016) Climatic dependency on the diversity and distribution of endophytic fungi from *Rauvolfia serpentina* of Western Ghats. Int J Curr Sci Res 2(2):296–307

Meneses EA, Durango DL, Carlos Y, García M (2009) Antifungal activity against postharvest fungi by extracts from colombian propolis. Quim Nova 32(8):2011–2017

Miles CO, Mena DME, Jacobs SWL, Garthwaite I, Lane GA, Prestige RA, Marshal SL, Wilkinson HH, Schardl CL, Ball OJP, Latch CM (1998) Endophytic fungi in indigenous Australasian grasses associated with toxicity to livestock. Appl Environ Microbiol 64:601–606

Muharni E, Munawar, Rizki (2012) Isolation of antioxidant compound from endophytic fungi *Acremonium* sp. from the twigs of Kandis Gajah. Makara J Sci 16(1):46–50

Musavi SF, Balakrishnan RM (2014) A study on the antimicrobial potentials of an endophytic fungus *Fusarium oxysporum* NFX 06. J Med Bioeng 3(3):162–166

Onelli E, Rivetta A, Giorgi A, Bignami M, Cocucci M, Patrignani G (2002) Ultrastructural studies on the developing secretory nodules of *Hypericum perforatum*. Flora 197:92–102

Ownley BH, Mary R, Griffin MR, Klingeman WE, Gwinn KD, Moulton JK, Pereira RM (2008) *Beauveria bassiana*: endophytic colonization and plant disease control. J Inver Pathol 98:267–270

Pavithra N, Sathish L, Ananda K (2012) Antimicrobial and enzyme activity of endophytic fungi isolated from tulsi. J Pharm Biomed Sci 16(12):1–6

Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunol Med Microbiol 48:367–372

Pieterse CM, Leon RA, Van dES, Van WSC (2009) Networking by small-molecule hormones in plant immunity. Nature Chem Biol 5:308–316

Prabavathy D, Nachiyar VC (2013) Cytotoxic Potential and Phytochemical Analysis of *Justicia beddomei* and its endophytic *Aspergillus* sp. Asian J Pharma Clin Res 6(5):159–161

Premjanu N, Jaynthy C (2015) Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Colletotrichum gloeosporioides* isolated from *Lannea corammenalica*. Int J Chem Tech Res 7(1):369–374

Prestige RA, Gallagher RT (1988) Endophyte conifers resistance to ryegrass: Argentine stem weevil larval studies. Ecologic Entomol 13:429–435

Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. J Nat Prod 68(12):1717–1719

Ranf S, Eschen LL, Pecher P, Lee J, Scheel D (2011) Interplay between calcium signaling and early signaling elements during defense responses to microbe- or damage-associated molecular patterns. Plant J 68:100–113

Rasmussen S, Parsons AJ, Newman JA (2009) Metabolomics analysis of the *Lolium perenne–Neotyphodium lolii* symbiosis: more than just alkaloids? Phytochem Rev. doi:10.1007/s11101-009-9136-6

Read JC, Camp BJ (1987) The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity and stand maintenance. Agron J 78:848–850

Redman RS, Sheehan KB, Stout RG, Rodrigues RJ, Henson JM (2002) Thermo tolerance generated by plant/fungal symbiosis. Science 298:181

Rehman AS, Shawla AK, Andrabi R, Sudan P, Sultana P, Verma V, Qazi GN (2008) An endophytic *Neurospora* sp. from *Nothapodytes foetida* Producing Camptothecin. Appl Biochem Microbiol 44(2):203–209

Roberts C, Andre J (2004) Tall fescue toxicosis and management. Crop Manag, doi:10.1094/cm-2004-0427-01-mg

Roberts CA, Benedict HR, Hill NS, Kjallenbach RL, Rottinghaus GE (2005) Determination of ergot alkaloid content in tall fescue by near-infected spectroscopy. Crop Sci 45:778–783

Rodriguez RJ, Henson J, Volkenburgh EV, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. Int Soc Microb Ecol 2:404–416

Rosa LH, Tabanca N, Techen N, Wedge DE, Pan Z, Bernier UR, Becne JJ, Agramonte NM, Walker LA, Moraes RM (2012) Diversity and biological activities of endophytic fungi

associated with micropropagated medicinal plant *Echinacea purpurea* (L.) Moench. Am J Plant Sci 3:1105–1114

Rubini MR, Silva RRT, Pomella AWV, Maki CS, Araujo WL, Santos DR, Azavedo JL (2005) Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipelli sperniciosa*, causal agent of Witches' Broon disease. Int J Biol Sci 1:24–33

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seed borne fungal endophytes. Ann Rev Plant Biol 55:315–340

Scherlach K, Hertweck C (2009) Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem 7:1753–1760

Schroeckh V, Scherlach K, Nutzmann HW, Shelest E, Schmidt HW, Schuemann J, Martin K, Hertweck C, Brakhage AA (2009) Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. Proc Nat Acad Sci USA 106:14558–14563

Schulz B, Boyle C, Draeger S, Rommert AK, Khrohn K (2002) Endophytic fungi: a source of noble biologically active secondary metabolites. Mycol Res 106(9):996–1004

Senthilkumar N, Murugesan S, Suresh BD, Rajeskannan C (2014) GC-MS analysis of the extract of endophytic fungus, *Phomopsis* sp. isolated from tropical tree species of India, *Tectona grandis* L. Int J Innov Res Sci. Eng Technol 3(3):10176–10179

Senyuva HZ, Gilbert J, Ozturkoglu S (2008) Rapid analysis of fungal cultures and dried figs for secondary metabolites by LC/TOF-MS. Analyt Chim Acta 617:97–106

Shiomi HF, Silva HSA, Melo IS, Nunes FV, Brttiol W (2006) Bioprospecting endophytic bacteria for biological control of coffee leaf rust. Sci Agric 63(1):32–39

Silvaa GH, Telesa HL, Trevisana HC, Bolzani VDS, Young MCM, Pfenning LH, Eberlind MN, Renato Haddad R, Neto CMC, Araujo AR (2005) New Bioactive Metabolites Produced by *Phomopsis cassiae*, an Endophytic Fungus in *Cassia spectabilis*. J Braz Chem Soc 16 (6):1463–1466

Sirvent T, Stuart B, Gibson DM (2003) Induction of hypericins and hyperforins in *Hypericum perforatum* in response to damage by herbivores. J Chem Ecol 29:2667–2681

Sobolev VS, Neff SA, Gloer JB (2008) Localized production of phytoalexins by peanut (*Arachis hypogaea*) kernels in response to invasion by *Aspergillus* species. J Agric Food Chem 56:1949–1954

Spiering MJ, Moon CD, Wilkinson HH, Schardl CL (2005) Gene cluster for insecticidal loline alkaloids in the grass-endophyte fungus *Neotyphodium uncinatum*. Genetics 169:1403–1414

Srinivasan K, Jagadish LK, Shenbhagaraman R, Muthumary J (2010) Antioxidant activity of endophytic fungus *Phyllosticta* sp. isolated from *Guazumato mentosa.* J Phytol 2(6):37–41

Stierle A, Strobel GA, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*. Science 260:214–216

Strayer C et al (2000) Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. Science 289(5480):768–771

Strobel GA (2002) Microbial Gifts from rain forests. Can J Plant Pathol 24:14–20

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel G, Yang X, Sears J, Kramer R, Sidhu RS, Hess WH (1996) Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallichiana*. Microbiology 142:435–440

Strobel GA, Hess WM, Li JY, Sears J, Sidhu RS, Summertell B (1997) *Pestalotiopsis guepinii*, a taxol-producing endophyte of the wollemi pine, *Wollemia nobilis*. Aust J Bot 45:1073–1082

Strobel GA, Miller RV, Miller C, Condron M, Teplow DB, Hess WM (1999) Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. Microbiology 45:1919–1926

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Tayung K, Barik BP, Jha DK, Deka DC (2011) Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of Himalayan yew. Mycosphere 2(3):203–2013

Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Shetty HS (2005) Endophytic Fungal Assemblages from inner bark and twig of *Terminalia arjuna* W. and A. (Combretaceae). World J Microbiol Biotechnol 2:1535–1540

Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS (2007) Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Divers 24:37–54

Terhonen E, Sipari N, Asiegbu FO (2016) Inhibition of phytopathogens by fungal root endophytes of norway spruce biological control. Biol Cont 99:53–63

Thrower LB, Lewis DH (1973) Uptake of sugars by *Epichloe typhina* (Pers. Ex Fr.) Tul, in culture and from its host, Agrostis stolonifer L. New Phytol 72:501–508

Toofanee SB, Dulymamode R (2002) Fungal endlophytes associated with *Cordemoya integrifolia*. Fungal Divers 11:169–175

Tucci M, Ruocco M, Masi LD, Palma MD, Lorito M (2011) The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype mpp. Mol. Plant Pathol 12(4):341–354

Turner WB (1976) In: Smith JE, Berry DR (ed) The filamentous fungi polyketides and related metabolites, vol 2. New York, pp 445–474

Vadassery J, Ranf S, Drzewiecki C, Mithofer A, Mazars C, Scheel D, Lee J, Oelmuller R (2009) A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of Arabidopsis seedlings and induces intracellular calcium elevation in roots. Plant J 59:193–206

Vega FE, Posada F, Aime MC, Ripoll MP, Infante F, Rehner ST (2008) Entomopathogenic fungal endophytes. Biologic Cont 46:72–82

Verma P, Prakash S (2010) Efficacy of *Chrysosporium tropicum* metabolite against mixed population of adult mosquito (*Culex quinquefasciatus*, *Anopheles stephensii*, and *Aedes aegypti*) after purification with flash chromatography. Parasitologic Res 107:163–166

Vinagre F, Vargas C, Schwarcz K, Cavalcante J, Nogueira EM, Baldani JI, Ferreira PC, Hemerly AS (2006) SHR5: a novel plant receptor kinase involved in plant-N_2-fixing endophytic bacteria association. J Exp Bot 57:559–569

Waller F, Aehatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, Wettstein DV, Franken P, Kogel K (2005) The endophytic fungus *Piriformospora indica* reprogram barley to salt stress tolerance, disease resistance and higher yield. Proc Nat Acad Sci 102(38):13386–13391

Wang J, Li G, Lu H, Zheng Z, Huang Y, Su W (2000) Taxol from *Tubercularia* sp. strain TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiol Lett 193:249–253

Waqas M, Khanc AL, Hamayun M, Shahzad R, Kanga SM, Kime JG, Lee IJ (2015) Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. J Plant Interac 10(1):280–287

Webber J (1981) A natural control of Dutch elm disease. Nature London 292:449–451

Webera D, Gorzalczanyb S, Martino V, Acevedo C, Sternerd O, Ankea T (2005) Metabolites from Endophytes of the Medicinal Plant *Erythrina crista-galli*. Naturforsch 60:467–477

Wicklow DT, Joshi BK, Gamble WR, Gloer JB, Dowd PF (1998) Antifungal metabolites (Monorden, Monocillin IV, and Cerebrosides) from *Humicola fuscoatra* Traaen NRRL 22980, a mycoparasite of *Aspergillus flavus* sclerotia. Appl Environ Microbiol 64:4482–4484

Winter JM, Behnken S, Hertweck C (2011) Genomics-inspired discovery of natural products. Curr Opin Chem Biol 15:22–31

Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N, Danwisetkanjana K, Ruangrungsi N, Meevootisom V (2004) Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World J Microbiol Biotechnol 20:265–272

Xia X, Lie TK, Qian X, Zheng Z, Huang Y, Shen Y (2011) Species diversity, distribution, and genetic structure of endophytic and epiphytic *Trichoderma* associated with banana roots. Microb Ecol 61:619–625

Xing X, Guo S, Fu J (2010) Biodiversity and distribution of endophytic fungi associated with *Panax quinquefolium* L. cultivated in a forest reserve. Symbiosis 51:161–166

Xing YM, Chen J, Cui JL, Chen XM, Guo SX (2011) Antimicrobial activity and biodiversity of endophytic fungi in *Dendrobium devonianum* and *Dendrobium thyrsiflorum* from vietman. Curr Microbiol 62(4):1218–1224

Young CA, Felitti S, Shields K, Spangenberg G, Johnson RD, Bryan GT, Saikia S, Scott B (2006) A complex gene cluster for indole-diterpene biosynthesis in the grass endophyte *Neotyphodium lolii*. Fungal Genet Biol 43:679–693

Yuan ZL, Zhang CL, Lin FC (2010) Role of diverse non-systemic fungal endophytes in plant performance and response to stress: progress and approaches. J Plant Growth Regul 29:116–126

Zhang Y, Brock M, Keller NP (2004) Connection of propinuy-CoA metabolism to polyketide biosynthesis in *Aspergillus nidulans*. Genetics 168:785–794

Chapter 5
Genomic Features of Mutualistic Plant Bacteria

Pablo R. Hardoim and Cristiane Cassiolato Pires Hardoim

Abstract Comparative genomics is a powerful technique to identify functional elements accountable for species competence that enables it to thrive in specific environmental niche and for species adaptation to implement particular lifestyles. It also allows insight into genomic island arising from genomic rearrangements. Here, the abundance profile of identified genes, protein families, metabolic pathways, and regulons were computed for endophytes (including nodule-forming plant symbionts), rhizosphere bacteria, and phytopathogens. The lifestyle of endophytes was characterized by significantly overrepresentation of genes encoding for nitrogenase as well as genes involved in the uptake of urea cycle components. The genomes of assigned endophytic bacteria revealed distinct signaling features that differed from those detected among rhizosphere bacteria and phytopathogens. Similar results were also observed for genes encoding proteins involved in transport and secretion systems as well as for transcriptional regulators. Genes involved in chemotaxis receptors are more abundantly represented among phytopathogens than endophytes. Likewise, distinct metabolic functions were enriched for the others plant-associated communities. There was no particular genomic feature that could inhabit common to all genomes in each investigated lifestyle, suggesting that multiple, rather than unique, key features are deployed by the symbionts as strategy to interact with the host plant statically.

Keywords Plant-microbe interactions · Functional characterization
Nitrogen metabolism · Redox-regulation · Type IV secretion system
Transport of polyamines

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_5

5.1 Introduction

Driven by human activities, the Earth atmosphere has been continuously altered. It is now believed that a new geological age termed "Anthropocene" has started with the population growth since 1950 (Steffen et al. 2015). This new age governed by human impact on the functioning of the Earth system is at least as important as other natural processes. Plants, as sessile organisms, will have to cope with ever-increasing environmental challenges. In the climate change scenario, droughts, occasional floods, and extreme temperatures have adversely affected food production globally. Some of these extreme weather disasters significantly reduced crop production up to 10% when compared to an estimated counterfactual global production without considering extreme weather disasters (Lesk et al. 2016). This result suggests that crops are not well adapted to new environmental challenges, and improved breeding strategies might be needed for superior phenotypic plasticity.

Plant domestication, which is one of the most important technological revolutions in human history, started around 13,000–11,000 years ago and was the linchpin of current human cultures (Purugganan and Fuller 2009). The constant selection of cultivars for high yields and improved disease and pest tolerance has led to considerable morphological, physiological, and biochemical changes. Many vegetative traits were unconsciously selected, and plant differentiation was artificially imposed according to the way they are used. This distinguishes several domesticated plant species from their wild ancestors in such extent that they might even be characterized as different species. It has been proposed that plant breeding under favorable conditions, for example copious fertilizer regimes, might have reduced the host capacity for selecting highly efficient mutualistic symbionts (Kiers et al. 2007), thus increasing the dependency of human inputs into the system. High crop yields have been largely accomplished by excessive application of chemical fertilizers and pesticides, which are often obtained from or with the use of non-renewable sources. On the other hand, a sustainable increase in agricultural productivity requires plant materials with improved yield potential that are more stress tolerant and more efficient in use of renewable resources. Microorganisms have been associated with plants from earlier ages (Cavalier-Smith 2010) and are well known for their capacity to participate in all nutrient cycling. Both improved and wild ancestors plants form associations with abundant and diverse microbial communities (Hardoim et al. 2015). The nature of these associations ranges from mutualism to pathogenicity. Similar to those vertebrate animals, plant also has innate immune system to control these associations (Jones and Dangl 2006).

Plant recognizes and responds accordingly to microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs) and to effector molecules. There is increasing evidence that at an initial stage, even beneficial microorganisms can trigger an immune response in plants similar to that of pathogens; however, later on, endophytes manage to escape host defense responses and therefore are able to thrive inside host plants (Zamioudis and Pieterse 2012). These immune responses

are also likely to be involved in mutualistic recognition, where sanctions and reciprocal rewards are crucial to stabilize the cooperation between plants and their symbionts (Kiers et al. 2011). Notably, the host plant is capable to detect, discriminate, and reward the best microbial symbionts. The symbiont enforced the cooperation by increasing nutrient transfer such as Nitrogen (N) and Phosphorus (P) only to those roots providing more carbohydrates (Kiers et al. 2003, 2011). This suggests that mutualistic cooperation is a bidirectional checkpoint mechanism where both partners assist each other for mutual benefits. One might speculate that when the benefits exceed costs, the host encourages mutualistic cooperation with the most efficient endosymbionts, hence favouring their growth. On the other hand, when costs exceed benefits, the host applies sanctions to diminish exploitative outcomes. Therefore, changes in biotic and abiotic conditions can tip the balance away. For instance, soil fertilization ameliorates the host nutrient limitation and might deplete host resource allocation to once beneficial mutualistic cooperation established (Kiers et al. 2010).

Microorganisms, in general, are known for their impressive metabolic and biochemical repertoire. Those organisms closely associated with plants interacting with the host are often capable to elicit drastic molecular, physiological, and morphological changes that modulate the growth and development (Conrath et al. 2006). For instance, bacterial endophytes have been shown to enhance plant growth by (i) improving the mobilization and uptake of nutrients; (ii) increasing stress tolerance to cold, heat, and water deficiency; (iii) production or (co)regulation of phytohormones; and (iv) enhancing plant disease resistance by antagonism, competition, or by inducing or priming the plant's systemic defense systems (Compant et al. 2010). Notably, it has been observed that mutualistic cooperation between the nitrogen-fixing *Klebsiella pneumonia* strain 342 and wheat *cv.* Trenton increased more than 300% the total N concentration in roots and shoots of the host plant when compared to uninoculated controls or wheat inoculated with a knockout *nifH* mutant of *K. pneumonia* 342 (Iniguez et al. 2004). In sugarcane, the contribution of Biological Nitrogen Fixation (BNF) varies greatly among host genotypes and might reach up to 210 kg N ha^{-1} in more efficient mutualistic associations (Dobëreiner et al. 2000). In addition to atmospheric N fixation, plant growth-promoting bacteria were also shown to modify the host synthesis of primary and secondary metabolites (Maheshwari 2010). For instance, inoculation of rice (*Oryza sativa*) with the endophyte *Azospirillum* sp. B510 promoted the production of phenolic compounds such as flavonoids, hydroxycinnamic acid derivatives, and alkylresorcinols that confer plant resistance against pathogenic fungi (Chamam et al. 2013). The induction of chilling tolerance in grapevine was attributed to the increased metabolism of trehalose after inoculation with the endophyte *Paraburkholderia phytofirmans* PsJN (Fernandez et al. 2012). Furthermore, bacteria are likely to be adapting to the presence and metabolization of complex organic molecules and therefore demonstrate interesting biodegradation activities (Sessitsch et al. 2012), due to production/secretion of novel enzymes and metabolites that are of interest for industrial applications.

Plants are constantly interacting with multiple archaeal, bacterial, fungal, and microeukaryotic players including both pathogens and mutualists; therefore, a dense multi-trophic networking is formed. How these interactions work are yet to be resolved; however, recognition, signal transduction, and response processes are highly important for the outcome (Friesen et al. 2011). Cherry genotypes characterized as easy- and difficult-to-propagate revealed distinct microbial communities of endophytes (Quambusch et al. 2014). The authors suggested that a specific set of microbiome is needed to stimulate plant growth. These cues between plants and their associated microorganisms often led to molecular, physiological, and morphological changes that influence plant metabolic pathways and phenotypes. Consequently, these changes may also affect the plant–host relationship with other associated microbes.

Microbes, including endophytes, are capable to directly antagonize plant pathogens. This might be achieved by constitutive biosynthesis of antimicrobial compounds or by the induction of sophisticated chemical communication signaling. Although the foliar (needle) fungal endophyte *Paraconiothyrium variabile* showed direct antagonism toward the phytopathogen *Fusarium oxysporum* in an in vitro dual culture assay, extracts from pure culture did not show any effects (Combès et al. 2012). Only when both endophyte and pathogen fungi are in proximity, the biosynthesis of competition-induced metabolites is induced. The fungal endophyte *P. variabile* synthesized a class of oxylipins metabolite that led to negative modulation of the biosynthesis of mycotoxin by the *Fusarium* pathogen (Combès et al. 2012). It is evident that communication mechanisms between endophytes and host plants are complex (Saikkonen et al. 2013), and it gets even more complex when chemical signaling and cross talk between microorganisms are taken into account, as the example illustrates. We are just beginning to glimpse the importance that multi-trophic metabolic interactions have on both plant hosts and their associated microorganisms (Schulz and Boyle 2005; Brader et al. 2014). Chemical interactions may also occur between fungal endophytes and bacteria that live within hyphae of fungal endophytes (endohyphal bacteria). Filamentous fungal endophytes frequently harbor diverse endohyphal bacteria, many of these bacteria have functions yet-to-be-identified (Hoffman and Arnold 2010). The endohyphal bacterium *Luteibacter* sp. BAC182 significantly enhances auxin (IAA) production of a foliar fungal endophyte identified as *Pestalotiopsis* sp. 9143, although the bacterium in pure culture does not exhibit IAA production under standard laboratory conditions (Hoffman et al. 2013). Another example of endofungal bacterial activity has on host plant is biosynthesis of toxin (rhizoxin) by *Paraburkholderia endofungorum* living within the fungus *Rhizopus microsporus* (Partida-Martinez and Hertweck 2005; Lackner et al. 2009). This toxin is responsible for the rice seedling blight phenotype. Other examples of multi-partner associations can be observed across bacteria, Arbuscular Mycorrhiza Fungi (AMF), and plants. Representatives of Mollicutes and "Candidatus *Glomeribacter,*" a group of *Burkholderia*-related Gram-negative species, have been demonstrated to live in hyphae and spores of AMF (Bonfante and Anca 2009; Naumann et al. 2010). These so-called mycorrhiza helper bacteria form tight relationship with AMF and most likely evolved along as the formation of

mycorrhizal structures in plant roots facilitate the host colonization of new niches (Garbaye 1994; Frey-Klett et al. 2007). Another example of tripartite interactions is provided by a phage-fungus-grass interaction. It was shown that a phage infecting the fungal endophyte *Curvularia protuberata* is capable to increase the tolerance of the geothermal grass *Dichanthelium lanuginosum* to high temperatures (Márquez et al. 2007; Rodriguez et al. 2008). Neither symbionts can tolerate temperatures above 40 °C when grown separately, but in symbiosis, the plant-fungus-phage combination is able to grow at soil temperatures as high as 65 °C.

Bacteria and fungi, including endophytes, are prone to phage infections. In principle, phages infecting these microorganisms can modulate the dynamic of endophytic communities (Márquez et al. 2007; Herrero et al. 2009). Several studies indicate that phages can play important roles in microbial community structuring (Blanquart and Gandon 2013; Koskella 2013). Bacteriophages infecting endophytes from a given horse chestnut tree were more virulent toward endophytes from the same tree than those of neighboring trees, indicating that coevolution forces operate concomitantly in bacteria and phage populations thriving in the same tree. All together, these examples demonstrate that neither host plants nor individual endophytes act independently and that host fitness is the outcome of multiple organism interactions within the biome.

Given the complexity of multiple host and bacterial genotypes, the selection for beneficial partnership are likely to be governed by both parties (Chamam et al. 2013). These genetic mechanisms involved in mutualistic cooperation lead to improve fitness are still poorly understood (Hardoim et al. 2015; Mitter et al. 2016). In this study, we have used comparative genomics to unravel deterministic molecular mechanisms, such as genes, protein families, metabolic pathways, and regulons of mutualistic bacterial communities (endophytic and rhizospheric bacteria compared to phytopathogen). The focus on genetic insights provides a clear picture of the selective pressures that accompany bacterial community interacting with plants. The view from this approach might increase understanding of the nature of mechanistic events that accompanies plant sustainability.

5.2 Methods of Analysis

5.2.1 Data set Collection and Comparative Analysis

Genomes from plant-associated communities, such as endosphere, rhizosphere, and phytopathogen have been compiled. Only genomes of bacterial strains published in peer-reviewed journals and deposited in the Pubmed repository (as of September 01, 2016) were used. The endosphere, rhizosphere, and phytopathogen data sets generated using the strings “endophyt* AND genome,” “rhizosph* AND genome,” and “phytopathog* AND genome,” respectively. These data sets have been further refined by strains available in the Integrated Microbial Genomes and Microbiome

samples (IMG/MER) database. This allowed an accurate comparative genome analysis, as the functional annotation of each strain was performed in similar standard (Markowitz et al. 2012). To avoid bacterial species redundancy, a pairwise genome-wide average nucleotide identity (gANI) was performed for each community data set. Genome sequences with more than 96.5% for gANI and an alignment fraction (AF) more than 0.6 were computed as an intraspecies cluster (Varghese et al. 2015). When more than one genome form a "cluster," a representative genome was selected using the sequence status "finished," the highest number of putative genes encoding proteins acted as priority. By removing intraspecies genome sequences, we aimed to reduce the community bias formed when a particular species is sequenced repeatedly. In addition, a hierarchical clustering (based on genus) profile of all genomes was prepared for display using the online application "Interactive Tree Of Life" (iTOL) (Letunic and Bork 2007). None of the investigated communities showed bias toward a specific genus (Fig. 5.1), suggesting that the community abundance of specific functional trait was not related to phylogenetic assignment. For the comparative genomic analysis sequences from genes encoding proteins of each genome was assigned to KEGG Ortholog (KO). A feature-by-sample contingency table, where properties with more than 15% abundance in at least one assigned community, was created. The assigned KO was normalized with the cumulative sum scaling (CSS) normalization, and a mixture model that implements a zero-inflated Gaussian distribution was enumerated and computed to detect differentially abundant properties with *metagenome Seq* package (Paulson et al. 2013).

5.3 Results and Discussion

5.3.1 Unraveling Distinct Features Within Plant-Associated Bacterial Communities

Comparative genomics is an important tool to identify genes and regulons that discriminate endophytes from other plant-associated communities (Wright et al. 2013) and have been used by several studies (Amadou et al. 2008; Taghavi et al. 2010; Tian et al. 2012; Mitter et al. 2013; Tisserant et al. 2013; Karpinets et al. 2014). To further expand on potential, functional, and mechanistic aspects of endophytes, a comparative analysis of the genomes of 108 well-described bacterial endophytes (obtained from published articles, accessed until September 1st, 2016) with those of 56 well-described plant bacterial pathogens (obtained from the Comprehensive Phytopathogen Genomics Resource, latest accessed until September 1st, 2016) and with those of 96 typical rhizosphere bacteria (obtained from published articles describing the genome, accessed until September 1st, 2016) was performed. The profile of molecular mechanisms and metabolic functions relevant in the process of host colonization and establishment was compared for

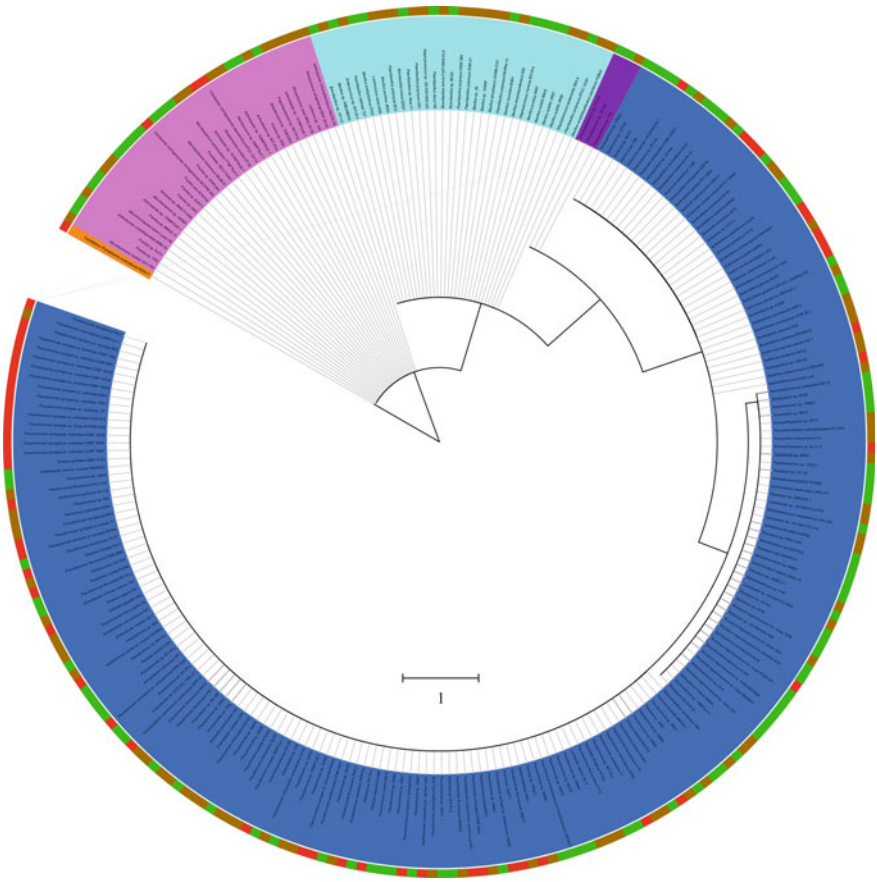

Fig. 5.1 Hierarchical clustering based on gene profile of endophytes (*out circle green*), phytopatogens (*out circle red*), and rizosphere (*out circle brown*) bacteria. The vast majority of investigated genomes were assigned by *Proteobacteria* (*blue*) followed by *Actinobacteria* (*magenta*), *Firmicutes* (*cyan*), *Bacteroidetes* (*purple*), and *Tenericutes* (*orange*)

each investigated group (i.e., phytopathogens and rhizosphere bacteria) to endophytes. We are aware of the fact that bacterial endophytes can have multiple colonization strategies. They might be encountered colonizing the rhizosphere soil or may even have a phytopathogenic lifestyle; however, the aim of this comparative genomics analysis was to obtain indications of potential typical endophytic properties, which are yet to be confirmed.

5.3.2 Sensing and Regulation

5.3.2.1 Chemotaxis and Motility

The ability to sense and respond to environmental cues is one of the major features driving competence of microorganisms. In general, for the investigated chemotaxis receptors, phytopathogens seem to have a better genetic potential to identify, locate, and navigate toward a suitable microenvironment in comparison to that of endophytes. Comparative genomics of features involved in chemotaxis and motility of bacteria suggest that the receptors aerotaxis Aer, plant-derived metabolites such as serine Tsr, aspartate Tar, ribose and galactose Trg, the uptake and metabolism of dipeptides DppA, and the response regulator protein CheB are more abundant among phytopathogens. Whereas, the response regulator proteins CheD, CheR, CheX, and CheC, and the flagellar assembly motor MotB are more abundant among endophytes (Fig. 5.2). These results suggest a clear functional distinction between plant pathogens and endophytes, the first being better equipped to survive in aerobic environments. Aerotaxis is considered to be a behavioral response to optimal metabolic activity driven by oxygen rather than a metabolism-dependent response (Rasche et al. 2006). In addition, several plant-produced metabolites such as serine, aspartate, and monosaccharides ribose and galactose as well as dipeptides seem to be largely used by phytopathogens as nutrient sources (Fig. 5.2). All of these further discriminates survival strategies of pathogens from that of endophytes. Interestingly, that using this approach, we only detected one protein putatively involved in the uptake and metabolism of dipeptides DppA as highly abundant among (free living) rhizobacteria, whereas those involved in uptake of ribose RbsB and metabolism Trg, the transducer of signaling protein CheA and the response regulators proteins CheB, CheR, CheW, CheY, and CheZ are more commonly detected among endophytes. These results also show distinct survival strategies of endophytes from that of free living rhizobacteria.

5.3.2.2 Signal Transduction

The two-component regulatory system (2CS), including quorum-sensing (QS) systems, is essential in the process of sensing and adapting to environmental cues. It is also involved in bacterial cell communication and synchronization of cooperative behavior (Hardoim et al. 2011; Ferrando et al. 2012). The 2CS proteins involved in global redox-regulation (RegB/RegA), activation of symbiotic genes (ChvG/ChvI), nitrogen regulation (NtrY/NtrX), and cell cycle progression and development (DivJ/DivK, CckA/CpdR, and PleC/PleD) are prominently detected among endophytes than any other investigated community (Table 5.1). The RegB-RegA regulon proteins function as a global regulatory system that activates numerous energy-generating and energy-utilizing processes such as photosynthesis, carbon fixation, nitrogen fixation, aerobic and anaerobic respiration, denitrification,

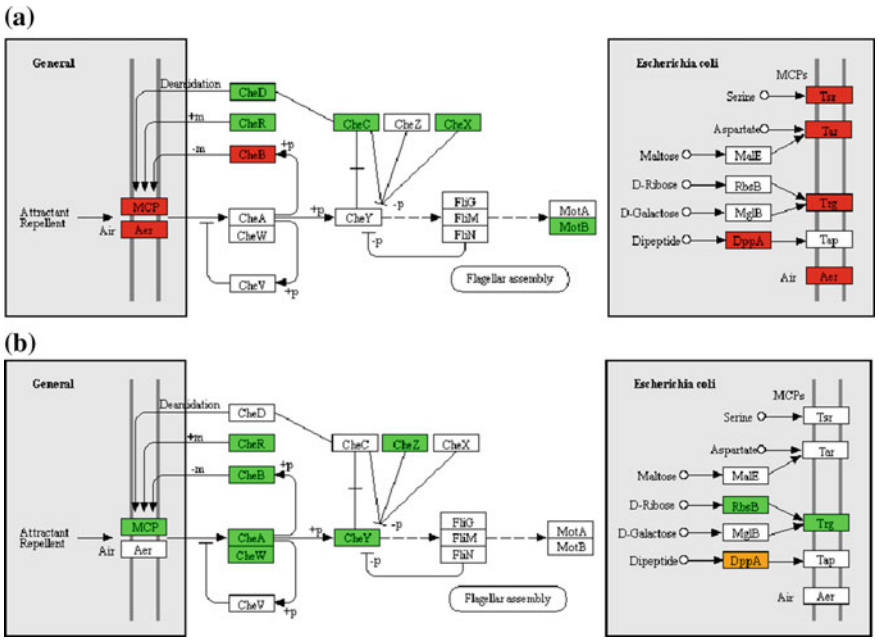

Fig. 5.2 KEGG pathway diagrams of chemotaxis and motility response between phytopathogens and endophytes (**a**) and between rhizosphere bacteria and endophytes (**b**). Genes encoding proteins more abundantly detected among endophytes are shown inside green boxes, whereas those more prominently detected among phytopathogens and rhizosphere bacteria are, respectively, shown in red and orange boxes. Figure modified from KEGG pathways Web site (http://www.genome.jp/kegg/kegg2.html) (ko02030)

and electron transport (Elsen et al. 2004). RegB as a membrane-spanning histidine kinase protein is capable of phosphorylate RegA, the associated cytosolic response regulator protein. Once phosphorylated, RegA might activate transcription of a number of genes, including the synthesis of the molybdenum nitrogenase (*nif*). Also, the transmembrane nitrogen sensor protein NtrY is capable to phosphorylate the response regulator protein NtrX, which induces the expression of *nif* genes (Pawlowski et al. 1991). Under nitrogen-limiting conditions, endophytes might not only fix nitrogen, but might also uptake diverse compounds from the host plant as nitrogen source. Therefore, mechanisms that allow bacteria to detect and respond to nitrogen conditions inside host plant seem to be important features for the fitness of endophytes.

The 2CS ChvG/ChvI is largely detected among alphaproteobacterial endosymbionts and pathogens of plants. These are devoted to the control of critical functions during parasitism of *Rhizobium radiobacter*, previously known as *Agrobacterium tumefaciens*, and rhizobia endosymbiosis. In the pathogenic *R. radiobacter*, ChvG/ChvI regulates the acid-induced expression of genes putatively encoding for an outer membrane protein that confers cell stability and tolerance to detergents,

Table 5.1 Summary of features putatively involved in quorum-sensing and transcriptional regulation from comparative genomics between phytopathogens and endophytes and between rhizosphere bacteria and endophytes

Class/ Family	Description	Phytopathogens	Rhizobacteria
2CS			
(*qseC-qseB*)	Quorum-sensing (*qseC*)	−0.188	
	Quorum-sensing (*qseB*)	−0.249	
(*resE-resD*)	Aerobic and anaerobic respiration (*resE*)	−0.853	0.240
	Aerobic and anaerobic respiration (*resD*)	−0.998	0.396
(*chvG-chvI*)	Activation of virulence genes upon acidic condition (*chvG*)	−0.925	−0.421
	Activation of virulence genes upon acidic condition (*chvI*)	−0.918	−0.383
(*kinB-spo0F*)	Sporulation and biofilm formation (*kinB*)	−0.930	0.542
	Sporulation and biofilm formation (*spo0F*)	−0.850	0.270
(*malK-malR*)	Malate metabolism (*malK*)	−1.022	0.390
	Malate metabolism (*malR*)	−1.068	0.352
(*liaS-liaR*)	Cell wall stress response (*liaS*)	−0.850	0.188
	Cell wall stress response (*liaR*)	−0.864	0.169
(*ntrY-ntrX*)	Nitrogen regulation (*ntrY*)	−0.939	−0.442
	Nitrogen regulation (*ntrX*)	−0.909	−0.438
(*pleC-pleD*)	Pole morphogenesis (*pleC*)	−1.307	−1.165
	Pole morphogenesis (*pleD*)	−0.970	−0.783
(*divJ-divK*)	Cell cycle progression and development (*divJ*)	−0.988	−0.887
	Cell cycle progression and development (*divK*)	−0.209	−0.387
(*cckA-ctrA/ rpdR*)	Cell cycle progression (*cckA*)	−0.928	−0.466
	Cell cycle progression (*cpdR*)	−0.892	−0.399
(*regB-regA*)	Oxidative phosphorylation (*regB*)	−0.150	−0.120
	Oxidative phosphorylation (*regA*)	−0.150	−0.147
(*chpC*)	Twitching motility (*chpC*)	0.124	
(*arcB-arcA*)	Anaerobic respiration (*arcB*)	0.241	
	Anaerobic respiration (*arcA*)	0.580	
(*rcsF-rcsD*)	Capsule polysaccharide synthesis (*rcsF*)	0.241	
	Capsule polysaccharide synthesis (*rcsD*)	0.236	
(*evgS-evgA*)	Antibiotic resistance (*evgS*)		0.609
	Antibiotic resistance (*evgA*)		0.473

(continued)

Table 5.1 (continued)

Class/ Family	Description	Phytopathogens	Rhizobacteria
TF			
abrB	Stage V sporulation protein T (*spoVT*)	−0.881	0.322
araC	Putative protein (*tetD*)	−0.474	
araC	Putative protein (*ygiV*)	−0.480	
araC	Putative protein (*desR*)	−0.851	0.272
araC	4-hydroxyphenylacetate 3-monooxygenase (*hpaA*)	−0.263	0.441
araC	Carnitine catabolism (*cdhR*)	−0.526	
araC	Ethanolamine operon (*eutR*)	−0.375	
araC	DNA-3-methyladenine glycosylase II (*alkA*)	0.365	
araC	Methylated-DNA-cysteine methyltransferase (*ada*)	−0.325	
araC	Methylphosphotriester-DNA methyltransferase (*adaA*)	−0.977	0.275
carD	CarD family (*carD*)	−0.551	
copG	Antitoxin EndoAI (*ndoAI*)	−0.853	0.135
CRP/FNR	Anaerobic regulatory protein (*fnr*)	−0.323	
deoR	Aga operon (*agaR*)	−0.843	
deoR	Fructose operon (*fruR*)	−0.415	
deoR	Glycerol-3-phosphate regulon repressor (*glpR*)		−0.276
deoR	Ula operon (*ulaR*)	−0.519	−0.269
deoR	Deoxyribonucleoside regulator (*deoR*)	−0.886	0.345
deoR	Stage III sporulation protein D (*spoIIID*)	−0.881	0.322
dtxR	Mn-dependent transcriptional regulator (*troR*)		0.313
fur	Iron response regulator (*irr*)	−1.081	−0.512
fur	Peroxide stress response regulator (*perR*)	−0.959	
gntR	Putative protein (*yurK*)	−0.409	
gntR	Putative protein (*ydhQ*)	−1.028	
gntR	Putative protein (*ytrA*)	−0.742	0.407
gntR	Aminotransferase family (*mocR*)	−0.519	0.268
gntR	Glc operon (*glcC*)	−0.757	0.151
gntR	Histidine utilization repressor (*hutC*)	−0.234	
gntR	Phosphonate transport system regulatory (*phnF*)		−0.427
gntR	Pyruvate dehydrogenase complex (*pdhR*)	−0.249	

(continued)

Table 5.1 (continued)

Class/ Family	Description	Phytopathogens	Rhizobacteria
gntR	Trehalose operon (*treR*)	−0.945	
iclR	Acetate operon repressor (*iclR*)	−0.273	
lacI	Asc operon repressor (*ascG*)	0.203	−0.194
lacI	Gluconate utilization system (*gntR*)		−0.398
lacI	Kdg operon repressor (*kdgR*)	−0.441	
lacI	Repressor for several operons (*cytR*)	−0.438	−0.311
lrp/asnC	Putative protein (*ybaO*)	0.425	
lrp/asnC	Leucine-responsive regulatory protein (*lrp*)	−0.311	
luxR	Maltose, positive regulatory protein (*malT*)	−0.283	
luxR	Quorum-sensing system regulator (*lasR*)	0.817	
luxR	Quorum-sensing system regulator (*sdiA*)	0.423	
luxR	Spore coat protein (*gerE*)	−0.881	0.345
lysR	Carnitine catabolism (*dhcR*)		0.143
lysR	Cyn operon (*cynR*)		0.192
lysR	Glycine cleavage system (*gcvA*)	−1.013	
lysR	MexEF-oprN operon (*mexT*)	−0.658	
lysR	Positive regulator for ilvC (*ilvY*)	0.241	
lysR	Gallate degradation pathway (*galR*)	−0.878	
marR	Catechol-resistance regulon repressor (*mhqR*)	−1.102	0.410
marR	Negative regulator of the multidrug (*emrR*)	0.453	
merR	Copper efflux regulator (*cueR*)	0.241	−0.098
merR	Glutamine synthetase repressor (*glnR*)	−0.756	0.158
merR	Redox-sensitive SoxR (*soxR*)	−0.255	
metJ	Methionine regulon repressor (*metJ*)	0.241	
ner	Ner family transcriptional regulator (*ner*)	0.699	
nifA	Nif-specific regulatory protein (*nifA*)	−0.664	−0.934
padR	Regulatory protein (*padR*)	−0.415	
rrf2	Cysteine metabolism repressor (*cymR*)	−0.852	0.130
rrf2	Iron-responsive regulator (*rirA*)	−1.016	−0.382
sgrR	Putative protein (*sgrR*)	0.241	
tetR/acrR	Putative protein (*slmA*)	0.366	
tetR/acrR	Fatty acid metabolism regulator protein (*ysiA*)	−0.795	0.151
tetR/acrR	MexCD-oprJ operon repressor (*nfxB*)	1.000	1.229

(continued)

Table 5.1 (continued)

Class/ Family	Description	Phytopathogens	Rhizobacteria
tetR/acrR	Putative protein (*rutR*)		0.399
Others	Central glycolytic genes regulator (*cggR*)	−0.852	0.145
Others	Cold shock protein (*cspA*)	−0.321	
Others	Heat-inducible (*hrcA*)	−0.204	
Others	HTH-type transcriptional regulator (*higA*)		0.269
Others	Mannitol operon repressor (*mtlR*)	0.173	
Others	Molybdate transport system regulator (*modE*)	−0.169	−0.186
Others	*N*-acetylglucosamine repressor (*nagC*)	0.241	
Others	Phenylacetic acid degradation (*paaX*)	−1.042	
Others	Prespore-specific regulator (*rsfA*)	−0.936	0.362
Others	Prophage regulatory protein (*alpA*)	0.653	
Others	Putative protein (*pspF*)	0.182	
Others	Purine catabolism regulatory protein (*pucR*)		0.495
Others	Putative protein (*yiaG*)		0.294
Others	Putative protein (*lanR*)	−0.597	0.380
Others	Redox-sensing (*rex*)	−0.423	
Others	Regulator of nucleoside diphosphate kinase (*rnk*)	0.182	
Others	Antitoxin (*relB*)	0.308	−0.251
Others	Sigma factor-binding protein (*crl*)	0.241	
Others	Thiaminase (*tenA*)	−1.143	
Others	Pleiotropic regulator of transition genes (*abrB*)	−1.471	0.457
Others	Stress and heat shock response (*ctsR*)	−0.756	0.158
Others	Trp operon repressor (*trpR*)	0.299	
Others	Aerobic/anaerobic benzoate catabolism (*boxR*)	−1.103	−0.924

Values shown are log2 fold change (FC) of features detected in the genome of endophytes (n = 108), phytopathogens (n = 56), and rhizosphere bacteria (n = 96). Only values with significant change (q-value threshold of 0.05) in features of investigated communities, phytopathogens/endophytes and rhizobacteria/endophytes, are shown

antibiotics and low pH, as well as type IV secretion system proteins (T4SS) involved in virulence to host cells (Zhu et al. 2000). The homologue system present in the endosymbiont *Sinorhizobium meliloti* (ExoS/ChvI) also controls the expression of the flagellum and the production of succinoglycan, an exopolysaccharide required for the colonization of legume plants and tolerance to drought stress (Cheng and Walker 1998). These results suggest that the ChvG/ChvI

regulatory system alters the physiology, morphology, and metabolism of the symbiont to initiate the invasion of host tissues.

The genes putatively encoding proteins involved in swimming motility regulated by quorum-sensing (*qseC*/*qseB*), aerobic and anaerobic respiration (*resE*/*resD*), sporulation and biofilm formation (*kinB*/*spo0F*), malate metabolism (*malK*/*malR*), and cell wall stress response (*liaS*/*liaR*) are more typical for endophytes than for phytopathogens, whereas those involved in twitching motility (*chpC*), anaerobic respiration (*arcB*/*arcA*), and capsule polysaccharide synthesis (*rcsF*/*rcsD*) are more relevant for phytopathogens than for endophytes (Table 5.1). On the other hand, genes putatively encoding proteins involved in aerobic and anaerobic respiration (*resE*/*resD*), sporulation and biofilm formation (*kinB*/*spo0F*), malate metabolism (*malK*/*malR*), cell wall stress response (*liaS*/*liaR*), and antibiotic resistance (*evgS*/*evgA*) are more prominently detected among rhizobacteria than endophytes. Overall, these results reveal distinct strategies that are suitable for plant-dwelling community to survive and thrive in different environmental niches and conditions.

5.3.2.3 Transcriptional Regulators

Rapid response to environmental cues is essential for bacterial fitness. Transcriptional regulators play major role by improving adaptation plasticity, cellular homeostasis, and colonization capabilities (Balleza et al. 2009). The genes putatively involved in the transcriptional regulation are detected in a significantly larger proportion among endophytes (56 proteins) than among phytopathogens (21 proteins), whereas only 13 proteins are detected in a significantly larger proportion among endophytes when compared to 28 proteins among rhizobacteria. These results suggest that rhizosphere soil is a more complex environment than the endosphere and that endophytes are more adapted to environmental challenges than phytopathogens. Regulatory genes related to specific carbon metabolism and stoichiometry of nitrogen might be of great importance for a life inside plants. The genes putatively involved in the repression of ascorbate metabolism (*ulaR*), anaerobic catabolism of benzoate (*bzdR*), nucleoside catabolism (*cytR*), nitrogen assimilation (*nifA*), and molybdate transport (*modE*) are detected in a significantly larger proportion among endophytes than among other investigated groups (Table 5.1).

In bacteria, the catabolism of ascorbate compounds occurs not only under anaerobic conditions but also in the presence of oxygen. It is regulated by the UlaR repressor. Ascorbic acid can be detected in relative high amounts, more than 10% of the soluble carbohydrate, in leaves, and together with glutathione enzymes, these are the most important antioxidant compounds in plants (Noctor and Foyer 1998). Aromatic compounds are also found in high abundance inside the host plant. The high abundance of BzdR repressor among endophytes suggested that this group of bacteria might utilize a variety of aromatic substrates as sole carbon sources under denitrifying conditions (Barragán et al. 2005). The catabolism of nucleosides seems to be important for the endosphere colonization as suggested by the highest

abundance of CytR repressor among endophyte group. Interestingly, in *Vibrio cholerae*, the homologue of *Escherichia coli* CytR repressor is also involved in the synthesis of exopolysaccharides (EPS) during biofilm development (Haugo and Watnick 2002). The authors showed that the uptake of nucleosides works as a signal to planktonic cells to join biofilm lifestyle. In plants, the development of biofilm by bacteria is limited to few groups of bacteria, mostly phytopathogens. Biofilm might cause disruption of nutrient supply to the host and thus promote the development of disease. It is still early to draw conclusions about the regulation of CytR protein on endophyte community, but it could be involved in signaling as well as carbohydrate catabolism.

Nitrogen is a limiting nutrient inside the host. Nitrogen fixation by bacteria is a well-studied mechanism in plant growth promotion. Many endophytes fix atmospheric nitrogen as evidenced by the transcriptional activator NifA, involved in activation of nitrogen-fixing (*nif*) operon, and regulator modE, involved sensing and uptake of molybdate at nanomolar concentrations (Gisin et al. 2010). Endophytes harboring the NifA activator are present in 26% of investigated community, whereas around 3% are detected among phytopathogens and rhizobacteria. These findings support the conclusion that endophytes have larger capacity to promote plant growth by the mechanism of nitrogen fixation. Whether or not this process is efficient inside the host is a matter of further discussion.

Iron is an essential micronutrient, and its availability is extremely depleted inside the host plant (Reinhold-Hurek and Hurek 2011). Siderophores are essential compounds for iron acquisition; however, the role of siderophore biosynthesis by endophytes in plant colonization is unknown. It has been suggested that these compounds play a role in induction of host Induced Systemic Resistance (ISR), as well as in biocontrol process by diminish the availability of iron to other members of the plant microbial community, such as pathogens. Diazotrophic bacteria have a special high demand for iron in symbiosis, since iron compounds are essential cofactors for many enzymes involved in the processes of nitrogen fixation. Nevertheless, iron in high concentrations inside cells can be harmful, leading to the formation of potentially damaging hydroxyl radicals via the Fenton reaction. Therefore, iron uptake is usually strictly regulated. A major regulation of iron uptake genes, RirA, and the repressor of heme biosynthesis (Irr) are detected in high abundance among endophytes than other groups (Table 5.1), suggesting that endophytes have a preference for particular mechanism to control iron homeostasis.

5.3.2.4 Secretion Systems

Protein secretion by the symbiont plays an important role in plant-bacterium interactions (Schnepf et al. 1998; Bodenhausen et al. 2013). The secretion systems type III and type VI are more typical for phytopathogens and for rhizosphere bacteria than for endophytes (Fig. 5.3). These secretion systems are more often

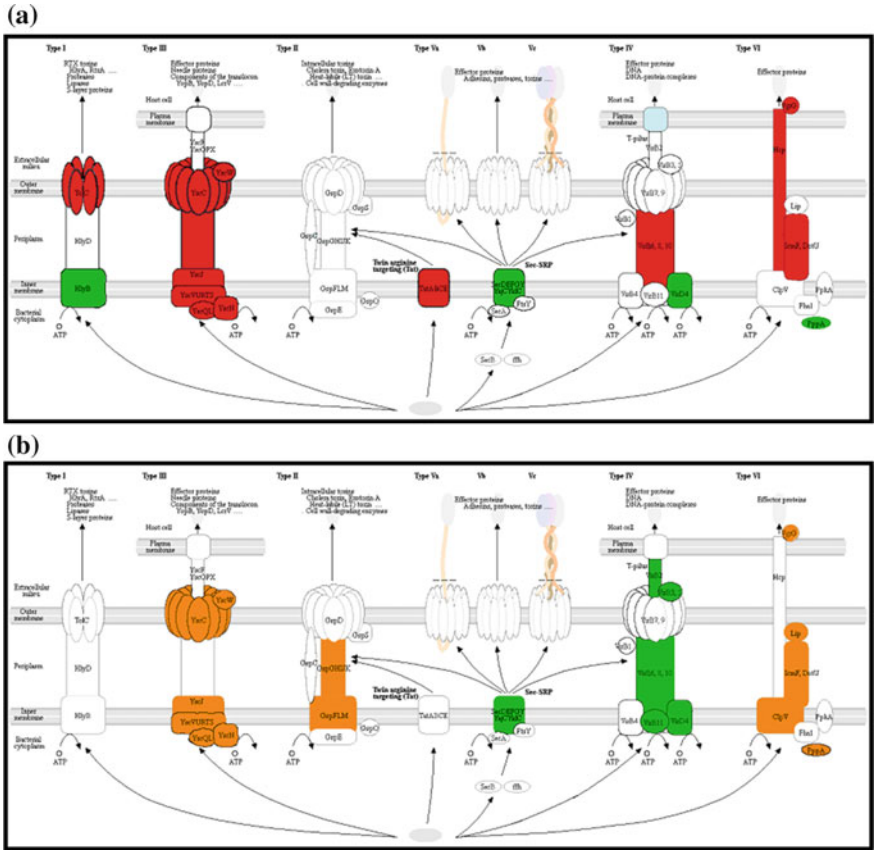

Fig. 5.3 KEGG pathway diagrams of bacterial secretion systems between phytopathogens and endophytes (**a**) and between rhizosphere bacteria and endophytes (**b**). Genes encoding proteins more abundantly detected among endophytes are highlighted in green color, whereas those more prominently detected among phytopathogens and rhizosphere bacteria are, respectively, shown in red and orange colors. Figure modified from KEGG pathways Web site (http://www.genome.jp/kegg/kegg2.html) (map03070)

employed by pathogens to manipulate host metabolism or to compete with other cells (van Overbeek et al. 2011; Shade et al. 2013). Conversely, genes putatively involved in type IV secretion system are more prominently detected among endophytes than among rhizosphere bacteria (Fig. 5.3). Type IV secretion system is likely to be involved in host colonization and conjugation of DNA (Unterseher et al. 2013). The specific function of type IV secretion among endophytes is unknown.

5.3.3 Transporters

Characterization of nutrient transporter genes can provide evidences of the commonly used source of nutrients by heterotrophic microorganisms, including those thriving inside plants (Taghavi et al. 2010; Mitter et al. 2013). The proportion of endophytes harboring genes for ATP-binding cassette (ABC) transporters, major facilitator superfamily (MFS), phosphotransferase system (PTS), and other transport systems largely varied in our analysis (Table 5.2).

Table 5.2 Summary of features putatively involved in nutrient transport from comparative genomics between phytopathogens and endophytes and between rhizosphere bacteria and endophytes

Class of transporters	Description	Phytopahogens	Rhizobacteria
ABC			
ABC-2	Lipopolysaccharide (*rfba*)	0.385	
Mineral and organic ion	Iron(iii) (*afua*)	−0.725	
	Spermidine/putrescine (*potd*)	−0.800	
	Putrescine (*potf*)	−0.472	0.540
	Nitrate/nitrite (*nrta*)	−0.165	
Monosaccharide	Glycerol 3-phosphate (*ugpe*)	−0.251	−0.324
	Ribose (*rbsb*)		−0.532
	Fructose (*frcb*)	−0.917	−0.402
	Rhamnose (*rhas*)	−0.643	−0.387
	Erythritol (*eryg*)	−0.560	−0.480
	Xylitol (*xltc*)	−0.063	
	Glucose/mannose (*gtsa*)	−0.498	−0.453
Oligosaccharide and lipid	Phospholipid/cholesterol (*mlad*)	−0.253	
	Raffinose/stachyose/melibiose (*msme*)	−1.080	
	Lactose/l-arabinose (*lace*)	−0.365	−0.330
	Alpha-glucoside (*agle*)	−0.831	−0.658
	Multiple sugar (*chve*)	−0.535	−0.405
Peptide	Microcin c (*yejb*)	0.236	
	Oligopeptide (*oppb*)	−0.332	
	Cationic peptide (*sapa*)	0.199	
Phosphate and aa	Branched-chain amino acid (*livk*)	−0.965	−0.818
	Phosphate (*psts*)	0.447	
	Phosphonate (*phnd*)	0.356	
	Arginine (*artj*)	0.172	−0.110
	Histidine (*hisj*)	−0.387	0.300
	Arginine/ornithine (*aotj*)	−0.883	
	L-cystine (*tcyk*)	−1.073	

(continued)

Table 5.2 (continued)

Class of transporters	Description	Phytopahogens	Rhizobacteria
Vitamin B12	Biotin (*bioy*)	−0.630	
MFS			
Fucose:H+symporter	L-fucose (*fucP*)	0.415	
Purine	xanthine/uracil (*pbuG*)	−0.244	0.202
Sugar porter	sugar:H+symporter (*Hxt*)	0.247	0.292
Anion:cation symporter	hexuronate (*exuT*)	0.338	
	tartrate (*ttuB*)	−0.496	−0.269
Aromatic acid:H +symporter	3-hydroxyphenylpropionic acid (*mhpT*)	−0.145	
	4-hydroxybenzoate (*pcaK*)	−0.494	
	benzoate (*benK*)	−0.660	
Cyanate porter	cyanate (*MFS.CP*)	−0.239	0.358
Metabolite:H +symporter	alpha-ketoglutarate permease (*kgtP*)	−0.232	0.244
	citrate/tricarballylate (*citA*)	−0.312	
Oxalate:formate antiporter	oxalate/formate (*oxlT*)	−0.542	−0.296
Phenylproprionate permease	3-phenylpropionic acid (*hcaT*)	−0.168	
Siderophore exporter	enterobactin (*entS*)	−0.278	
Aromatic compound/ drug	multidrug resistance protein (*yitG*)	−1.003	0.358
Drug:H+antiporter-1	inner membrane (*ydhP*)	−0.322	
	multidrug resistance protein (*mdtG*)	−0.511	
	multidrug/chloramphenicol (*mdfA*)	−0.256	
	purine base/nucleoside efflux (*pbuE*)		0.325
	purine ribonucleoside efflux (*nepI*)	0.607	
	putative efflux (*ybcL*)	−0.982	0.401
	lincomycin resistance protein (*lmrB*)	−0.778	
Drug:H+antiporter-2	methylenomycin A resistance (*mmr*)	−0.369	
	multidrug resistance protein (*emrB*)	−0.717	
Drug:H+antiporter-3	macrolide efflux (*mef*)	0.926	
Fosmidomycin resistance	fosmidomycin resistance (*fsr*)	−0.152	

(continued)

Table 5.2 (continued)

Class of transporters	Description	Phytopahogens	Rhizobacteria
Others	putative metabolite protein (*yaaU*)		0.864
	putative metabolite:H + symp (*ydjE*)	−0.344	
	putative signal transducer (*ybtX*)	0.909	−0.139
	putative transporter (*yqgE*)	−0.881	0.322
	UMF1 family (*umf1*)	−0.866	
	UMF2 family	0.241	
PTS			
Enzyme I	phosphotransferase I system (*ptsP*)	−0.064	
Phosphocarrier protein HPr	phosphocarrier protein (*ptsH*)	0.254	
	phosphocarrier protein (*ptsA*)	−0.327	
Nitrogen regulatory II	nitrogen regulatory IIA comp (*ptsN*)	−0.186	
Cellobiose-specific II	cellobiose IIC component (*celB*)	−0.599	
Others			
	GABA permease (*gabP*)	−0.327	0.357
	S-adenosylmethionine uptake (*sam*)	−0.502	−0.414
	succinoglycan biosynthesis (*exoP*)	−1.024	−0.423
	ammonium transporter (*amtB*)	−0.212	
	cellulose synthase (*bcsA*)	−0.478	−0.483

Values shown are log2 fold change (FC) of features detected in the genome of endophytes (n = 108), phytopathogens (n = 56), and rhizosphere bacteria (n = 96). Only values with significant change (q-value threshold of 0.05) in features of investigated communities, phytopathogens/endophytes and rhizobacteria/endophytes, are shown. GABA, gamma-aminobutyric acid

Genes putatively involved in the uptake of branched-chain amino acids–isoleucine–valine (*livK*), basic polar amino acid histidine (*hisJ*), and the non-polar amino acid cysteine (*tcyK*) are more prominently detected among endophytes than among phytopathogens. In nitrogen-fixing symbioses, the transport of host-derived branched-chain amino acids is important for the mutualistic interaction. Many nitrogen-fixing symbionts become symbiotic auxotrophs for the synthesis of branch-chain amino acids, whereas genes encoding for the transport of branch-chain amino acids (LIV) from the host are upregulated during symbiotic nitrogen exchange (Prell et al. 2009; Alloisio et al. 2010).

Genes encoding for a general basic amino acid transport system for arginine and ornithine (AOT) are more abundantly detected among endophytes than among phytopathogens, whereas proteins involved in the uptake of arginine-specific system (ArtJ) are more abundant among phytopathogens. The gene encoding for arginine/ornithine substrate-binding transporter (*aotJ*) is subjected to arginine regulation and is induced by exogenous arginine (Lu 2006). Arginine is an important storage form of N and is one of the precursors of polyamines such as putrescine and spermidine. Genes involved in the transport of putrescine and spermidine (*pot*) are also more abundantly detected among endophytes than phytopathogens. Putrescine and spermidine as well as arginine can be used for bacterial growth as the sole N-source (Lugtenberg et al. 2001). Polyamines are protonated at physiological pH and bind various cellular macromolecules such as DNA, RNA, chromatin, and proteins by electrostatic linkage, which might cause change of conformation and thus stabilization and destabilization of molecules. In plants, polyamines are involved in various cellular functions and biochemical processes, including regulation of gene expression, translation, modulation of cell signaling, cell proliferation, growth regulator, morphogenesis, differentiation, membrane stabilization, and programed cell death (Kusano et al. 2008). An accumulation of polyamines has been observed under various abiotic conditions, namely salt stress, water deficit, oxidative stress, ammonium nutrition, and mineral K deficiency (Gerendás 2007). Stress tolerance is associated with the production of conjugated and bound polyamines and stimulation of polyamine oxidation, which alleviate the stress (Bouchereau et al. 1999). However, putrescine is toxic for the vegetative growth of the plant, and accumulation for extend period might result in similar detrimental effects to those induced by stress. The severity of altered phenotype is correlated with putrescine content, a clear indication that putrescine homeostasis is required for proper plant growth. Studies on characterizing the mobility of polyamines within plants are scarce. Nevertheless, polyamines have been identified in phloem and xylem sap of several plant species, and polyamine oxidases were collected from apoplast. Experiments conducted with *Vicia faba* (broad bean) revealed a strong accumulation of free putrescine in the apoplast of ammonium-grown plants, but not observed in plants grown with nitrate (Mühling and Läuchli 2001). The result suggests that apoplastic polyamine contents of broad bean are influenced by the form and concentration of N and K supplied. Here, we postulate that the product of the nitrogen fixation is exported to the host plant in form of ammonium where it might contribute to increase polyamine components and their precursors in the cells and apoplast. These metabolites might be imported back by specialized bacteria to be used as nutrient source for their own growth (Fig. 5.4). In addition, the beneficial effect of bacterial uptake of polyamines might also be exacerbated when the plant is growing under continuous stress challenges as observed for the modulation of ethylene metabolism (Glick 2014). Indeed, the gene involved in the uptake of S-adenosyl methionine (SAM), the precursors involved in the synthesis of higher polyamines spermidine as well as in the synthesis of the phytohormone

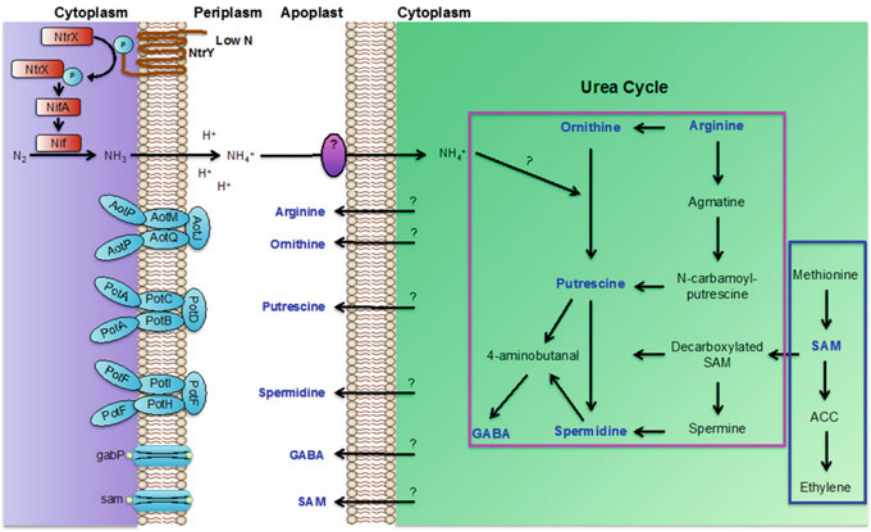

Fig. 5.4 A conceptual modulation model of plant urea cycle (*magenta box*) and ethylene biosynthesis (*blue box*) by endophytes. Low Nitrogen (N) content in the plant apoplast is sensed by bacterial transmembrane nitrogen sensor kinase protein NtrY. The autophosphorylation of NtrY leads to subsequent phosphotransfer to NtrX, the response regulator of N homeostasis inside bacterial cytoplasm (*purple cell*). NtrX activates RpoD-dependent *nifA* gene promoter, which in turn activates the RpoN-dependent *nif* operon promoter allowing the bacteria to fix atmospheric Nitrogen (N_2). The secreted ammonia (NH_3) is protonated in the bacterial periplasm, and ammonium (NH_4^+) is uptaken by the plant host in an unknown mechanism. Upon ammonium addition, the urea cycle inside the host cell is stimulated, and a strong accumulation of free polyamines is observed in the plant cytoplasm. Some of these polyamines will also accumulate in the apoplast by unknown mechanisms. The biosynthesis of ethylene in the plant cytoplasm is correlated with high polyamines biosynthesis (spermidine and spermine). S-adenosylmethionine (SAM) components can be decarboxylated and used as precursor of these polyamines. Biotic and abiotic environmental factors might also positively regulate the plant urea cycle and ethylene biosynthesis pathways. Polyamines and ethylene are involved in plant stress tolerance; however, putrescine is toxic when accumulated for extended period of time (Bouchereau et al. 1999). Specialized bacterial endophytes might use the plant polyamines and their precursors (arginine and ornithine) present in the apoplast for provision of nutrients (uptake by ABC transporter proteins AOT and POT). By keeping the plant polyamines homeostasis and ethylene synthesis on balance, bacterial endophytes might improve plant stress tolerance in adverse environmental conditions. Catabolism of polyamines inside the host cell produces gamma-aminobutyric acid (GABA), which might be oxidized to succinic acid and incorporated into the Krebs cycle or uptaken by specialized bacterial endophytes as nutrient source. All components of urea cycle and ethylene biosynthesis that are more prominently detect among endophytes than other investigated communities are shown in *blue*

ethylene, is also detected more abundantly among endophytes than among other investigated groups, suggesting that endophytes might actively modulate the intensity of stress the host plant is subjected under challenge conditions.

Nitrogen contents inside bacterial cells are affected by N transporters and the membrane permeability. For instance, it is assumed that ammonia, the main product of N_2 fixation, is passively diffused across the bacteroid membrane as ammonia and then converted by protonation to ammonium in the acidic peribacteroid space (Udvardi and Poole 2013). The N transporters detected in high abundance among endophytes than phytopathogens are the genes putatively involved in the uptake of ammonium (*amtB*) and nitrate (*nrtA*) as well as the nitrogen regulatory system II (*ptsN*). The expression of the protein transporter channel AmtB is upregulated only under nitrogen limitation and is absent from nitrogen-fixing cells. The protein NrtA is a key regulator metabolite for N_2 fixation, whereas the protein PtsN is involved in post-translational inhibition of ABC transporters.

Genes putatively involved in the uptake of saccharides, such as alpha-glucoside, glucose/mannose, fructose, rhamnose, erythritol, lactose/L-arabinose, multiple sugar, succinoglycan, and glycerol 3-phosphate, and those involved in the uptake of organic acids, such as oxalate and tartrate, are more prominently detected among endophytes than in the other investigated groups. These results reveal how complex nutrient transport systems of endophytes are and might reflect their lifestyle strategies for acquiring nutrients inside plants.

5.3.4 Genes Involved in Plant Growth Promotion

The nitrogenase (*nifH*) gene putatively involved in the fixation of atmospheric N_2 is detected in a significantly larger proportion among endophytes than among phytopathogens and rhizospheric bacteria (Table 5.3). Surprisingly, 26% of the investigated endophytic prokaryotic group harbors this gene, indicating that it has an important function to improve plant productivity under N limitation (see above). Gene putatively involved in biosynthesis of plant hormone such as salicylic acid; jasmonic acid; abscisic acid; brassinosteroid; ethylene; gibberellin; cytokinine; auxin; and volatile organic compounds (VOC); and encoding 1-aminocyclopropane-1-carboxylate deaminase (*acdS*) are found among endophytes as well as among phytopathogens and rhizosphere/soil colonizers but are not characteristic for one of these groups in particular. A recent analysis of bacterial endophyte genomes suggests that ACC deaminase is not as widely spread among endophytic bacteria as previously thought (Mitter et al. 2013).

Table 5.3 Summary of plant growth promoting features from comparative genomics between phytopathogens and endophytes and between rhizosphere bacteria and endophytes

Features	Description	Phytopathogens	Rhizobacteria
Auxin	Tryptophan 2-monooxygenase (*iaaM*)		0.279
	Nitrile hydratase (*nthA*)	−0.938	
Ethylene	ACC deaminase (*acdS*)	0.339	
VOC	Acetolactate synthase II (*ilvM*)	0.462	
	Acetoin synthase (*ribBA*)	0.198	
	Butanediol dehydrogenase (*butA*)	−0.443	
	Butanediol dehydrogenase (*butB*)		0.390
Vitamin B	Phosphomethylpyrimidine synthase (*thiC*)	−0.066	
	Thiamine-phosphate pyrophosphorylase (*thiE*)	−0.229	−0.203
	Hydroxyethylthiazole kinase (*thiM*)	−0.285	
QQ	Amidase (*amiE*)	−0.481	
Nitrogen	Nitrogenase (*nifH*)	−1.003	−0.691

Values shown are log2 fold change (FC) of features detected in the genome of endophytes ($n = 108$), phytopathogens ($n = 56$), and rhizosphere bacteria ($n = 96$). Only values with significant change (q-value threshold of 0.05) in features of investigated communities, phytopathogens/endophytes and rhizobacteria/endophytes, are shown. *Abbreviations ACC* deaminase 1-aminocyclopropane-1-carboxylate; *VOC* volatile organic compounds; and *QQ* quorum quenching

5.4 Concluding Remarks

Comparative genomics is an important tool to identify genes and regulons that allow endophytes to colonize and thrive inside the host plants. Specific features discriminating endophytes from those of closely related non-endophytic strains have been previously found (Amadou et al. 2008; Taghavi et al. 2010; Tian et al. 2012; Mitter et al. 2013; Tisserant et al. 2013; Karpinets et al. 2014). The -omics technologies have greatly improved our understanding of how host plant interacts with its microbiome. Nowadays, we are better capable to discriminate important features for each specific community associated with plants, such as the so-called endophytes, phytopathogens, and rhizosphere dwelling microorganisms. Although in an ecological context the boundaries between these groups are not always clear, these technologies will enable us to unravel distinct features unique for a specific group of interest. Because in nature multi-trophic interactions among plants and microbial players are the rule rather than the exception, these technologies will enable us to unravel complex complementary functions that allow the holobiome to thrive. Genomic studies will also provide information of which genetic machineries and molecular mechanisms are minimally required to successfully colonize the plant endosphere. And mostly important, what are their functions inside the host plants? We must learn more about yet unknown roles of the so-called commensal endophytes (i.e., groups that apparently do not cause effects on plant performance

but that live on the metabolic costs of host plants), which, in quantity, is the most dominant functional group inside the host plants. Hidden functions are expected among this functional group of endophytes, and by exploring their genome sequences in particular, we might glimpse unforeseen features that can resolve the complexity of microbial interactions within plants. It is yet to be observed more about the mechanisms of interaction between endophytes and plants as well as between endophytes and their partners. It will be highly relevant to elucidate the molecular mechanisms for growth of endophytes, because the physiological conditions inside the host plant differ drastically from those in soil, in a Petri dish, or even inside other host. By implementing new technologies and multi-disciplinary approaches to tackle complex systems such as plant biome, we hope to understand the ecology and biology of endophytes to foster our knowledge on the plant holobiome.

References

Alloisio N, Queiroux C, Fournier P, Pujic P, Normand P, Vallenet D et al (2010) The *Frankia alni* symbiotic transcriptome. Mol Plant Microb Interac 23:593–607

Amadou C, Pascal G, Mangenot S, Glew M, Bontemps C, Capela D et al (2008) Genome sequence of the ß-rhizobium *Cupriavidus taiwanensis* and comparative genomics of rhizobia. Genome Res 18:1472–1483

Balleza E, Lopez-Bojorquez LN, Martinez-Antonio A, Resendis-Antonio O, Lozada-Chavez I, Balderas-Martinez YI et al (2009) Regulation by transcription factors in bacteria: beyond description. FEMS Microbiol Rev 33:133–151

Barragán MJL, Blázquez B, Zamarro MT, Mancheño JM, García JL, Díaz E et al (2005) BzdR, a repressor that controls the anaerobic catabolism of benzoate in *Azoarcus* sp. CIB, is the first member of a new subfamily of transcriptional regulators. J Biol Chem 280:10683–10694. doi:10.1074/jbc.M412259200

Blanquart F, Gandon S (2013) Time-shift experiments and patterns of adaptation across time and space. Ecol Lett 16:31–38. doi:10.1111/ele.12007

Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. Plos One 8:e56329. doi:56310.51371/journal.pone.0056329

Bonfante P, Anca I-A (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Ann Rev Microbiol 63:363–383

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. Plant Sci 140:103–125

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37. doi:10.1016/j.copbio.2013.09.012

Cavalier-Smith T (2010) Deep phylogeny, ancestral groups and the four ages of life. Phil Trans Royal Soc B: Biologic Sci 365:111–132. doi:10.1098/rstb.2009.0161

Chamam A, Sanguin H, Bellvert F, Meiffren G, Comte G, Wisniewski-Dye F et al (2013) Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum-Oryza sativa* association. Phytochemistry 87:65–77

Cheng H-P, Walker GC (1998) Succinoglycan production by *Rhizobium meliloti* is regulated through the ExoS-ChvI two-component regulatory system. J Bacteriol 180:20–26

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. doi:47310.41371/journal.pone.0047313

Compant S, Clement C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Conrath U, Beckers GJM, Flors V, Garcia-Agustin P, Jakab G, Mauch F et al (2006) Priming: getting ready for battle. Mol Plant Microb Interac 19:1062–1071

Döbereiner J, Baldani VLD, Reis VM (2000) The role of biological nitrogen fixation to bioenergy programmes in the tropics. In: Rocha-Miranda E (ed) Transition to global sustainability: the contribution of Brazilian science. Academia Brasileira de Ciências, Rio de Janeiro, Brazil, pp 195–208

Elsen S, Swem LR, Swem DL, Bauer CE (2004) RegB/RegA, a highly conserved redox-responding global two-component regulatory system. Microbiol Mol Biol Rev 68:263–279. doi:10.1128/MMBR.68.2.263-279.2004

Ferando L, Mañay JF, Scavino AF (2012) Molecular and culture-dependent analyses revealed similarities in the endophytic bacterial community composition of leaves from three rice (*Oryza sativa*) varieties. FEMS Microbiol Ecol 80:696–708

Fernandez O, Vandesteene L, Feil R, Baillieul F, Lunn JE, Clement C (2012) Trehalose metabolism is activated upon chilling in grapevine and might participate in *Burkholderia phytofirmans* induced chilling tolerance. Planta 236:355–369

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36. doi:10.1111/j.1469-8137.2007.02191.x

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E (2011) Microbially mediated plant functional traits. Ann Rev Ecol Evol Syst 42:23–46. doi:10.1146/annurev-ecolsys-102710-145039

Garbaye J (1994) Helper bacteria - a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210. doi:10.1111/j.1469-8137.1994.tb04003.x

Gerendás J (2007) Significance of polyamines for pectin-methylesterase activity and the ion dynamics in the apoplast. The apoplast of higher plants: compartment of storage, transport and reactions. Springer, The Netherlands, pp 67–83

Gisin J, Müller A, Pfänder Y, Leimkühler S, Narberhaus F, Masepohl B (2010) A *Rhodobacter capsulatus* member of a universal permease family imports molybdate and other oxyanions. J Bacteriol 192:5943–5952

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39

Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, van Overbeek LS, van Elsas JD (2011) Rice root-associated bacteria: insights into community structures across 10 cultivars. FEMS Microbiol Ecol 77:154–164

Hardoim PR, van Overbeek LS, 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. doi:10.1128/MMBR.00050-14

Haugo AJ, Watnick PI (2002) *Vibrio cholerae* CytR is a repressor of biofilm development. Mol Microbiol 45:471–483

Herrero N, Sanchez Marquez S, Zabalgogeazcoa I (2009) Mycoviruses are common among different species of endophytic fungi of grasses. Arch Virol 154:327–330. doi:10.1007/s00705-008-0293-5

Hoffman MT, Arnold AE (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. Appl Environ Microbiol 76:4063–4075

Hoffman MT, Gunatilaka MK, Wijeratne K, Gunatilaka L, Arnold AE (2013) Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. PLoS ONE 8(9):e73132. doi:10.1371/journal.pone.0073132

Iniguez AL, Dong Y, Triplett EW (2004) Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol Plant Microbe Interac 10:1078–1085
Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329
Karpinets TV, Park BH, Syed MH, Klotz MG, Uberbacher EC (2014) Metabolic environments and genomic features associated with pathogenic and mutualistic interactions between bacteria and plants. Mol Plant Microb Interac 27:664–677
Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume-rhizobium mutualism. Nature 425:78–81
Kiers ET, Hutton MG, Denison RF (2007) Human selection and the relaxation of legume defences against ineffective rhizobia. Proc Royal Soc London B: Biologic Sci 274:3119–3126. doi:10.1098/rspb.2007.1187
Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective. Ecol Lett 13:1459–1474
Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E et al (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882
Koskella B (2013) Phage-mediated selection on microbiota of a long-lived host. Curr Biol 23:1256–1260
Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381. doi:10.1007/s00425-008-0772-7
Lackner G, Partida-Martinez LP, Hertweck C (2009) Endofungal bacteria as producers of mycotoxins. Trend Microbiol 17:570–576
Lesk C, Rowhani P, Ramankutty N (2016) Influence of extreme weather disasters on global crop production. Nature 529:84–87. doi:10.1038/nature16467
Letunic I, Bork P (2007) Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–128
Lu (2006) Pathways and regulation of bacterial arginine metabolism and perspectives for obtaining arginine overproducing strains. Appl Microbiol Biotechnol 70:261–272
Lugtenberg BJJ, Dekkers L, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Ann Rev Phytopathol 39:461–490
Maheshwari DK (2010) Plant growth and health promoting bacteria. Springer, Netherlands, p 448
Markowitz VM, Chen IMA, Palaniappan K, Chu K, Szeto E, Grechkin Y et al (2012) IMG: the integrated microbial genomes database and comparative analysis system. Nucl Acid Res 40: D115–D122. doi:10.1093/nar/gkr1044
Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ (2007) A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science 315:513–515
Mitter B, Petric A, Shin MW, Chain PSG, Hauberg-Lotte L, Reinhold-Hurek B, et al (2013) Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. Front Plant Sci 4:120 doi:110.3389/fpls.2013.00120
Mitter B, Pfaffenbichler N, Sessitsch A (2016) Plant-microbe partnerships in 2020. Microb Biotechnol. doi:10.1111/1751-7915.12382
Mühling KH, Läuchli A (2001) Influence of chemical form and concentration of nitrogen on apoplastic pH of leaves. J Plant Nut 24:399–411
Naumann M, Schussler A, Bonfante P (2010) The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the Mollicutes. ISME J 4:862–871
Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Ann Rev Plant Physiol Plant Mol Biol 49:249–279. doi:10.1146/annurev.arplant.49.1.249
Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884–888
Paulson JN, Stine OC, Bravo HC, Pop M (2013) Differential abundance analysis for microbial marker-gene surveys. Nat Methods 10:1200–1202
Pawlowski K, Klosse U, Debruijn FJ (1991) Characterization of a novel *Azorhizobium caulinodans* ORS571 2-component regulatory system, NtrY/NtrX, involved in nitrogen-fixation and metabolism. Mol Gen Genet 231:124–138

Prell J, White JP, Bourdes A, Bunnewell S, Bongaerts RJ, Poole PS (2009) Legumes regulate *Rhizobium* bacteroid development and persistence by the supply of branched-chain amino acids. Proc Nat Acad Sci 106:12477–12482

Purugganan MD, Fuller DQ (2009) The nature of selection during plant domestication. Nature 457:12. doi:10.1038/nature07895

Quambusch M, Pirttilä AM, Tejesvi MV, Winkelmann T, Bartsch M (2014) Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunus avium* genotypes. Tree Physiol 34:524–533

Rasche F, Velvis H, Zachow C, Berg G, van Elsas JD, Sessitsch A (2006) Impact of transgenic potatoes expressing anti-bacterial agents on bacterial endophytes is comparable with the effects of plant genotype, soil type and pathogen infection. J Appl Ecol 43:555–566

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14:435–443

Rodriguez RJ, Henson J, van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Saikkonen K, Gundel PE, Helander M (2013) Chemical ecology mediated by fungal endophytes in grasses. J Chem Ecol 39:962–968. doi:10.1007/s10886-013-0310-3

Schnepf E, Crickmore N, van Rie J, Lereclus D, Baum J, Feitelson J et al (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev 62:775–806

Schulz B, Boyle C (2005) The endophytic continuum. Mycoloic Res 109:661–686

Sessitsch A, Hardoim PR, Döring J, Weilharter A, Krause A, Woyke T et al (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant Microbe Interac 25:28–36

Shade A, McManus PS, Handelsman J (2013) Unexpected diversity during community succession in the apple flower microbiome. mBio 4:e00602–00612 doi:00610.01128/mBio.00602-00612

Steffen W, Broadgate W, Deutsch L, Gaffney O, Ludwig C (2015) The trajectory of the Anthropocene: the great acceleration. Anthrop Rev 2:81–98. doi:10.1177/2053019614564785

Taghavi S, van der Lelie D, Hoffman A, Zhang YB, Walla MD, Vangronsveld J, et al (2010) Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp 638. Plos Genetics 6:e1000943 doi:1000910.1001371/journal.pgen.1000943

Tian CF, Zhou YJ, Zhang YM, Li QQ, Zhang YZ, Li DF et al (2012) Comparative genomics of rhizobia nodulating soybean suggests extensive recruitment of lineage-specific genes in adaptations. Proc Nat Acad Sci USA 109:8629–8634. doi:10.1073/pnas.1120436109

Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R et al (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Nat Acad Sci USA 110:20117–20122. doi:10.1073/pnas.1313452110

Udvardi M, Poole PS (2013) Transport and metabolism in legume-Rhizobia symbioses. Ann Rev Plant Biol 64:781–805

Unterseher M, Gazis R, Chaverri P, Guarniz CFG, Tenorio DHZ (2013) Endophytic fungi from peruvian highland and lowland habitats form distinctive and host plant-specific assemblages. Biodivers Conser 22:999–1016

van Overbeek LS, Franke AC, Nijhuis EHM, Groeneveld RMW, da Rocha UN, Lotz LAP (2011) Bacterial communities associated with *Chenopodium album* and *Stellaria media* seeds from arable soils. Microbiol Ecol 62:257–264

Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, et al (2015) Microbial species delineation using whole genome sequences. Nucl Acid Res 657

Wright KM, Chapman S, McGeachy K, Humphris S, Campbell E, Toth IK et al (2013) The endophytic lifestyle of *Escherichia coli* O157:H7: quantification and internal localization in roots. Phytopathology 103:333–340

Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microb Interac 25:139–150. doi:10.1094/MPMI-06-11-0179

Zhu J, Oger PM, Schrammeijer B, Hooykaas PJJ, Farrand SK, Winans SC (2000) The bases of crown gall tumorigenesis. J Bacteriol 182:3885–3895

Chapter 6
Endophytism in Cupressoideae (Coniferae): A Model in Endophyte Biology and Biotechnology

Jalal Soltani

Abstract Plants live in a close association with microorganisms in below ground soil and above ground air. Versatile endophytic communities of microorganisms often shape symbiotic relationships with host plants, enter the foliar and root tissues, and promote host's health. Evidence suggests that Cupressoideae subfamily of Cupressaceae (Coniferae) harbors beneficial distinct fungal and bacterial endophytic communities. Besides, the fungal endophytic community in Cupressoideae harbors endohyphal bacteria which indirectly enhance the host plant's health through interaction with their endophytic fungal hosts. Moreover, data from different experiments suggest that the endophytic communities of Cupressoideae could find applications in agroforestry for plant protection against biotic and abiotic stresses. The endophytic microorganisms isolated from the cupressaceous plants are also being regarded as a novel source of biomolecules with immediate significance in medicine and agroforestry. Thus, Cupressoideae, as an underexplored niche, exhibits great promises for endophyte biology and chemistry, as well as evolutionary studies, with potential uses in pharmaceutical, agricultural and biotechnological industries.

Keywords Cupressoideae, Cupressaceae · Endophyte · Endohyphal bacteria Endofungal · Podophyllotoxin · Taxol · Pezizomycotina

6.1 Introduction

Endophyte biology and biotechnology have become a hot topic in recent studies in modern biology, but still remains without a comprehensive understanding of the nature of endophytes and endophytism. This is partly due to the lack of efficient methodologies and reductionism which might not be the case in endophyte biology. Historically, plant pathology precedes the endophyte biology, and it is increasingly

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_6

becoming evident that many phytopathogens could adapt endophytic lifestyle inside the alternative hosts (Arnold et al. 2009; Kusari et al. 2012). Thus, to understand the biodiversity of endophyte biology, it would help to have a look at the biodiversity of phytopathogens first. Indeed, both cellular and non-cellular organisms are introduced as phytopathogens. The non-cellular phytopathogens, known to this date, comprise viroids and viruses. The cellular phytopathogens include both prokaryotic and eukaryotic organisms. Among the prokaryotes, the eubacteria dominate in pathogenicity in plants, while no evidence is available on pathogenicity of archaea. Among the eukaryotes, the fungi are dominant pathogenic and endophytic colonizers of plants. The parasitic plants, nematodes, and protozoans are the other groups of organisms capable of pathogenicity in plants (Agrios 2005). Except for the parasitic plants, the other groups of pathogens could in part or complete of their life cycle enter the plant tissues and exhibit an endophytic lifestyle (Arnold 2007; Rodrigues et al. 2009). Endophytes can vertically or horizontally traverse between alternative hosts (Rodrigues et al. 2009), and their entity could induce disease in the susceptible hosts or possibly remain non-pathogenic inside the alternative hosts (Kusari et al. 2012).

Among eukaryotes, endophytic fungi are classified as class 1 (Clavicipitaceous) and class 2, 3, and 4 (non-Clavicipitaceous) endophytes (Rodrigues et al. 2009), but there is no such classification for other endophytic entities. It is recently observed that the archaea could colonize non-harsh environments such as human body (Aminov 2013; Lurie-Weinberger and Gophna 2015) and internal plant tissues (Ma et al. 2013; Oliveira et al. 2013; Müller et al. 2015). Furthermore, persistent or cryptic viruses are highly common in plants and fungi, and transmit vertically, thus are being considered as beneficial endophytes (Roossinck 2011, 2014, 2015).

Together, endophyte biology in any plant lineage should consider and explore all cultivable and non-cultivable non-cellular and cellular organisms living inside the respective plants. Moreover, shedding light on the complex interrelationship among the endophyte communities occupying the same niche might be of high value for understanding the plant health (Hoffman et al. 2013; Pakvaz and Soltani 2016). In this respect, endophyte biology in Cupressoideae subfamily of Cupressaceae has become a pioneering model. Thus, in this chapter, the current state of the art of endophyte biology and biotechnology in the members of Cupressoideae is comprehensively highlighted.

6.2 Cupressoideae (Cupressaceae, Coniferae)

The Cupressaceae family, also known as cypress family, is a member of the order Pinales (Coniferales), class Pinopsida, division Pinophyta (Coniferae; Conifers) of the kingdom Plantae. Currently, Coniferae comprises seven families, i.e., Araucariaceae, Cephalotaxacae, Cupressaceae, Pinaceae, Podocarpaceae, Sciadopityaceae, and Taxaceae, with a total of 65–70 genera. Among those families, Cupressaceae with 27–30 genera has a nearly global distribution. In addition to

their great ecological significance, coniferous plants are of economic importance for timber production, landscape, and ornamental uses, as well as in perfumery, flavoring beverages, and in medicine. The cypress family includes seven subfamilies, i.e., Athrotaxidoideae, Callitroideae, Cunninghamhioideae, Cupressoideae, Sequoioideae, Taiwanioideae, and Taxodioideae (Gadek et al. 2000; Farjon 2005; Mao et al. 2012). These subfamilies comprise over 30 plant genera, among which *Calocedrus, Chamaecyparis, Cupressus, Fokienia, Juniperus, Microbiota, Platycladus (Thuja), Tetraclinis, Thujopsis,* and *Xanthocyparis* belong to Cupressoideae (Farjon 2005; Jagel and Dörken 2015). The majority of endophyte research in this subfamily has been focused on the genera *Cupressus* (cypress) (Fig. 6.1), *Juniperus* (juniper), and *Platycladus* (syn. *Thuja*) (thuja or arborvitae) which will be discussed in the next sections.

Fig. 6.1 *Cupressus sempervirens* L., a representative species of Cupressoideae. The photo represents Sarv-e-Abarkouh or Sarv-e-Zoroastyria, a 4000 years old cypress tree growing in Abarkouh, Yazd, Iran.

6.3 Endophyte Biology in Coniferae

Among the seven Conifer families, endophyte biology is mainly investigated in Cupressaceae, Pinaceae, and Taxaceae. Currently, research in Taxaceae, and to some extent in Araucariaceae, is focused on exploration and industrial exploitation of taxol-producing endophytes (Zhou et al. 2010). Pinaceae, the other prolific source of bioactive endophytes, has delivered a large number of promising endophytes for application in agriculture and biopharmacy (Stierle and Stierle 2015). Beside these, Cupressaceae is emerging as a promising niche inhabiting diverse endophytes with great potentials for application in biotechnology (Hoffman and Arnold 2008, 2010; Soltani and Hosseyni Moghaddam 2015; Pakvaz and Soltani 2016; Soltani et al. 2016). Indeed, the subfamily Cupressoideae is being emerged as a pioneering model in biology and biotechnology of endophytes and endohyphal bacteria of fungal endophytes.

6.3.1 Biodiversity of Fungal Endophytes in Cupressoideae

Various studies have revealed that the foliar tissues of healthy cupressaceous plants harbor a diverse range of prokaryotic and eukaryotic endophytic microorganisms. Until now, most efforts have investigated the cultivable endophytic fungi and bacteria and mainly in the genera *Calocedrus, Chamaecyparis, Cupressus, Juniperus, and Platycladus (Thuja)* (Cupressoideae). Also, the endofungal (endohyphal) bacteria of fungal endophytes of Cupressoideae have recently attracted considerable attentions and are becoming a pioneering model in the context of biology of endofungal bacteria. However, so far, uncultivable or fastidious endophytes have not been studied in this subfamily.

The most studied endophytic microorganisms in Cupressoideae are cultivable fungi. This fungal community represents a versatile number of taxa from subphylum Pezizomycotina of Ascomycota. It is, currently, evident that the classes Dothideomycetes, and Sordariomycetes dominate in colonizing the plants of Cupressoideae, but fungal species from Eurotiomycetes, Leotiomycetes, and Pezizomycetes classes, from Pezizomycotina, are also common colonizers of these plants. Indeed, this pattern of endophytism is the case for the plant genera *Chamaecyparis*, *Cupressus*, *Juniperus,* and *Thuja* (Carroll and Carroll 1978; Petrini and Carroll 1981; Petrini 1982; Bills and Polishook 1992; Hoffman and Arnold 2008; Ellsworth et al. 2013; Hosseyni Moghaddam 2013; Hosseyni Moghaddam et al. 2013; Hosseyni Moghaddam and Soltani 2014a, b; Soltani and Hosseyni Moghaddam 2014a, b, 2015). In *Calocedrus*, the endophytic Sordariomycetes and *inserta cedis* isolates from Pezizomycotina have been documented (Petrini and Carroll 1981). However, to my knowledge, other plant species of Cupressoideae have not been investigated for the presence of endophytic fungal communities so far.

Subjective studies in USA and Iran have revealed significant similarities in fungal endophytes colonizing Cupressoideae in spite of vast differences in two geographical regions. Indeed, the first investigation on the fungal endophytism in *Cupressus*, *Juniperus,* and *Thuja* in Arizona and North Carolina in USA, revealed that *Alternaria, Ascochyta, Aureobasidium, Botryosphaeria, Cladosporium, Guignardia, Kabatina, Leptosphaerulina, Monodictys, Phoma, Phyllosticta, Preussia, Rhizosphaera, Stagonospora* (Dothideomycetes), *Bartalinia, Biscogniauxia, Chaetomium, Cordyceps, Diaporthe, Lecythophora*, *Nemania, Pestalotiopsis, Phomopsis*, *Pestalotia, Xylaria, Thielavia,* (Sordariomycetes), *Paecilomyces, Penicillium* (Eurotiomycetes), *Morchella,* and *Peziza* (Pezizomycetes) associated with healthy foliage of those plant genera (Hoffman and Arnold 2008). Subsequent research in our laboratory on the same cupressaceous genera growing at four distinct locations in Iran (i.e., Fars, Guilan, Hamedan, and Markazi Provinces) revealed that *Alternaria, Aureobasidium, Bipolaris, Cladosporium, Embellisia, Didymella, Leptosphaeria, Phoma, Pleospora, Pyrenochaeta,* (Dothideomycetes), *Coniochaeta, Cytospora*, *Fusarium, Thielavia* (Sordariomycetes), *Aspergillus, Penicillium*, *Talaromyces* (Eurotiomycetes), and *Ascorhizoctonia* (Pezizomycetes) associated the foliage of Cupressoideae (Hosseyni Moghaddam 2013; Hosseyni Moghaddam et al. 2013; Hosseyni Moghaddam and Soltani 2014a, b; Soltani and Hosseyni Moghaddam 2014a, b, 2015). Thus, those studies indicated the dominance of Dothideomycetes and Sordariomycetes in colonizing cupressaceous trees, and that both geographic locality and host plant identity affected the biodiversity and bioactivity of the recovered endophytes. Other studies, performed in different geographic regions i.e., India, Egypt, Canada and Oregon in USA, are in agreement with these findings (Petrini and Carroll 1981; Vujanovic and St-Arnaud 2003; Chandrasekar et al. 2013; Gherbawy and Elhariry 2014), with the exemption of the recovery of different subset or frequency of fungal genera.

In healthy foliage of *Chamaecyparis*, dominance of endophytic association of *Coniochaeta, Gelasinospora, Glomerella, Harknessia, Microdochium, Mycoleptodiscus, Nodulisporium, Pestalotiopsis, Phomopsis, Tubercularia, Xylaria*, (Sordariomycetes), and *Alternaria, Diplodia, Cladosporium, Epicoccum, Hormonema, Phyllosticta, Pleurophoma*, and *Sporidesmium* (Dothideomycetes) is observed (Petrini and Carroll 1981; Bills and Polishook 1992). Besides, a number of infrequent Eurotiomycetes, Pezizomycetes and sterile fungi have also been recovered.

In addition, some fungi from Leotiomycetes (Pezizomycotina), such as *Chloroscypha* and *Cryptosporiopsis*, are introduced as the frequent endophytes of *Chamaecyparis* and *Thuja* (Petrini and Carroll 1981; Petrini 1982; Bills and Polishook 1992). Also, *Leotiomycetes* sp. and *Lophodermium* (Leotiomycetes) are documented as endophytes of *Juniperus* (Ellsworth et al. 2013). The fungus *Retinocyclus* from Lecanoromycetes (Pezizomycotina) has been observed as the common endophyte of *Juniperus* (Petrini and Carroll 1981). However, endophytism or frequencies of these genera in the respected host plants are not reproduced during further investigations.

Taking all together, dominance of Dothideomycetes and Sordariomycetes in endophytic colonization of the healthy foliage of Cupressoideae appears to be a repeated pattern, even in distinct geographic regions. This may be an indicative of host-endophyte coevolution in these plant and fungal lineages. Endophytic dominance of distinct fungal classes in certain plant lineages are also observed in other plant–endophyte associations, e.g., Sordariomycetes in Fagaceae (*Quercus* spp.) and Leotiomycetes in Pinaceae (*Pinus ponderosa*) as reviewed by Arnold (2007).

6.3.1.1 Bioactivity of Cupressoideae's Fungal Endophytes

Increased resistance of human pathogens to antibiotics has urged for intensified anti-infective molecule discovery from microorganisms (Fair and Tor 2014). Most cupressaceous genera are medicinal plants and used in folk and ethnomedicine. It was suggested that some bioactive metabolites obtained from medicinal plants may be of endophytic microorganism's origin (Strobel and Daisey 2003). Thus, analyzing untapped or underexplored niches to discover novel microbial strains for novel anti-infective and anticancer drugs has gained considerable attention by various scientists. In this context, endophytic microorganisms offer a potentially prolific source of unique secondary metabolites due to their immense biodiversity in unexplored niches (Aly et al. 2010, 2011).

The endophytic fungi of Cupressoideae, isolated from *Cupressus, Juniperus,* and *Thuja* trees, possess antagonistic activities and produce secondary metabolites with potent antifungal, antibacterial, and anti-proliferative activities against plant pathogenic microorganisms (Hosseyni Moghaddam 2013; Hosseyni Moghaddam et al. 2013; Hosseyni Moghaddam and Soltani 2014a, b; Soltani and Hosseyni Moghaddam 2014a, b, 2015). Endophytic fungi recovered from *Juniperus* trees have also exhibited antifungal and antibacterial activities against human pathogenic microorganisms (Ellsworth et al. 2013; Gherbawy and Elhariry 2014). Moreover, endophytic *Aspergillus* from *Chamaecyparis lawsoniana* showed antimicrobial and termiticidal activities (Sun et al. 2015).

Thus, Cupressoideae hosts highly bioactive endophytic fungi that could be used as antagonistic agents against fungal and bacterial pathogens. Those endophytes can also serve as a prolific source of novel chemical compounds to be used as biopesticide in organic agriculture or as biopharmaceuticals.

6.3.1.2 Chemo-Diversity and Pharmaceutical Significance of Cupressoideae's Fungal Endophytes

Endophytic fungi from coniferous plants have demonstrated the ability of producing potent pharmaceutical and agrochemical compounds as reviewed by Stierle and Stierle (2015). It has become evident that some endophytes of medicinal plants are capable of independently synthesizing bioactive molecules similar to their host (Kusari et al. 2012). Thus, besides synthesizing a vast array of biomolecules, the

endophytic communities of Cupressoideae would also possibly biosynthesize some similar biomolecules produced by their host lineage (Kusari et al. 2012).

Indeed, some endophytic fungi isolated from Cupressoideae are reported as producers of antimitotic compounds such as the anticancer drug paclitaxel (taxol). Taxanes, such as taxol, are abundantly produced by the members of the coniferous family Taxaceae (Wang et al. 2011). It is claimed that a number of fungal endophytes isolated from yew trees (*Taxus* spp., Taxaceae) produce taxol, in vitro (Zhou et al. 2010). Likewise, *Phyllosticta spinarum*, an endophytic fungus of *Cupressus* sp. has been reported as a producer of taxol (Kumaran et al. 2008). Presence of the key genes of taxane biosynthesis pathway in some fungal endophytes of Cupressoideae and in vitro taxane production by them is recently confirmed in our lab (Sheikh-Ahmadi 2016). Furthermore, podophyllotoxin, an aryl tetralin lignan, is a prominent anticancer molecule biosynthesized by the plant *Podophyllum* and its endophytic fungus *Phialocephala fortinii* (Stähelin and von Wartburg 1991; Eyberger et al. 2006). Notably, podophyllotoxin and its prodrug deoxy podophyllotoxin have been obtained from the cultures of the *Juniperus*'s endophytic fungi *Fusarium oxysporum* and *Aspergillus fumigatus*, respectively (Kour et al. 2008; Kusari et al. 2009).

Investigating fungal metabolites of *Nodulisporium* from *Juniperus* revealed seven new chemicals (Dai et al. 2006). Also, the natural furanones, cis-gregatin B, graminin C, and pulvinulin A, antibacterial in nature, have been isolated from *Pulvinula* sp., an endophytic fungus of *Cupressus arizonica* (Wijeratne et al. 2015). Furthermore, a variety of terpene compounds have been identified in essential oil of endophytic *Xylaria* sp. isolated from *Cupressus lusitanica*. These terpenes include monoterpenes, sesquiterpenes, and diterpenes, which were also co-produced by their host plant (Amaral and Rodrigues-Filho 2010; Santos Filho et al. 2011). Recently, an endophytic *Alternaria* of *Thuja* has shown to comprise phytotoxic compounds with strong inhibition of seed germination in monocotyledonous plants (Hao et al. 2015).

6.3.2 *Endohyphal Bacteria of Cupressoideae's Fungal Endophyte Community: A Pioneering Model in Endophyte's Endosymbiont Biology*

Research over the past two decades suggested widespread intimate fungal–bacterial interactions in nature (Bonfante and Anca 2009; Kobayashi and Crouch 2009). For example, a major symbiont community of plant roots is endo- and ecto-mycorrhizae. It is well documented that such plant-associated fungi harbor "helper" endohyphal bacteria. Most of such fungi, harboring endohyphal bacterial symbionts, are Zygomycetous fungi such as the members of Mucoromycotina and Glomeromycota, which establish arbuscular-mycorrhizal (AM) associations with plant roots (Bianciotto et al. 1996, 2000; Levy et al. 2003; Lumini et al. 2007). Such bacterial symbionts influence the physiology and development of the host fungi and their interactions with the host plant (Ruiz-Lozano and Bonfante 1999; Lumini

et al. 2007; Mirabal-Alonso et al. 2007). This, in turn, influences the plant growth and health in diverse ways (Bonfante and Anca 2009; Kobayashi and Crouch 2009). However, bacterial endosymbiosis in Zygomycetous fungi is not only restricted to mycorrizal associations, but also involves phytopathogic fungi such as *Rhizopus microsporous* (Mucoromycotina) (Partida-Martinez and Hertweck 2005). In this symbiosis model, it is the *Burkholderia* bacterium that produces phytotoxin, responsible for pathogenicity of the host fungus in the host plant (Partida-Martinez et al. 2007a, b). In addition, bacterial endosymbiosis is also observed in ectomycorhizal fungi from Ascomycotina and Basidiomycotina (Barbieri et al. 2000, 2005, 2007; Bertaux et al. 2003, 2005; Sharma et al. 2008). Those intriguing findings encouraged to search for endohyphal bacteria in fungal endophyte communities. Such investigations highlighted the endohyphal association of a diverse bacterial community with endophytic fungi colonizing the foliage of Cupressoideae (Hoffman and Arnold 2010; Pakvaz and Soltani 2016).

6.3.2.1 Biodiversity of Cupressoideae's Endofungal (Endohyphal) Bacteria

The pioneering research on endosymbiosis of bacteria in fungal endophytes observed the presence of bacteria in hyphae in all four Pezizomyconia classes (Dothideomycetes, Eurotiomycetes, Pezizomycetes, and Sordariomycetes) colonizing cupressaceous trees (Hoffman and Arnold 2010). The bacterial community included the Gram-negative bacteria of α-Proteobacteria (*Sphingomonas* from Sphingomonadaceae, Sphingomonadales), β-Proteobacteria (several unknown bacteria from Burkholderiaceae and Oxalobacteriaceae; *Variovorax* from Comamonadaceae; all from Burkholderiales), γ-Proteobacteria (*Acinetobacter* from Moraxellaceae, Pseudomonadales; *Pantoeae* from Enterobacteriaceae, Enterobacteriales; and several unknown bacteria from Pasturellaceae, Pasteurellales and Xanthomonadaceae, Xanthomonadales) (Hoffmar and Arnold 2010). Also, a small fraction of bacteria was Gram-positive Firmicutes, which included Bacilli i.e., *Bacillus* (Bacillaceae, Bacillales) and *Paenibacillus* (Paenibacillaceae, Bacillales). It was interesting to observe that about 35% of the fungal isolates harbored endohyphal bacteria. However, most of the host fungi lost endosymbiotic bacteria over subculturing, indicating a facultative association (Hoffman and Arnold 2010). Recent finding suggests that low-nutrient conditions favor maintenance of endohyphal bacteria in the host fungi (Arendt et al. 2016).

Recently, bacterial endosymbiosis in fungal endophyte community of the Mediterranean cypress *Cupressus sempervirens* has been highlighted (Pakvaz and Soltani 2016). It has been observed that about 31% of *C. sempervirens*'s endophytic fungi, from the same four classes of Pezizomyconia, harbored bacterial endosymbionts. The bacteria were recovered from fungal hyphae, and a non-obligatory (or facultative) symbiotic lifestyle was observed. The bacteria included Gram-negative members of α-Proteobacteria, i.e., *Sphingomonas* (Sphingomonadaceae, Sphingomonadales) from the fungus *Ascorhizoctonia* sp. and the Gram-positive

Firmicutes, i.e., *Bacillus* spp. (Bacillaceae, Bacillales, and Bacilli) from *Ascorhizoctonia*, *Leptosphaeria,* and *Pyrenochaeta* fungal genera (Pakvaz and Soltani 2016). Each fungal isolate harbored only one endohyphal bacterial species. Moreover, in contrast to the former finding (Hoffman and Arnold 2010), the bacteria were stably maintained in symbiosis over subculturing, and the length of time in culture did not adversely affect their endosymbiotic associations.

An interesting observation in endohyphal bacterium–host fungus interaction is that the fungus can be cured of its bacterium by using antibiotics (Partida-Martinez and Hertweck 2005; Hoffman et al. 2013; Arendt et al. 2016). Further, the axenic bacteria can then be reintroduced into the hyphae of the symbiont-free fungal host or novel hosts from different classes (Partida-Martinez and Hertweck 2005; Arendt et al. 2016).

6.3.2.2 Bioactivity of Endofungal (Endohyphal) Bacteria

Endofungal bacteria are introduced as a source of chemical compounds (Partida-Martinez and Hertweck 2005; Lackner et al. 2011). It was suggested that the endofungal bacteria of AM fungi might be involved in vitamin B12 supply for the host fungi (Ghignone et al. 2012). Further, the endosymbiotic *Burkholderia* of the rice pathogenic fungus *Rhizopus microspores* (Zygomycota) synthesizes antimitotic macrolides (Scherlach et al. 2006), upon which the phytotoxin rhizoxin is produced (Scherlach et al. 2012).

The endofungal bacteria of endophytic fungi are bioactive and produce secondary metabolites and volatile compounds with significant antifungal and antibacterial properties in vitro (Pakvaz and Soltani 2016). The axenic endofungal bacteria of Cupressoideae showed antagonistic activities against the fungal pathogens, and the endophytic microbiome of cupressaceous tress (Pavaz and Soltani 2016). However, the bioactivity of axenic endofungal bacteria seemed to be weak as compared to the endophytic microbiome of Cupressoideae (Soltani and Hosseyni Moghaddam 2015; Pakvaz and Soltani 2016; Soltani et al. 2016). These findings suggest a complicated interrelationship among the host plants, their endophytic microbiome and the endofungal bacteria, which may be of high significance in evolutionary, environmental, agricultural, and pharmaceutical sciences. The observation that every investigated plant hosts endophytic fungi suggests a comprehensive research on bio- and chemo-diversity of endofungal bacteria inhabiting endophytic fungi. Recent findings in our lab indicate the presence of diterpenoid biosynthesis pathway genes and production of such metabolites by Cupressoideae's endofungal bacteria in axenic cultures, in vitro (Tamjid 2015). Therefore, a profound research on biosynthetic pathways and chemical repertoire of such bacteria may discover novel bioactive compounds.

6.3.2.3 Effect of Bacterial Endosymbionts of Fungal Endophyts on the Host Fungi

Association of endofungal bacteria with mycorrhizae plays pivotal roles in fungal host development and its interaction with the host plant (Frey-Klett et al. 2011; Scherlach et al. 2013). Also, it has become evident that *Burkholderia* bacteria serve as the arsenal for the rice pathogenic fungus *Rhizopus microspores* to infect the host plant (Partida-Martinez and Hertweck 2005). Currently, little is known about the functions of the endosymbiotic bacteria in association with endophytic fungi and its effect on the host plant. A recent investigation on the endophytes of Cupressoideae has found that the fungal endophyte *Pestalotiopsis* from the foliage of *Platycladus* produces indole-3-acetic acid (IAA), having stimulatory role in plant growth and development (Hoffman et al. 2013). Interestingly, the fungus harbored a facultative endohyphal bacterium identified as *Luteibacter* (Xanthomonadales) which enhanced IAA production in the host fungus . Such findings suggest that facultative endofungal bacteria, whether independent or in association with their endophytic fungi, play significant roles in their associations and influence the host plant's health. This provides a new framework to explore such bacteria, which may serve as pioneering models for biotechnology and agroforestry.

6.3.3 Biodiversity of Cupressoideae's Bacterial Endophyte Community

Bacterial endophyte communities play pivotal roles in plant health and its growth promotion (Chebotar et al. 2015). Such beneficial effects are mainly mediated by a range of different types of bacterial metabolites (Brader et al. 2014). Furthermore, similar to the endophytic fungi, natural products of endophytic bacteria have shown great potentials in combating human and plant pathogens (Christina et al. 2013). Thus, endophytic bacteria are viewed as prolific sources of novel bioactive compounds.

Advances in Cupressoideae's endophyte biology have shown that bacteria comprise a versatile endophyte community in this plant subfamily. Initial investigation documented the endophytic association of several *Bacillus* and an *Erwinia* species with the foliage of *Cupressus arizonica* and *Juniperus* spp. (Hoffman and Arnold 2010). The dominance of *Bacillus* and *Paenibacillus* bacterial species in endophytic colonization of tissues of *Thuja plicata* was also demonstrated (Bal et al. 2012). Further, a diverse and bioactive bacterial community associates the members of Cupressoideae, i.e., *Cupressus, Juniperus,* and *Thuja* in Iran (Soltani et al. 2016). Sixty-nine bacterial strains of Proteobacteria, Bacilli, and Actinobacteria from healthy foliage of those host plants have been isolated. The initial bioassays in our lab screened superior bacterial strains of the highest antifungal activities. The superior strains belonged to the Gram-negative genera

Brevundimonas (Caulobacteraceae, α-Proteobacteria), and *Stenotrophomonas* (Xanthomonadaceae, γ-Proteobacteria), and the Gram-positive genera *Bacillus* (Bacillaceae, Bacilli), and *Microbacterium* (Microbacteriaceae, Actinobacteria). Although *Bacilli* seem to be a major component of the bacterial endophyte community of Cupressoideae, the dominant genus in our assay was *Stenotrophomonas,* representing 63.6% of the superior strains (Soltani et al. 2016).

6.3.3.1 Bioactivity and Pharmaceutical Significance of Cupressoideae's Bacterial Endophytes

Bacterial endophyte community of Cupressoideae has exhibited potent bioactivities in vitro. Endophytic bacterial strains showed antifungal activity as demonstrated by inhibiting the mycelia growth of *Pyricularia oryzae* causing blast disease of rice (Hosseyni Moghaddam and Soltani 2013; Soltani et al. 2016). Twenty such strains demonstrated more than 50% radial growth inhibition of the mycelia of the test fungi. The selected strains contained high capability in producing antifungal secondary metabolites and volatile organic compounds (Soltani et al. 2016). However, the chemo-diversity of the bioactive compounds from Cupressoideae's endophytic bacteria, as well as the possibility of using such bacteria in plant health management, is yet to be investigated.

Furthermore, in addition to endophytic fungi, several reports have indicated in vitro taxol production by endophytic bacteria isolated from yew trees (Page and Landry 1996; Page et al. 2000; Caruso et al. 2000). Various genera namely *Bacillus, Curtobacterium, Pantoea, Sphingomonas* (Page and Landry 1996; Page et al. 2000), and the actinobacteria *Kitasatospora, Micromonospora*, and *Streptomyces* (Caruso et al. 2000) produced varying degrees of the active compound. Data from our laboratory indicate that endophytic bacteria from cupressaceous trees harbor the key genes of taxane biosynthesis pathways and produce taxanes, in vitro (Tamjid 2015; Sheikh-Ahmadi 2016). Also, *Streptomyces ambofaciens*, an endophyte of *Thuja*, has shown to produce the telomycin-like cyclic depsipeptide, ambobactin (Wei et al. 2015). It is also observed that *Bacillus subtilis*, an endophytic bacterium of *Juniperus virginiana*, produces antitermite compounds such as α-terpinol (Zhao et al. 2011).

For future gain of knowledge in this area of lines and understanding the potential of these endophytic bacteria, a profound research on their chemo-diversity is needed.

6.4 Enhancing Host Plant's Tolerance to Abiotic Stress by Cupressoideae Endophytic Microbiome

Endophytic microorganisms often elicit physiological changes in the host plant and modulate its growth, development, and tolerance to abiotic stresses (Conrath et al. 2006; Van Volkenburgh et al. 2008). In Cupressoideae, it is observed that an endophytic diazotroph *Paenibacillus polymyxa* accounted for 36% foliar nitrogen derivation from atmosphere and significant enhanced growth of *Thuja plicata* in a nitrogen-limited soil (Aand and Chanway 2013). It was also observed that exogenous culture filtrate of the endophytic fungus *Pestalotiopsis* (isolated from *Platycladus*) harboring *Luteibacter* sp., enhanced the growth of tomato in comparison with the filtrate of the fungus alone (Hoffman et al. 2013). Further, seed application of Cupressoideae's endophytic and endofungal microbiome enhanced the growth and yield of wheat and tomato, especially under drought stress conditions (Tamjid 2015; Sheikh-Ahmadi 2016). Thus, the endophytic and endofugal microorganisms of Cupressoideae positively affect the host and non-host plant's physiology and enhance their tolerance to abiotic stresses.

6.5 Conclusions and Future Prospects

In spite of its great promises, we have just started to understand endophyte biology and biotechnology in Cupressoideae. The findings indicate that this plant subfamily hosts a versatile community of bioactive endophytic fungi and bacteria. Endofungal bacteria, living inside the hyphae of endophytic fungi, represent the third bioactive community in these plants. These communities exhibit huge potential for biotechnological applications. However, further investigation is needed to explore endophytic archaea, viruses, viroids, and protozoans in cupressaceous trees, as well as possible endosymbionts of endophytic microorganisms, such as endofungal bacteria, mycoviruses, and bacteriophages. Understanding the microbe–microbe interactions among those communities and host plant–microbe interactions and their outcomes for plant health may be of immense importance in evolutionary and applied sciences. Besides, systematic approaches are needed to further characterize the realm of bioactive chemicals produced by those endophytic and endofungal communities. It is now clearly indicated that Cupressoideae is an untapped niche with a huge promise for delivering novel endophytic microorganisms for use in drug and agrochemical discovery programs, and in plant health management.

Acknowledgements The author gratefully acknowledges Bu-Ali Sina University of Hamedan (BASU), Iran, for its supporting research grants. This work is dedicated to Mohammad-Reza Soltani.

References

Agrios GN (2005) Plant Pathology, 5th edn. Elsevier Academic Press, Burlington, MA, p 922

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41:1–16

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90:1829–1845

Amaral LS, Rodrigues-Filho E (2010) Two novel Eremophilanes Sesquiterpenes from an endophytic fungus isolated from leaves of *Cupressus lusitanica*. J Braz Chem Soc 21:1446–1450

Aminov RI (2013) Role of archaea in human disease. Front Cell Infect Microbiol 3:42

Anand R, Chanway C (2013) N2-fixation and growth promotion in cedar colonized by an endophytic strain of *Paenibacillus polymyxa*. Biol Fert Soils 49:235–239

Arendt KR, Hockett KL, Araldi-Brondolo SJ, Baltrus DA, Arnold AE (2016) Isolation of endohyphal bacteria from foliar Ascomycota and in vitro establishment of their symbiotic associations. Appl Environ Microbiol 82:2943–2949

Arnold AE (2007) Understanding the diversity of foliar fungal endophytes: progress, challenges, and frontiers. Fungal Biol Rev 21:51–66

Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD et al (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst Biol 58:283–297

Bal A, Anand R, Berge O, Chanway CP (2012) Isolation and identification of diazotrophic bacteria from internal tissues of *Pinus contorta* and *Thuja plicata*. Can J For Res 42:807–813

Barbieri E, Potenza L, Rossi I, Sisti D, Giomaro G, Rossetti S et al (2000) Phylogenetic characterization and in situ detection of a Cytophaga-Flexibacter-Bacteroides phylogroup bacterium in *Tuber borchii* vittad ectomycorrhizal mycelium. Appl Environ Microbiol 66:5035–5042

Barbieri E, Bertini L, Rossi I, Ceccaroli P, Saltarelli R, Guidi C et al (2005) New evidence for bacterial diversity in the ascoma of the ectomycorrhizal fungus *Tuber borchii* Vittad. FEMS Microbiol Lett 247:23–35

Barbieri E, Guidi C, Bertaux J, Frey-Klett P, Garbaye J, Ceccaroli P et al (2007) Occurrence and diversity of bacterial communities in *Tuber magnatum* during truffle maturation. Environ Microbiol 9:2234–2246

Bertaux J, Schmid M, Chemidlin Prevost-Boure N, Churin JL, Hartmann A, Garbaye J, Frey-Klett P (2003) In situ identification of intracellular bacteria related to *Paenibacillus* spp in the mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238 N. Appl Environ Microbiol 69:4243–4248

Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, Frey-Klett P (2005) Occurrence and distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238N. Environ Microbiol 7:1786–1795

Bianciotto V, Bandi C, Minerdi D, Sironi M, Tichy HV, Bonfante P (1996) An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl Environ Microbiol 62:3005–3010

Bianciotto V, Lumini E, Lanfranco L, Minerdi D, Bonfante P, Perotto S (2000) Detection and identification of bacterial endosymbionts in arbuscular mycorrhizal fungi belonging to the family *Gigasporaceae*. Appl Environ Microbiol 66:4503–4509

Bills GF, Polishook JD (1992) Recovery of endophytic fungi from. Chamaecyparis thyoides. Sydowia 44:1–12

Bonfante P, Anca IA (2009) Plants, mycorrhizal fungi, and bacteria: A network of interactions. Ann Rev Microbiol 63:363–383

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37

Carroll GC, Carroll FE (1978) Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Can J Bot 56:3032–3043

Caruso M, Colombo AL, Fedeli L, Pavesi A, Quaroni S, Saracchi M et al (2000) Isolation of endophytic fungi and actinomycetes taxane producers. Ann Microbiol 50:3–13

Chandrasekar S, Thiyagarajan S, Sridhar R, Ambethkar B (2013) Diversity of endophytic mycobiota colonizing the aerial tissues of *Thuja plicata* (Donn ex. D. Don.). Int J Curr Microbiol Applied Sci 2:176–183

Chebotar V, Malfanova N, Shcherbakov A, Ahtemova G, Borisov A, Lugtenberg B, Tikhonovich I (2015) Endophytic bacteria in microbial preparations that improve plant development. Appl Biochem Microbiol 51:271–277

Christina A, Christapher V, Bhore SJ (2013) Endophytic bacteria as a source of novel antibiotics. Pharmacogn Rev 7:11–16

Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab G, Mauch F et al (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071

Dai J, Krohn K, Flörke U, Draeger S, Schulz B, Kiss-Szikszai A, et al. (2006) Metabolites from the endophytic fungus *Nodulisporium* sp. from *Juniperus cedre*. Eur J Org Chem 3498–350610

Ellsworth KT, Clark TN, Gray CA, Johnson JA (2013) Isolation and bioassay screening of medicinal plant endophytes from eastern Canada. Can J Microbiol 59:761–765

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J Nat Prod 69:1121–1124

Fair RJ, Tor Y (2014) Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 6:25–64

Farjon A (2005) Monograph of Cupressaceae and Sciadopitys. Royal Botanic Gardens, Kew, p 648

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75:583–609

Gadek PA, Alpers DL, Heslewood MM, Quinn CJ (2000) Relationships within Cupressaceae sensu lato: a combined morphological and molecular approach. Am J Bot 87:1044–1105

Gherbawy YA, Elhariry HM (2014) Endophytic fungi associated with high-altitude *Juniperus* trees and their antimicrobial activities. Plant Biosyst 11:1–10

Ghignone S, Salvioli A, Anca I, Lumini E, Ortu G, Petiti L, Cruveiller S, Bianciotto V, Piffanelli P, Lanfranco L, Bonfante P (2012) The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions. ISME J 6:136–145

Hao SH, Wei Y, Wang J, Zhou YM (2015) Allelopathy and the active metabolites of the endophytic fungus *Alternaria* J46 from *Platycladus orientalis*. Weed Biol Manag 15:95–101

Hoffman MT, Arnold AE (2008) Geographic locality and host identity shape fungal endophyte communities in Cupressaceous trees. Mycol Res 112:331–334

Hoffman MT, Arnold AE (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. Appl Environ Microbiol 76:4063–4075

Hoffman MT, Gunatilaka M, Wijeratne EMK, Gunatilaka AAL, Arnold AE (2013) Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. PLoS One 8:e73132

Hosseyni Moghaddam, MS (2013) Study on some biological effects of natural products from endophytes of cypress. MSc thesis, Bu-Ali Sina University of Hamedan, Iran, 180pp

Hosseyni Moghaddam MS, Soltani J (2013) An Investigation on the effects of photoperiod, aging and culture media on vegetative growth and sporulation of rice blast pathogen *Pyricularia oryzae*. Prog Biol Sci 3:135–143

Hosseyni Moghaddam MS, Soltani J (2014a) Bioactivity of endophytic *Trichoderma* fungal species from the plant family Cupressaceae. Ann Microbiol 64:753–761

Hosseyni Moghaddam MS, Soltani J (2014b) Psycrophilic endophytic fungi with bioactivity inhabit Cupressaceae plant family. Symbiosis 63:79–86

Hosseyni Moghaddam MS, Soltani J, Babalhavaeji F, Hamzei J, Nazeri S, Mirzaei S (2013) Bioactivities of endophytic Penicillia from Cupressaceae. J Crop Prot 2:421–433

Jagel A, Dörken V (2015) Morphology and morphogenesis of the seed cones of the Cupressaceae - part II. Cupressoideae. In: Bulletin of the Cupressus Conservation Project 4:51–78

Kobayashi DY, Crouch JA (2009) Bacterial/Fungal interactions: from pathogens to mutualistic endosymbionts. Ann Rev Phytopathol 47:63–82

Kour A, Shawl AS, Rehman S, Sultan PH, Qazi PH, Suden P, Khajuria RK, Verma V (2008) Isolation and identification of an endophytic strain of *Fusarium oxysporum* producing podophyllotoxin from *Juniperus recurva*. World J Microbiol Biotechnol 24:1115–1121

Kumaran RS, Muthumary J, Hur BK (2008) Production of taxol from *Phyllosticta spinarum*, an endophytic fungus of *Cupressus* sp. Eng Life Sci 4:438–446

Kusari S, Lamshoft M, Spiteller M (2009) *Aspergillus fumigatus* fresenius an endophytic fungus from *Juniperus communis* L Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. Appl Microbiol 107:1364–5072

Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19:792–798

Lackner G, Moebius N, Partida-Martinez L, Hertweck C (2011) Complete genome sequence of *Burkholderia rhizoxinica*, an endosymbiont of *Rhizopus microsporus*. J Bacteriol 193:783–784

Levy A, Chang BJ, Abbott LK, Kuo J, Harnett G, Inglis TJJ (2003) Invasion of spores of the arbuscular mycorrhizal fungus *Gigaspora decipiens* by *Burkholderia* spp. Appl Environ Microbiol 69:6250–6256

Lumini E, Bianchiotto V, Jargeat P, Noveno M, Salvioli A et al (2007) Presymbiotic growth and sporal morphology are affected in the arbuscular mycorrhizal fungus *Gigaspora margarita* cured of its endobacteria. Cell Microbiol 9:1716–1729

Lurie-Weinberger MN, Gophna U (2015) Archaea in and on the Human Body: Health Implications and Future Directions. PLoS Pathog 11(6):e1004833

Ma B, Lv X, Warren A, Gong J (2013) Shifts in diversity and community structure of endophytic bacteria and archaea across root, stem and leaf tissues in the common reed, *Phragmites australis*, along a salinity gradient in a marine tidal wetland of northern China. Antonie Van Leeuwenhoek 104:759–768

Mao K, Milne RI, Zhang L, Peng Y, Liu J, Thomas P, Mill RR, Renner SS (2012) Distribution of living Cupressaceae reflects the breakup of Pangea. Proc Nat Acad USA 109:7793–7798

Mirabal-Alonso L, Ortega-Delgado E (2007) Phosphate solubilizing bacteria isolated from the inside of *Glomus mosseae* spores from Cuba. First international meeting on microbial phosphate solubilization. In: Velazquez E, Rodriguez-Barrueco C (eds) Developments in plant and soil sciences, vol 102 [Reprint of Plant Soil 287:1–84]

Müller H, Berg C, Landa BB, Auerbach A, Moissl-Eichinger C, Berg G (2015) Plant genotype-specific archaeal and bacterial endophytes but similar *Bacillus* antagonists colonize Mediterranean olive trees. Front Microbiol 6:138

Oliveira MN, Santos TM, Vale HM, Delvaux JC, Cordero AP, Ferreira AB et al (2013) Endophytic microbial diversity in coffee cherries of *Coffea arabica* from southeastern Brazil. Can J Microbiol 59:221–230

Page M, Landry N (1996) Bacterial mass production of taxanes with Erwinia. US Patent No. 5561055A

Page M, Landry N, Boissinot M, Helie MC, Harvey M, Gagne M (2000) Bacterial mass production of taxanes and paclitaxel. US Patent No. 6030818A

Pakvaz S, Soltani J (2016) Endohyphal bacteria from fungal endophytes of the Mediterranean cypress (*Cupressus sempervirens*) exhibit in vitro bioactivity. Forest Pathol 46:569–581

Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884–888

Partida-Martinez LP, de Looss CF, Ishida K, Ishida M, Roth M et al (2007a) Rhizonin, the first mycotoxin isolated from the zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. Appl Environ Microbiol 73:793–797

Partida-Martinez LP, Monajembashi S, Greulich KO, Hertweck C (2007b) Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. Curr Biol 17:773–777

Petrini O (1982) Notes on some species of *Chloroscypha endophytic* in Cupressaceae of Europe and North America. Sydowia 35:206–222
Petrini O, Carroll GC (1981) Endophytic fungi in foliage of some Cupressaceae in Oregon. Can J Bot 59:629–636
Rodriguez R, White J, Arnold AE, Redman R (2009) Fungal endophytes: diversity and ecological roles. New Phytol 182:314–330
Roossinck MJ (2011) The good viruses: viral mutualistic symbioses. Nat Rev Microbiol 9:99–108
Roossinck MJ (2014) Metagenomics of plant and fungal viruses reveals an abundance of persistent lifestyles. Front Microbiol 5:e787
Roossinck MJ (2015) A new look at plant viruses and their potential beneficial ropes in crops. Mol Plant Pathol 16:331–333
Ruiz-Lozano MJ, Bonfante P (1999) Identification of a putative P-transporter operon in the genome of a *Burkholderia* strain lyving inside the arbuscular mycorrhizal fungus *Gigaspora margarita*. J Bacteriol 181:4106–4109
Santos-Filho FC, Amaral LS, Rodrigues-Filho E (2011) Composition of essential oils from *Cupressus lusitanica* and a xylariaceous fungus. Biochem Syst Ecol 39:485–490
Scherlach K, Partida-Martinez LP, Dahse H-M, Hertweck C (2006) Antimitotic rhizoxin derivatives from a cultured bacterial endosymbiont of the rice pathogenic fungus *Rhizopus microsporus*. J Am Chem Soc 128:11529–11536
Scherlach K, Busch B, Lackner G, Paszkowski U, Hertweck C (2012) Symbiotic cooperation in the biosynthesis of a phytotoxin. Angew Chem Int Ed Engl 51:9615–9618
Scherlach K, Graupner K, Hertweck C (2013) Molecular bacterial-fungal interactions with impact on the environment, food and medicine. Ann Rev Microbiol 67:375–397
Sharma M, Schmid M, Rothballer M, Hause G, Zuccaro A et al (2008) Detection and identification of bacteria intimately associated with fungi of the order Sebacinales. Cell Microbiol 10:2235–2246
Sheikh-Ahmadi A (2016) Exploring the genes involved in taxane diterpenoid biosynthesis pathway in cypress endophytic fungi *Alternaria* and *Trichoderma*, and drought stress tolerance induced by those fungi in wheat. MSc thesis, Bu-Ali Sina University of Hamedan, Iran 190 pp
Soltani J, Hosseyni Moghaddam MS (2014a) Antiproliferative, antifungal and antibacterial activities of endophytic *Alternaria* species from Cupressaceae. Curr Microbiol 69:349–356
Soltani J, Hosseyni Moghaddam MS (2014b) Diverse and bioactive endophytic Aspergilli inhabit Cupressaceae plant family. Arch Microbiol 196:635–644
Soltani J, Hosseyni Moghaddam MS (2015) Fungal endophyte diversity and bioactivity in the Mediterranean cypress *Cupressus sempervirens*. Curr Microbiol 70:580–586
Soltani J, Zaheri-Shoja M, Hamzei J, Hosseyni Moghaddam MS, Pakvaz S (2016) Diversity and bioactivity of endophytic bacterial community of Cupressaceae. Forest Pathol 46: 353–361
Stähelin HF, von Wartburg A (1991) The chemical and biological route from podophyllotoxin glucoside to etoposide: ninth Cain memorial award lecture. Cancer Res 51:5–15
Stierle AA, Stierle DB (2015) Bioactive secondary metabolites produced by the fungal endophytes of Conifers. Nat Prod Commun 10:1671–1682
Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502
Sun L, Hse CY, Shupe T, Sun M, Wang X, Zhao K (2015) Isolation and characterization of an endophytic fungal strain with potent antimicrobial and termiticidal activities from Port-Orford-Cedar. J Econ Entomol 108:962–968
Tamjid SS A (2015) Exploring the genes involved in taxane diterpenoid biosynthesis pathway in bacterial endophytes of cypress. MSc thesis, Bu-Ali Sina University of Hamedan, Iran, 136 pp
Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416
Vujanovic V, St-Arnaud M (2003) A new species of *Pseudorobillarda*, an endophyte from *Thuja occidentalis* in Canada, and a key to the species. Mycologia 95:955–958
Wang YF, Shi QW, Dong M, Kiyota H, Gu YC, Cong B (2011) Natural taxanes: developments since 1828. Chem Rev 111:7652–7709

Wei S, Zhang W, Ji Z (2015) Structure and antibacterial activity of ambobactin, a new telomycin-like cyclic depsipeptide antibiotic produced by *Streptomyces ambofaciens* F3. Molecules 20:16278–16289

Wijeratne EM, Xu Y, Arnold AE, Gunatilaka AA (2015) Pulvinulin A, graminin C, and cis-gregatin B–new natural furanones from *Pulvinula* sp. 11120, a fungal endophyte of *Cupressus arizonica*. Nat Prod Commun 10:107–11

Zhao K, Liu J, Li Z, Chang Z, Shi P, Ping W, Zhou D (2011) *Bacillus subtilis* subspecies *virginiana*, a new subspecies *of* antitermitic compound-producing endophytic bacteria isolated from *Juniperus virginiana*. J Econ Entomol 104:1502–1508

Zhou X, Zhu H, Liu L, Lin J, Tang K (2010) A review: recent advances and future prospects of taxol-producing endophytic fungi. Appl Microbiol Biotechnol 86:1707–1717

Chapter 7
Potential Role of Endophytes in Sustainable Agriculture-Recent Developments and Future Prospects

Pranay Jain and Ram Kumar Pundir

Abstract Discovery of new solutions for the establishment of sustainable agricultural is essential that may avoid the heavy use of fertilizers and pesticides as a reliance of productivity booster. Plant associative beneficial microbes are expected to harness their contribution in integrated pest management schemes over the coming decades. There is global ever growing demand for implanting ecologically compatible and ecofriendly practices in agriculture, capable of providing adequate solutions for improving agriculture productivity. For these reasons, the endophytes prove to be an important alternative practice for long. The term endophyte is used to define those microorganisms which colonize in the plant tissues. These microorganisms induce plant growth using several mechanistic approaches such as biological nitrogen fixation, phytohormone production, phosphate solubilization, inhibition of ethylene biosynthesis, and tolerance to abiotic stresses by inducing resistance in plant to counteract against pathogenic attacks or by the release of secondary metabolites such as enzymes, siderophore, and antibiotics. The major factor that is contributing in sustainable agriculture involves choice of the plant, its age, and endophytic microorganisms which could adapt themselves in the plant tissues to be inhabited in. The basic knowledge of this kind of symbiotic relationship would assist in increasing crop production by using them as bioinoculants. The research on the ecology of endophytic bacteria will be most important contributing factor to capitalize on the agricultural returns from these microbes.

Keywords Agriculture sustainability · Beneficial microorganisms
Endophytes · Plant growth-promoting potential

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_7

7.1 Introduction

Agricultural intensification in the twentieth century has been largely achieved through the use of farm equipment, high-yielding crop varieties, intensive tillage, irrigation, fertilizers, pesticides, and other manufactured inputs (Foley et al. 2005). However, detrimental effects of the agricultural practices on soil ecology have been recognized. Therefore, new ecofriendly approaches have to be employed to maintain sustainable agricultural production and to overcome threats that lead to loss of crop yield, including plant stresses associated with unfavorable environmental conditions, such as drought, temperature extremes or soil salinity, as well as biotic stress induced by pathogens and pests. In this context, harnessing the contribution of beneficial bacteria for agricultural management in general and more particularly for integrated nutrient and pest management now became utmost need in the current scenario (Singh et al. 2011).

Endophyte refers to those organisms inhabiting within the living and functional tissues of plants. Bacon and White (2000) have postulated that microorganisms colonize internal tissues of plant, live in, and does not overt any apparent negative onset and systemic symptoms. These are the microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects (Bacon and White 2000). Microorganisms like bacteria, fungi, and actinobacteria inhabit intra- and intercellular plant tissues. Endophytes are capable to colonize leaves, petioles, stems, twigs, bark, root, fruit, flower, and seeds. These microorganisms represent positive plant–microbe interaction and association of different plant species with microbes including bacteria and fungi. The interaction is such a complex yet to be fully understood. Due to its great impact on the different crops, it is considered as a best alternative of different agro-chemicals used in the field of agriculture.

Initially, the endophytic microorganisms were considered harmless to plants, but from ‘70s onwards their importance was realized (Azevedo et al. 2002). There are several positive effects attributed to endophytic microorganisms, such ability to enhance plant growth (Okon and Labandera-Gonzalez 1994), control of pests and plant diseases (Mariano et al. 2004), biological fixation of nitrogen (Dobereiner and Boddey 1981), systemic resistance induction (Halmann et al. 1997), production of siderophore (Burd et al. 1998), and antibiotics (Strobel and Daisy 2003). The plant growth-promoting ability owes the secretion of phytohormones. *Gluconacetobacter, Azosprillium, Herbaspirillum, Erwinia, Pseudomonas,* and *Pantoea* have been identified as phytohormone producing endophytes (Kuklinsky-Sobral et al. 2004; Maheshwari et al. 2015). Symbioses of mycorrhiza with the root of legumes have been accounted as determining factor to stimulate root growth, nodulation, and nitrogen fixation (Redecker et al. 1997).

Plant–microbe symbiotic relationships have been known for decades (Peterson et al. 2008). The symbiotic association of endophytic fungi with crops may responsible for an increase in crop growth and yield without supplementing

extensive fertilizers. On the other hand, subsequent to this the symbiont improves the plant abilities to resist against biotic and abiotic stresses (Rodriguez et al. 2008). Thus, endophytic fungi provide solutions of modern agricultural constraints and increase food production thereby. Sessitsch et al. (2002) considered soil rhizosphere as huge reservoir of root endophytes, also found in free form in this region. The endophytic invasion accomplishes through root infection (Gough et al. 1997), and further colonization promotes plant growth in several means including biocontrol (Waller et al. 2005). On the other hand, production of phytohormone is also considered as a significant contribution to enhancement of plant growth (Zou and Tan 1999) and nutrients uptake (Malinowski and Belesky 1999; Reis et al. 2000; Zhang et al. 2013).

Endophytes colonize majority of plants and coordinate with wide array of ecological roles to be actively participated in mutualism to parasitic interactions (Saikkonen et al. 1998). Colonization by endophytic fungi promotes plant growth by protecting against several fungal and bacterial borne diseases, assisting in the uptake of available phosphorus or improving the ecological adaptation abilities of the host by providing tolerance to counteract against biotic and abiotic stresses (Schulz et al. 1999; Sieber 2002; Schulz and Boyle 2005). Endophytic fungi have been classified into Clavicipitales, with few hosts within the monocots (Bischoff and White 2005), and nonclavicipitaceous species inhabiting both monocots and eudicots (Carroll 1988; Van Bael et al. 2005) which probably represent the majority of microbial symbionts which interact with plants, with a great diversity occurring both at taxonomical and at functional levels. In spite of this, the importance of this group of fungi has been largely neglected until recent years, probably due to their facultative mutualism within plants which is often difficult to establish. However, their ability to grow saprophytically in the absence of host plants make them amenable for biotechnological purposes, since they can be isolated and grown in culture media. Unsuitability of some mycorrhizal fungi for mass production is one of the main problems to incorporate these valuable symbionts into mainstream agricultural production (Hart and Trevors 2005).

The activation of internal plant defense mechanism exhibits via the production of wide array of elicitors against biotic and abiotic stresses. Though fungicides could be an alternative method of protecting plants from disease, various side effects cannot be ignored (Walters et al. 2005). Thus, beneficial fungal endophytes are the alternative having properties of increasing plant fitness by convening abiotic and biotic stress tolerance, plant growth, and yield by increasing nutrient uptake (Barka et al. 2002; Tanaka et al. 2005; Vega et al. 2008). These fungal endophytes also provide immune system to host plant to defend against phytopathogenic organisms by regulating plant physiology (Giménez et al. 2007). The systemic acquired resistance is most common immune system of plant. Besides this, major economic losses on an annual basis have been reported due to pathogenic filamentous fungi (Pennisi 2001, Muńoz et al. 2013).

7.2 Diversity of Endophytic Microflora in Agricultural Crops

Endophytic microbes are found in most plant species. Their entry in the plants is mainly through wounds or epidermal conjunctions on root hairs. Endophytic microbes aggressively pierce plant tissues by secreting variety of hydrolytic enzymes such as pectinase and cellulase. The commonly encountered endophytic bacteria belong to Acidobacteria, Actinobacteria, Ascomycota, Bacteroidetes, Basidiomycota, Deinococcus-Thermus, and Firmicutes (Posada and Vega 2005; Brader et al. 2014).

Endophytic bacteria have been reported from woody tree species, such as oak and pear, to herbaceous crop plants such as sugar beet and maize. Endophytic microorganisms play multifunctional role in ecosystems and plant physiology, and these bacteria colonize intercellular and intracellular spaces of inner tissue. The endophytic habitat offers protection to those microorganisms which colonizes and establishes in intercellular spaces in plants including seeds (Miche and Balandreau 2001; Posada and Vega 2005).

The bacteria genera of *Bacillus* and *Pseudomonads* are identified as frequently occurring in agricultural crops (Souza et al. 2013). The occurrence of different endophytes depends mostly on plant host and bacteria genetic makeup, biotic–abiotic environmental factors. Meanwhile, a single host plant species comprises several genera, and species of endophytes, the tissue type of plant, or season of isolation may determine the extent of the endophytic population (Rosenblueth and Martinez-Romero 2006). Endophytic species mostly encountered are α, β, γ-proteobacteria subgroups which are closely related to epiphytic species (Kuklinsky-Sobral et al. 2004). The γ-proteobacteria group is the most diverse and dominant. It has been reported that most of Gram-negative endophytes act as biocontrol agents (Kobayashi and Palumbo 2000), while the dominant Gram-positive endophytic bacteria are *Bacillus* species (Bacon and Hinton 2007). Most of the culturable isolated endophytic bacteria species belong to *Proteobacteria*, while Firmicutes, Actinobacteria, and also Bacteroides are less common (Reinhold-Hurek and Hurek 2011). Recently, a number of workers have focused on identification of unculturable endophytes using novel metagenomic analysis approaches (Hawksworth 2004; Gaiero et al. 2013; Akinsanya et al. 2015). Direct amplification of microbial DNA from plant tissue samples and application of modern bioinformatics tools allow analysis of a bacterial community composition and its phylogenetic structure inside a variety of plant organs or tissues (Manter et al. 2010).

Most endophytic fungi isolated from plants are members of the Ascomycota, or their anamorphs, with only a few reports of basidiomycetous endophytes, often belong to orchid mycorrhizas (Rungjindamai et al. 2008). Basidiomycetous morphotypes have been obtained from the oil palm *Elaeis guineensis* which were further characterized by molecular analysis using rDNA sequences (Rungjindamai et al. 2008). For the first time ever, the microorganism species *Acremonium*

terricola, *Monodictys castaneae*, *Penicillium glandicola*, *Phoma tropica,* and *Tetraploa aristata* were isolated as endophytic fungi (Bezerra et al. 2012). As stated earlier, fungal endophytes have been categorized into two major groups based on phylogenetic traits as clavicipitaceous endophytes, which colonize grasses, and the nonclavicipitaceous endophytes, which colonize nonvascular plants, ferns and allies, conifers, and angiosperms (Rodriguez et al. 2009). Nonclavicipitaceous endophytes have three major groups based on colonization and transmission in host plant, *in planta* biodiversity, and plant growth traits deliberated to hosts, while the clavicipitaceous group has just one class.

7.3 Mechanism of Action of Endophytes

Microorganisms that reside inside the plant tissues without doing substantive harm or gaining benefits are considered as endophytes. The main action includes increase in the availability of nutrients, suppression of plant pathogens and insects, phytohormone production, phytoremediation and rhizoremediation, and by conferring stress resistance to host plants. Different endophytes have the ability to fix, solubilize, and mobilize the micro- and macro-elements for plant. Phosphate solubilization among endophytic bacteria isolated from soybean was reported by Kuklinsky-Sobral et al. (2004). Several nitrogen-fixing microbes associated with sugarcane can fix atmospheric nitrogen from 30 to 80 kg N/ha/year (Boddey et al. 1995). Different grasses growing in the nitrogen-deficient soil harbor several endophytic bacteria viz. *Pseudomonas, Stenotrophomonas,* and *Burkholderia* that can fix atmospheric nitrogen. Endophytic bacteria are known to induce plant growth and productivity by acting as a biocontrol agent (Shiomi et al. 2006). In the past, several natural methods of crop growing have been reviewed for moving toward sustainable development of agriculture and environment. It is an emerging biotechnological trend which aims to reduce chemical fertilizers in plant production, in the context of sustainable horticulture and agriculture (Bjornberg et al. 2015).

7.3.1 Availability of Nutrients

Endophytes assist in the uptake of essential nutrients by plants. They elicit different modes of action in tall fescue adaptation to phosphorus deficiency (Malinowski et al. 2000) and induce increased uptake of nitrogen (Arachevaleta et al. 1989). In the past, application of bacterial endophytes efficiently accomplishing nitrogen necessity of host plants such as cereal crops has increased plant yield in sustainable fashion (Varma et al. 1999). Certain endophytic rhizobial found to be associated with nonlegume plants as free-living bacteria (Rothballer et al. 2008). Endophytic bacteria are considered to be better in fixing nitrogen more efficiently comparable to

rhizospheric bacteria as they directly provided nitrogen in fixed form to their host plant due to lower oxygen pressure in the plant tissues in comparison with soil environment (Marella 2014).

The positive correlation between biological nitrogen fixation and accumulation of total nitrogen in plant has strong relationship with the endophytic association of diazotroph. Boddey (1995) reported that different varieties such as CB45-3, SP70-1143, and Krakatau of sugarcane accumulated about 60–80% nitrogen through biological nitrogen fixation. Muthukumarasamy et al. (2005) reported that *Gluconoacetobacter diazotrophicus* fix about 150 kg N $ha^{-1}yr^{-1}$ in sugarcane. Ladha and Reddy (2000) reported another nitrogen-fixing endophyte, *Azoarcus,* associated with the roots of kallar grass (*Leptochloa fusca*) which could enhance yield up to 20–40 t ha^{-1} yr^{-1} without the addition of supplementing any nitrogen fertilizer under saline sodic soil conditions.

Phosphate is the second most limiting compound for plant growth. It is generally found in insoluble form and not utilized by plants. Plant growth-promoting bacteria with phosphate solubilizing ability have been isolated generally belonging to *Azotobacter, Burkholderia Citrobacter, Enterobacter*, *Pantoea,* and *Pseudomonas* (Park et al. 2010). Few endophytic bacteria solubilize organic form of phosphate into inorganic phosphate by involving enzymes, namely phytase, C-P lyase, and nonspecific phosphatases. Involvement of various organic acids such as gluconate, ketogluconate, acetate, lactate, oxalate, tartrate, succinate, citrate, and glycolate is reported responsible for phosphate solubilization (Khan et al. 2009a; Sharma et al. 2013). Biochemical and biological phosphorous also influences phosphate solubilization by these endophytes (McGill and Cole 1981; Sharma et al. 2013). The various factors influencing phosphate solubilizing ability of endophytes are concentration of iron ore, temperature, and carbon and nitrogen sources. Ammonium salts have been found to be the best nitrogen source utilized by endophytes followed by asparagine, sodium nitrate, potassium nitrate, urea, and calcium nitrate (Ahuja et al. 2007).

Anuar et al. (2015) isolated *Hendersonia Amphinema* and *Phlebia* fungi from trunk and root tissues of oil palms and observed that *Phlebia* could serve as a biofertilizer promoting the oil palm seedlings eventually. These are used as empty fruit bunches (EFB) powder and real strong bioorganic fertilizer (RSBF) with *Phlebia* as formulation. It was observed that after eight months, the ratio of 30 g of EFB powder to 30 g of *Phlebia* (30:30 g) and the ratio of 10 g of RSBF to 50 g of *Phlebia* (10:50 g) were found to be the suitable ratios for the in vitro study and application in the field.

Endophytic fungi like *Acremonium terricola*, *Aspergillus japonicas*, *Cladosporiumcladosporioides*, *Cladosporium sphaerospermum*, *Fusarium lateritium*, *Penicillium glandicola, Pestalotiopsis guepinii,* and *Xylaria* sp. and many other unidentified species in *Opuntia ficus-indica* Mill. have indicated their potential for production of pectinases, cellulases, xylanases, and proteases (Bezerra et al. 2012). An endophyte, *Acremonium zeae*, isolated from maize produced the enzyme hemicellulose extracellularly which could be utilized for bioconversion of lignocellulosic biomass into fermentable sugars (Bischoff et al. 2009).

The simulations of plant growth executed by plant growth promoters could be attributed in terms of tolerance to biotic and abiotic stresses and improved plant nutrition (Machungo et al. 2009). It has been reported that *Festuca rubra* when inoculated with the fungal endophyte *Epichloe festucae* resulted in the increase in the uptake and concentration of phosphorus (Zabalgogeazcoa et al. 2006; Pineda et al. 2010). Similarly, the root endophyte *Heteroconium chaetospira* has been shown to significantly increase the biomass production of Chinese cabbage due to nitrogen transfer (Usuki and Narisawa 2007).

Srivastava et al. (2011) suggested that mycorrhiza such as arbuscular mycorrhizal fungi (AMF) is of potential significance in sustainable agricultural production. Though AMFs are considered as unique biofertilizers, they are difficult to mass multiply as they are biotrophs and difficult to get their propagation under axenic condition. An alternate bioagent, i.e., *Fusarium pallidoroseum*, has a significant positive role in plant growth and development. Inoculation of tomato seeds with *F. pallidoroseum* enhanced proline content; acid and alkaline phosphomonoesterase activity; and peroxidase activity. The fungus enhanced shoot dry weight and shoot length of wheat, maize, marigold, okra, moongbean, and brinjal over control (Srivastava et al. 2011).

Ngamau et al. (2014) suggested that endophytes increase plant growth in non-leguminous crops such as bananas and plantains through BNF, P solubilization, or siderophore production (iron chelation). Nigris et al. (2013) characterized the endophytes associated with *Vitis vinifera* L. cv. *Glera* (Prosecco) for their application in plant growth and health promotion along with nutritional improvement in soil and reviewed attentive researches carried out on small cyclic lipopeptides (LPs) belonging to fengycin, surfactin, and mycosubtilin families, with known antimicrobial potential.

Piriformospora indica, a new basidiomycetous endophyte, has gained substantial attention as a plant growth-promoting agent. The fungus colonizes the roots both inter- and intracellularly and forms coils or round bodies and branches in the cortex (Varma et al. 1998, 1999) without any colonization of the host stele. This endophyte has a broad host range including various agricultural crops as stated by Varma et al. (1999 and Singh et al. (2000). In a study, Barazani et al. (2005) confirmed the growth increase in *N. tobaccum* due to *P. indica* and showed that the growth promotion was related to better aptness, as enhanced seed production was observed in treated plants. Rai et al. (2001) also presented similar results of *Spilanthes calva* and *Withania somnifera,* whereas Waller et al. (2005) of *Hordeum vulgare*. *Piriformospora* has been shown to serve as a model to describe the mechanisms of host growth promotion. A lot of studies have shown *Piriformospora indicia* as phosphorus mobilizer (Singh et al. 2000). Furthermore, Sherameti et al. (2005) observed nitrogen accumulation in the shoots of *N. tobaccum* and *A. thaliana* ().

Nath et al. (2012) studied *Penicillium* species isolated from tea leaves as phosphate solubilizer. It was revealed from the study that there was remarkable phosphorous solubilizing activity by *Penicillium* up to eight days with an increase in acidity of the medium.

7.3.2 Suppression of Plant Pathogens and Insects

Plant diseases and pests are considered as major factor for restraining agricultural development. Conventionally, diseases and pests are managed by the application of pesticides which could cause environmental pollution as well as animal and human health-related problems. Endophytes synthesize compounds that are needed for defense against plant pathogens. Several natural products from endophytes including alkaloids, terpenoids, flavonoids, and steroids have been reported which are known to have various roles such as antibiotics, immunosuppressants, anti-cancer compounds, and biocontrol agents (Joseph and Priya 2011).

Biocontrol of plant diseases can be defined as the use of microbial antagonists to suppress diseases and typically involves an active human role. Biocontrol agents are ecofriendly, cheap, and improve the soil physicochemical properties to sustain natural soil flora. The biocontrol agent should be active under varied conditions of pH, temperature, and concentrations of different ions. Biocontrol agents have the potential to limit growth of pathogen as well as few nematodes and insects. Antagonistic substances, competition for iron, detoxification or degradation of virulence factors, or by indirectly inducing systemic resistance in plants against certain diseases are major constrains of biological control (Lugtenberg and Kamilova 2009; Maheshwari 2013).

The endophytic bacterial components eliciting induction of ISR are flagella, lipopolysaccharides, siderophores, antibiotics, and quorum-sensing molecules (van Loon 2007). Mechanisms of ISR in bacteria such as *Pseudomonas* spp. have been studied extensively and were reviewed by Jankiewicz and Kołtonowicz (2012). Development of induced systemic response (ISR) regulation of various genes contributes to strengthen the host involving plant cell wall strength, alteration of host physiology or metabolic responses, enhanced synthesis of plant defense, pathogenicity-related protein enzymes, etc. (Niu et al. 2011).

Endophytes may inhibit growth of fungal pathogens by the production of antibiotics, siderophore, and lytic enzymes. Lugtenberg and Kamilova (2009) reported that *Pseudomonas* could produce HCN, pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, and phenazines which could serve as antimicrobial substances. Some of the biocontrol agents have been shown to secrete siderophore, which chelates available iron of the soil, thereby depriving pathogenic microorganisms from iron (Compant et al. 2005).

Biocontrol organisms have the ability to control such harmful organisms in the agriculture and ultimately solve environmental and health-related issues by reducing or minimizing use of toxic chemicals in the agriculture (Azevedo et al. 2000). Various studies have proposed different possible mechanisms of action of endophytes. However, the knowledge of the mechanism behind endophytic plant pathogen suppression is still in the early age. The possible mechanism includes direct effect, indirect effect, and ecological effects (Castillo et al. 2002). In the direct effect, endophytes inhibit pathogens by antibiosis, secreting lytic enzymes. Application of several endophytic bacterial isolates in banana seedling at earlier age

can reduce the 60% incidences of banana bunchy top viruses as compared to control plants (Castillo et al. 2002).

The endophytes which provide indirect defense against herbivores may arise from mutualistic root endophyte associations and the evolution of entomopathogenic fungi into plant endophytes (Baverstock et al. 2005; Vega et al. 2008; Gómez-Vidal et al. 2009).

It has been reported that chemical defense was thought itself mechanisms of plant which later been understood as mechanisms of endophytes. The endophyte–plant mutualisms to spoor up defense against insects have been extensively studied in the perennial ryegrass and indole diterpenes, ergot alkaloids, and peramine (Rutschmann and Stadler 1978; Betina 1984; Rowan et al. 1986). Certain alkaloids were reported to induce defense signals counteract upon the toxic metabolites secreted by phytophagous insects (Zhang et al. 2009). Terpenoids and ketones provide protection from herbivores in higher plants (Akiyama and Hayashi 2001).

Fungal resistance to herbivores has experienced reasonable success in agricultural applications due to an environmentally sustainable alternative to pesticides (West and Gwinn 1993). Infected crops of soybean (Rabin and Pacovsky 1985), ribwort plantain (Gange and West 1994), cabbage, banana (Akello et al. 2008), coffee bean (Vega et al. 2008), and tomato (Jallow et al. 2004) reveal markedly lower rates of herbivore damage compared to uninfected plants.

An endophytic fungus *Beauveria bassiana* has been found to control the borer insects in coffee seedlings (Posada and Vega 2006) and sorghum (Tefera and Vidal 2009), respectively. The fungal pathogen *Botrytis cinerea* causes severe rotting on tomato fruits during storage. The endophytic bacteria *B. subtilis*, isolated from *Speranskia tuberculata* (Bge.) Baill, was found antagonistic to the pathogen *B. cinerea* in in vitro studies carried out by Wang et al. (2009). A new strain of *Burkholderia pyrrocinia* JK-SH007 and *B. cepacia* has been identified as potential biocontrol agent against poplar canker (Ren et al. 2011). Not only naturally occurring endophytes are used as biocontrol agents but also they are genetically engineered to express antipest proteins like lectins (Fahey 1988). Fungal endophyte of *Chaetomium globosum* YY-11 with antifungal activities, isolated from rape seedlings, and bacterial endophytes of *Enterobacter* sp. and *B. subtilis* isolated from rice seedlings have been shown to express *Pinellia ternate* agglutinin (*PtA*) gene (Zhao et al. 2010). These recombinant endophytes expressing *PtA* gene were found to control the population of sap-sucking pests in several crop seedlings. Similarly, recombinant endophytic bacteria *E. cloacae* expressing *PtA* gene proved to be a bioinsecticide against white-backed planthopper, *Sogatellafurcifera* (Zhang et al. 2011). Use of recombinant endophytes as biocontrol agents expressing different antipest proteins becomes a promising technique for control of plant pests because of their aggressive colonization within different crop plants.

Endophytes colonize the ecological niche similar to the pathogens which might favor endophytes to be used as biocontrol agents (Carroll 1986; Azevedo et al. 2000). Griffith and Hedger (1994) isolated endophytic fungi from *Theobroma cacao* and evaluated their ability to inhibit *Moniliophtora perniciosa*, which was reported to be a major pathogen of cacao plant. The results revealed that fungus

Gliocladium catenulatum was able to reduce disease incidence in cacao seedlings (Rubini et al. 2005). It was also revealed that *M. perniciosa* could also act as an endophyte (Lana et al. 2011). Bing and Lewis (1991, 1992) studied *Beauveria bassiana* from maize (*Zea mays*) which was found to be able to control the European corn borer (*Ostrinia nubilalis*).

Kloepper and Ryu (2006) studied the role of endophytes in systemic acquired resistance. It has been found that clavatol synthesized by *Aspergillus clavatonanicus* from *Torreya mairei*, lactones from *Phomopsis* sp. and *Xylaria* sp., Xularosides produced by *Xylaria* sp., jesterone from *Pestalotiopsis jesteri*, javanicin from *Chloridium* sp., and phomoenamide, phomonitroester, and deacetylphomoxanthone B from endophytic fungus *Phomopsis* sp. have been found to exhibit the strong antifungal activities both against pathogenic fungi (Jalgaonwala et al. 2011).

Qadri et al. (2013) revealed that the fungi from Western Himalayas belonged to Basidiomycota and ascomycetous fungi. *Cedrus deodara*, *Pinus roxburgii,* and *Abies pindrow* harbored the most diverse fungi. Several fungal extracts prepared from the fermented broth of these fungi demonstrated strong inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* and *Candida albicans*. It was also observed that endophytes inhibited phytopathogens by at least 50% in co-culture. Extracts from such fungi also possessed immunomodulatory activities as demonstrated by the in vitro lymphocyte proliferation assay.

7.3.3 Phytohormome Production

Endophytic microorganisms have been found to produce phytohormones such as auxins, cytokinins, ethylene, abscisic acid, and gibberellic acid. In a study by Xin et al. (2009), *Burkholderia vietnamiensis*, an endophytic diazotroph isolated from wild cottonwood (*Populus trichocarpa*), produced indole acetic acid (IAA), which promotes the growth of the plant. Hamayun et al. (2009a) isolated *Cladosporium sphaerospermum* from the roots of *Glycine max* (L) Merr., which showed the presence of bioactive GA_3, GA_4, and GA_7.

The endophytes isolated from medicinal plants have been found to exhibit induced plant growth and development. Waqas et al. (2012) studied the endophytic fungi *Phoma glomerata* and *Penicillium* sp. in growth promotion of shoot and allied vegetative growth and other attributes of GAs-deficient dwarf mutant Waito-C and Dongjin-byeo rice. Therefore, if cultured endophytes produce the same rare and important bioactive compounds as their host plants, this would diminish harvesting of slow-growing rare plants, and will also help to restore the world's biodiversity (Waqas et al. 2012).

Endophytic fungi have been found to exert their effect on plants at seed germination stages. Jerry (1994) revealed that during seed germination, the symbiotically associated endophytic fungi degrade cuticle cellulose and make available carbon for seedling which improves seed germination, vigor, and establishment.

Endophytes have the ability to produce plant growth regulators and thereby promote seed germination in crop plants (Bhagobaty and Joshi 2009).

Plant growth promotion is the major contribution of fungal symbiosis (Hassan et al. 2013). However, fungal endophytes enhance plant growth by the production of ammonia and plant hormones, particularly indole acetic acid (IAA) (Bal et al. 2013). IAA acts as plant growth promoter which enhances both cell elongation and cell division, and is essential for plant tissues differentiation (Taghavi et al. 2009). The ability of soil microorganisms to involve in the production of IAA in culture plates and in soil has been recorded (Spaepen and Vanderleyden 2011). The endophytic microorganisms isolated from various plants have showed high IAA production level compared to those isolated from root-free soil (Spaepen et al. 2007). The functional role of IAA in plant growth in addition to the capacity of fungal endophytes to produce IAA has gained great attention due to their impact on the quantity and distribution of IAA in plant tissues.

Gibberellins also play an important role in plant growth and development. Only a few fungi associated with plants have been reported as gibberellin producers (MacMillan 2002; Kawaide 2006; Vandenbussche et al. 2007) such as *Cladosporium sphaerospermum,* and *Penicillium citrinum* (Khan et al. 2008; Hamayun et al. 2009a). Gibberellin-producing fungi have potential to increase crop yields. Hamayun et al. (2010) investigated gibberellin production and growth-promoting potential of a novel fungal strain belonging to *Cladosporium* sp. isolated from the roots of cucumber.

You et al. (2012) demonstrated the plant growth-promoting activity of endophytic fungus *Penicillium* sp. isolated from the roots of halophytes using Waito-C rice seedlings. Khan et al. (2008) isolated *Penicillium citrinum* which showed the growth promotion activity on dune plants due to the presence of bioactive gibberellins in the filtrate of fungi (Khan et al. 2008). Hasan (2002) revealed the growth promotion activity of endophytic *Phoma herbarum* and *Chrysosporium pseudomerdarium* on Soybean and proved that some endophytes are host specific. Nadeem et al. (2010) studied the plant growth-promoting activity and stress resistance capability of endophytic *Penicillium* sp. and *Aspergillus* sp., which were shown to produce physiologically active gibberellins.

Many fungal endophytes such as *Neurospora crassa* (Rademacher 1994), *Sesamum indicum* (Choi et al. 2005), *Penicillium citrinum* (Khan et al. 2008), *Scolecobasidium tshawytschae* (Hamayun et al. 2009a), *Arthrinium phaeospermum* (Khan et al. 2009b), *Chrysosporium pseudomerdarium* (Hamayun et al. 2009b), *Cladosporium sphaerospermum* (Hamayun et al. 2009c), *Cladosporium* sp. (Hamayun et al. 2009c), *Gliomastix murorum* (Khan et al. 2009c), *Fusarium fujikuroi, Sphaceloma manihoticola* (Shweta et al. 2010), *Phaeosphaeria* sp. (Kawaide 2006), *Phaeosphaeria* sp., *Penicillium* sp. (Hamayun et al. 2010), *Aspergillus fumigatus* (Khan et al. 2011a), *Exophiala* sp. (Khan et al. 2011b), and *Penicillium funiculosum* (Khan et al. 2011c) have been reported as gibberellin producers. Hasan (2002) demonstrated gibberellin production by molds such as *Aspergillus flavus, A. niger, Penicillium corylophilum, P. cyclopium, P. funiculosum,* and *Rhizopus stolonifera.*

Plants inoculated with endophytes are often healthy (Bacon and White 2000; Khan et al. 2008), which may be attributed to the secretion of indole acetic acid (Kawaguchi and Sydn 1996) and gibberellins (Khan et al. 2008). Marina et al. (2011) showed that *Aspergillus ustus* synthesizes IAA-related indoles (auxins) and gibberellins in submerged conditions in Arabidopsis plants. Sirrenberg et al. (2007) reported the production of indole acetic acid in submerged culture of *Piriformospora indica* when colonized with *Arabidopsis thaliana*.

Ming and coworkers (2013) reported that an endophyte *Trichoderma atroviride* D16 from the root of *Salvia miltiorrhiza* promoted the growth of hairy roots of *S.* Mahmoud and Narisawa (2013) studied fungal endophyte, *Scolecobasidium humicola,* which is able to enhance growth and biomass of tomato plant.

Janarthine and Eganathan (2012) isolated endophytic bacterium *Sporosarcina aquimarina* from mangrove plant *Avicennia marina* which produced indole acetic acid and siderophore. The bacterium could also solubilize phosphorous and fix atmospheric nitrogen in the host plants.

Haddad et al. (2013) evaluated endophytic *Pseudomonas* spp. and *Bacillus* spp. for their ability to promote tomato plant growth. It was found that the endophytic bacteria positively affected seed germination and stimulated early seedling growth in vitro and in greenhouse. Tomato seedlings treated with the bacterial filtrates in vitro and plants from bacterized seeds exhibited an increase in all vegetative and reproductive plant growth parameters.

El-Tarabily and Sivasithamparam (2006) suggested that endophytic actinomycetes employ fungal antagonism due to siderophores and chitinolytic enzymes, especially chitinase and β-1,3-glucanase. It has also been revealed by several researchers that the siderophores produced by endophytes promote the growth and biocontrol phytopathogen (Cao et al. 2005; Tan et al. 2006; Rungin et al. 2012). El-Shatoury et al. (2009) reported actinobacteria from *Achillea fragrantissima* capable of producing chitinases and siderophores, which exhibited inhibitory activity against plant pathogenic fungi.

El-Tarabily (2003) and El-Tarabily and Sivasithamparam (2006) reported that chitinases produced by the endophytic *Actinoplanes missouriensis* cause hyphal lysis and loss in conidial germination of fungal phytopathogens. El-Tarabily et al. (2010) studied potential use of endophytic *Actinoplanes campanulatus*, *Micromonospora chalcea,* and *Streptomyces spiralis* for biocontrol of *Pythium aphanidermatum* to reduce seedling damping-off, root and crown rot of cucumber plants, and suggested that these strains could serve as biological control agents. Gangwar et al. (2014) revealed hydroxamate and catechol type of siderophore produced by actinobacteria isolated from *Aloe vera, Mentha arvensis,* and *Ocimum sanctum*.

Fouda et al. (2015) isolated *Penicillium chrysogenum*, *Alternaria alternata,* and sterile hyphae from *Asclepias sinaica*. It was observed that these endophytes had the ability to produce several extracellular enzymes including amylase, pectinase, cellulase, gelatinase, xylanase, and tyrosinase. In addition, these isolates were found to improve root growth.

Thus, rich and cost-effective significance of the endophytic actinobacteria is to be harnessed as agro-based biological agents. It is desirable to use agent to protect the crops and avoid the problems of cross-resistance.

7.3.4 Endophytes in Bioremediation

Many endophytic microorganisms possess genetic machinery for the degradation of toxic and recalcitrant molecules present in the rhizosphere region and other contaminates sites. Barac et al. (2004) reported the application of genetically modified *Burkholderia cepacia* for enhanced phytoremediation so as to promote plant resistance against toluene.

Few reports have shown that endophytes play a pivotal role in biodegradation of the litter of its host plants (Muller et al. 2001). During biodegradation, the endophytes colonize initially within the plants (Thormann et al. 2003) and facilitate the saprophytes to act on through antagonistic interaction, thus increasing the litter decomposition (Terekhova and Semenova 2005). It was demonstrated that endophytes could decompose organic components, including lignin, cellulose, and hemicellulose (He et al. 2012).

Nutrient cycling is a very important phenomenon to balance the existing nutrients and for making it available for every ecosystem component. Biodegradation of the dead flora and fauna became major step in it to bring back the utilized nutrients back to the ecosystem. Bioremediation is defined as elimination of pollutants from the environment using microorganisms. A group of researchers studied the role of endophytes in bioremediation in *Nicotiana tabaccum*. Mastretta et al. (2009) inoculated *Nicotiana tabaccum* with endophytes which resulted in improved biomass under cadmium stress due to beneficial effects of seed endophytes. Russell et al. (2011) screened several endophytic fungi and found them efficient to degrade polyurethane (PUR) in both solid and submerged conditions. It was also suggested that an enzyme serine hydrolase is mainly responsible for degradation of PUR.

Newman and Reynolds (2005) suggested that there are many benefits of using endophytes on improving xenobiotic remediation as these microbes are easier to manipulate than plants where genetic engineering of a xenobiotic degradation pathway is required. They also suggested that quantitative gene expression of pollutant catabolic genes within the endophytic populations could be a useful monitoring tool for assessing the efficiency of the remediation process.

7.3.5 Endophytes in Stress Tolerance

Drought tolerance is the adaptation that can provide plants to withstand huge water deficits. Three major mechanisms of drought tolerance have been categorized by various researchers viz accumulation and translocation of assimilates, maintenance

of cell wall elasticity, and osmotic adjustment. These mechanisms are generally affected by the endophytic microorganisms. In one study carried out by Richardson et al. (1992), endophyte-infected plant produced more soluble sugars such as glucose and fructose in their leaf blades, which indicated evidence of first mechanism. Endophytes may direct the plant metabolism for the secretion of soluble sugars, amino acids such as proline, polyols, and alkaloids that confer wall elasticity, and osmotic adjustment during drought condition. Several endophytes have the capacity to secrete enzyme ACC deaminase that reduces level of ethylene, which is more during drought (Richardson et al. 1992).

Fernandez et al. (2013) reported that *Burkholderia phytofirmans* strain PsJN enhanced chilling tolerance of grapevine plants and found higher concentrations of carbohydrates before chilling exposure under bacterial treatment. However, upon chilling, several defense-related genes as well as priming of the key cold regulator *VvCBF*4 gene was expressed i bacterized plants. Similar positive effect of the bacterium on metabolic balance and reduced effect of drought stress were demonstrated in wheat grown under reduced irrigation conditions (Naveed et al. 2014). Endophytic bacteria *Pseudomonas pseudoalcaligenes* was shown by Jha et al. (2011) to induce accumulation of higher concentrations of glycine betain-like compounds leading to improved salinity tolerance in rice. Cohen et al. (2009) demonstrated that water stress tolerance in maize plants was alleviated by accumulation of the abscisic acid (ABA) produced by endophytic *Azospirillum* spp., and the effects were further enhanced by IAA and gibberellins.

Panka et al. (2013) reported *Neotyphodium* and *Epichloë* endophytes as grass symbionts which improved the plant's growth and its ability to resist biotic and abiotic stresses and found that volatile organic compounds have shown to be important in plant's response to stress factors.

Many endophytes are known to have wide range of activity within hosts. In one study, endophytic microbes were found to have herbicidal activity along with antimicrobial activity (Li et al. 2012). *Bacillus* sp. SLS18, known as a PGP endophyte, owes its potential due to the presence of IAA, siderophore, and ACC deaminase activity. Luo et al. (2012) studied the role of SLS18 strain in the biomass production and manganese and cadmium uptake by *Sorghum bicolorowes* L., *Phytolacca acinosa* Roxb., and *Solanum nigrum* L. and displayed multiple heavy metals and antibiotics resistances.

7.4 Future Prospectives

The interaction between fungi/bacteria and plant in terms of saprophytic or symbiotic relationships could be detrimental or beneficial. Most of these microbes remain in the rhizospheric soil or rhizoplane, but a small subpopulation of them, designated as "endophytes," is able to penetrate and live within plant tissues. Some endophytes affect plant growth and plant responses to pathogens, herbivores, and environmental changes or produce important secondary metabolites. Most

endophytes are unculturable; therefore, the analysis of their diversity and the molecular basis of their interactions with the plant are revealed by using molecular approaches. The study of endophytes is a broad field of investigation and is entirely open to new findings and discoveries. Endophytic microbes are able to fix atmospheric nitrogen, solubilize, and mineralize nutrient such as phosphate, zinc, potassium including trace elements, production of phytohormones, ammonia, volatile hydrogen cyanide, and nonvolatile siderophores acted in antagonistic activity. Thus, a novel means of relations and interactions between endophytes and

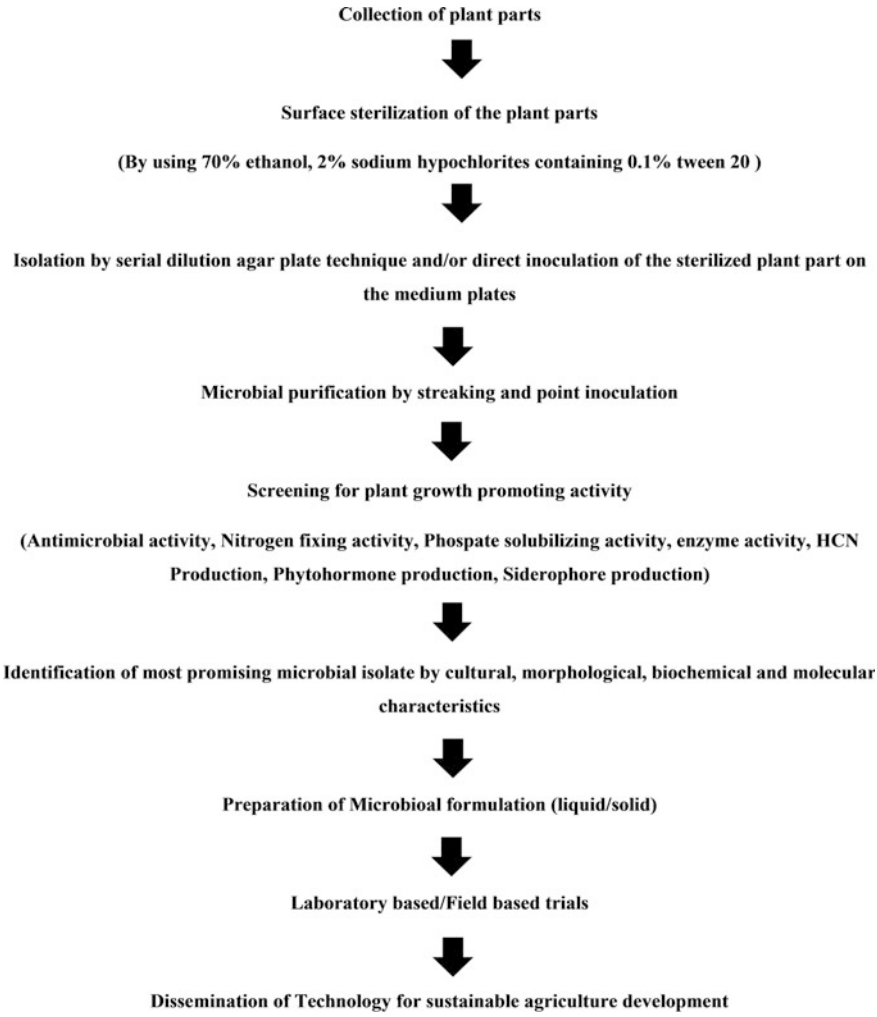

Fig. 7.1 Protocol for isolation, purification, identification, characterization of most promising microbial isolate/s having plant growth-promoting activities for the purpose of sustainable agriculture development

their hosts could be studied to boost agricultural production. Innovative biotechnological tools could become farmer's aid to provide strength for agricultural economy.

In void of appropriate methodologies, constrained advances and poor understanding limit the benefits that could be harnessed from plant–microbe interactions. With the use of genomics, the biotechnological potential of efficient plant–microbe partnerships could be achieved.

The major challenge lies in the method of selection of plant genotype and age and compatible associative endophyte. Understanding of these gaps can help us to enhance productivity by using specific strain using as bio-inoculant. In addition, the endophytic colonization mechanism is still preliminary. In-depth analysis of molecular studies could enhance colonization process and increase plant growth properties.

Endophytic community structure is influenced by plant genotype, abiotic and biotic factors such as environment conditions, microbe–microbe interactions, and plant–microbe interactions. Agricultural practices, such as soil tillage, irrigation, use of pesticides, and fertilizers, have a major effect on function and structure of endophytic microbial populations. Therefore, the use of agricultural practices that maintain natural diversity of plant endophytic bacteria is becoming an important element of sustainable agriculture that could ensure plant productivity and quality of agricultural production.

7.5 Conclusions

Agriculture in the twenty-first century is facing huge task of satisfying the food demand for all, and thus, concerning over alternatives of conventional agriculture provides multiple benefits to agriculture system. Advanced knowledge of plant–microbe symbioses can provide several ways to spoor up of the sustainable agriculture ensuring enough food for every needy. Microbial applications in plant rhizosphere as inoculant ensure improved crop performance under cold, draft, or contaminated soil stress conditions or enhanced disease resistance. The association known as endophytism represents a new horizon of research broading its boundaries on the account of benefits from mutualistic interactions between host crops and nonpathogenic microorganisms. The diverse endophytic microbial communities play integral and unique role in the development of sustainable agriculture. (Fig. 7.1)

Acknowledgements The authors are grateful to the Hon'ble Vice-Chancellor, Kurukshetra University, Kurukshetra, for providing infrastructural facilities to carry out research on endophytes and Science and Engineering Research Board, Department of Science and Technology, New Delhi, for providing necessary funds. The authors are also grateful to Director, University Institute of Engineering and Technology (UIET), Kurukshetra University, Kurukshetra, Haryana (India), and the Management of Ambala College of Engineering and Applied Research (ACE), Devsthali, Ambala, Haryana (India), for their valuable support.

References

Ahuja A, Ghosh SB, D'Souza SF (2007) Isolation of a starch utilizing, phosphate solubilizing fungus on buffered medium and its characterization. Bioresour Technol 98:3408–3411

Akello J, Dubois T, Coyne D, Kyamanywa S (2008) Endophytic *Beauveria bassiana* in banana (*Musa* spp.) reduces banana weevil (*Cosmopolites sordidus*) fitness and damage. Crop Protec 27(11):1437–1441

Akinsanya MA, Goh JK, Lim SP, Ting ASY (2015) Metagenomics study of endophytic bacteria in *Aloe vera* using next-generation technology. Genom Data 6:159–163

Akiyama K, Hayashi H (2001) Arbuscular mycorrhizal fungus-promoted accumulation of two new triterpenoids in cucumber roots. Biosci Biotech Biochem 66:762–769

Anuar EN, Nulit R, Idris AS (2015) Growth promoting effects of endophytic fungus *Phlebia GanoEF3* on oil palm (*Elaeis guineensis*) seedlings. Int J Agric Biol 17:135–141

Arachevaleta M, Bacon CW, Hoveland CS, Radcliffe DE (1989) Effect of tall fescue endophyte on plant response to environmental stress. Agron J 81:83–90

Azevedo JL, Maccheroni WJr, Pereira JO, Araujo WL (2000) Endophytic microorganisms: a review oninsect control and recent advances on tropical plants. Elect J Biotechnol 3:40–65

Azevedo JL, Maccheroni JW, Araujo WL (2002) Microorganisms endophytic and its role in tropical plants. In: Biotechnology: Adv Agric Agribus 233–268

Bacon CW, Hinton DM (2007) Bacterial endophytes: the endophytic niche, its occupants, and its utility. In: Gnanamanickam SS (ed.) Plant associated bacteria. Springer Science and Business Media, pp 155–194

Bacon CW, White JF (2000) Microbial endophytes. Marcel Deker Inc., New York, pp 99–101

Bal HB, Subhasis D, Tushar KD, Tapan KA (2013) ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. Bas Microbiol 53(12):972–984

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Barazani O, Benderoth M, Groten K, Kuhlemeier C, Baldwin IT (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia 146:234–243

Barka AE, Gognies S, Nowak J, Audran J-C, Belarbi A (2002) Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biol Contr 24:135–142

Baverstock J, Elliot SL, Alderson PG, Pell JK (2005) Response of the entomopathogenic fungus Pandora neoaphidis to aphid-induced plant volatiles. J Invert Pathol 89(2):157–164

Betina V (1984) Indole derived tremorgenic toxins. In: Betina V (ed). Mycotoxins production, isolation, separation and purification. Developments in food science, vol 8. Elsevier, New York, p 415

Bezerra JDP, Santos MGS, Svedese VM (2012) Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill. (Cactaceae) and preliminary screening for enzyme production. World J Microbiol Biotechnol 28(5):1989–1995

Bhagobaty RK, Joshi SR (2009) Promotion of seed germination of Green gram and Chick pea by *Penicillium verruculosum* RS7PF, a root endophytic fungus of *Potentilla fulgens* L. Adv Biotechnol 16–18

Bing LA, Lewis LC (1991) Suppression of *Ostrinia nubilalis*(Hubner) (Lepidoptera: Pyralidae) by endophytic*Beauveria bassiana* (Balsamo) Vuillemin. Environ Entomol 20:1207–1211

Bing LA, Lewis LC (1992) Temporal relantionshipsbetween *Zea mays, Ostrinia nubilalis* (Hubner) (Lep: Pyralidae) and endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Entomophaga 37:525–536

Bischoff JF, White JF Jr (2005) Evolutionary development of the *Clavicipitaceae*. In: Dighton J, White JF, Oudemans P (eds) The fungal community: its organization and role in the ecosystem. CRC Press, Boca Raton

Bischoff KM, WicklowDT Jordan D et al (2009) Extracellular hemi cellulolytic enzymes from the maize endophyte *Acremoniumzeae*. Curr Microbiol 58(5):499–503

Bjornberg KE, Jonas E, Marstorp H, Tidaker P (2015) The role of biotechnology in sustainable agriculture: views and perceptions among key factors n the Sweedish food supply chain. Sustainability 7(6):7512–7529

Boddey RM (1995) Biological nitrogen fixation in sugarcane: a key to energetically viable biofuel production. Crit Rev Plant Sci 14:209–266

Boddey RM, de Oliveira OC, Urquiaga S, Reis VM, Olivares FL, Baldani VLD, Döbereiner J (1995) Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. Plant Soil 174:195–209

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotech 27:30–37

Burd GI, Dixon DG, Glick BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Cao L, Qiu Z, You J, Tan H, Zhou S (2005) Isolation and characterization of endophytic *Streptomyces*antagonists of *Fusarium* wilt pathogen from surface sterilized banana roots. FEMS Microbiol Lett 247:147–152. doi:10.1016/j.femsle.2005.05.006

Carroll G (1986) Fungal associates of woody plants asinsect antagonists in leaves and stems. In: Inarbosa P, Krischik VA, Jones CG (eds) Microbial mediation of plant herbivore interactions. Wiley, New York, pp 253–271

Carroll GC (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69:2–9

Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robison R, Condron MA, Teplow DB, Stevens D, Yaver D (2002) Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. Microbiology 148(9):P2675–P2685

Choi WY, Rim SO, Lee JH, Lee JM, Lee IJ, Cho KJ (2005) Isolation of gibberellins-producing fungi from the root of several *Sesamum indicum* plants. J Microbiol Biotechnol 15:22–28

Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87(5):455–462

Compant S, Duffy B, Nowak J, Clément C, Ait Barka E (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microb 71:4951–4959

Dobereiner J, Boddey RM (1981) Nitrogen fixation in association with graminae. Current perspectives in nitrogen fixation. Aust Acad Sci 305–312

El-Shatoury S, El-Kraly O, El-Kazzaz W, Dewedar A (2009) Antimicrobial activities of Actinomycetes inhabiting *Achillea fragrantissima* (Family: *Compositae*) Egypt J Nat Toxins 6(2):1–15

El-Tarabily KA (2003) An endophytic chitinase-producing isolate of *Actinoplanes missouriensis*, with potential for biological control of root rot of lupine caused by *Plectosporium tabacinum*. Aust J Bot 51:257–266. doi:10.1071/BT02107

El-Tarabily KA, Sivasithamparam K (2006) Nonstreptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Soil Biol Biochem 38:1505–1520. doi:10.1016/j.soilbio.2005.12.017

El-Tarabily KA, Hardy GE, St J, Sivasithamparam K (2010) Performance of three endophytic actinomycetes in relation to plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber under commercial field production conditions in the United Arab Emirates. Eur J Plant Pathol 128:527–539. doi:10.1007/s10658-010-9689-7

Fahey W (1988) Endophytic bacteria for the delivery of agrochemicals to plants. In: Cutler HG (ed) Biologically active natural products. American Chemical Society, Washington, DC, pp 120–128

Fernandez O, Theocharis A, Bordiec S, Feil R, Dhont-Cordelier S, Bailleuil F, Clement C, Fontaine F, Ait Barka E (2013) Interaction between *Burkholderia phytofirmans* and grapevine: link between modulation of carbohydrate metabolism and induced tolerance to low temperatures. In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art. Deutsche Phytomedizinische Gesellschaft, Braunschweig

Foley JA, Defries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK (2005) Global consequences of land use. Science 309(5734):570–574

Fouda AH, El-Din Hassan S, Eid AM, El-Din Ewais E (2015) Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss). Ann Agric Sci 60 (1):95–104

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750

Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. New Phytol 128:79–87

Gangwar M, Dogra S, Gupta UP, Kharwar RN (2014) Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. Afr J Microbiol Res 8(2):184–191. doi:10.5897/AJMR2012.2452

Giménez C, Cabrera R, Reina M, Coloma-González A (2007) Fungal endophytes and their role in plant protection. Curr Org Chem 11:707–720

Gómez-Vidal S, Salinas J, Tena M, Lopez-Llorca LV (2009) Proteomic analysis of date palm (*Phoenix dactylifera* L.) responses to endophytic colonization by entomopathogenic fungi. Electrophoresis 30(17):2996–3005

Gough C, Galera C, Vasse J, Webster G, Cocking EC, Denarie J (1997) Specific flavonoids promote intercellular root colonization of *Arabidopsis thaliana* by *Azorhizobium caulinodans* ORS571. Mol Plant Microbe Interact 10:560–570

Griffith GW, Hedger JN (1994) The breeding biology of biotypes of the witches' broom pathogen of cacao, *Crinipellis perniciosa*. Heredity 72:278–289

Haddad N, Krimi Z, Raio A (2013) Endophytic bacteria from weeds promotes growth of tomato plants in vitro and in greenhouse. In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art. Deutsche Phytomedizinische Gesellschaft, Braunschweig

Halmann J, Quadt-Hallmann A, Mahaffee WF (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hamayun M, Khan SA, Ahmad N, Khan AL, Rehman G, Sohn EY, Kim SK, Joo GJ, Lee I-J (2009a) *Phoma herbarum* as a new gibberellin producing and plant growth-promoting fungus. J Microbiol Biotechnol 19:1244–1249

Hamayun M, Khan SA, Ahmad N, Tang DS, Kang SM, Sohn E-Y, Hwang YH, Shin DH, Lee BH, Kim JG, Lee I-J (2009b) *Cladosporium sphaerospermum* as a new plant growth promoting endophyte from the roots of *Glycine max* (L.) Merr. World J Microbiol Biotechnol 25:627–632

Hamayun M, Khan SA, Khan MA, Khan AL, Kang SM, Kim SK, Joo GJ, Lee IJ (2009c) Gibberellin production by pure cultures of a new strain of *Aspergillus fumigatus*. World J Microbiol Biotechnol 25:1785–1792

Hamayun M, Khan SA, Khan AL, Rehman G, Kim Y-H, Iqbal I, Hussain J, Sohn E-Y, Lee I-J (2010) Gibberllin production and plant growth promotion from pure cultures of *Cladosporium* sp.MH-6 isolated from cucumber (*Cucumis sativus* L.). Mycologia 102(5):989–995

Hart MM, Trevors JT (2005) Microbe management: application of mycorrhyzal fungi in sustainable agriculture. Front Ecol Environ 3:533–539

Hasan HAH (2002) Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity-calcium interaction. Rostlinná Výroba 48:101–106

Hassan SED, Liu A, Bittman S, Forge TA, Hunt DE, Hijri M, St-Arnaud M (2013) Impact of 12-year field treatments withorganic and inorganic fertilizers on crop productivity and mycorrhizalcommunity structure. Biol Fertil Soil 49:1109–1121

Hawksworth DL (2004) Fungal diversity and its implications for genetic resource collections. Stud Mycol 50:9–18

He X, Han G, Lin Y et al (2012) Diversity and decomposition potential of endophytes in leaves of a *Cinnamomum camphora* plantation in China. Ecol Res 27(2):273–284

Jalgaonwala RE, Mohite BV, Mahajan RT (2011) A review: natural products from plant associated endophytic fungi. J Microbiol Biotech Res 1(2):21–32

Jallow MFA, Dugassa-Gobena D, Vidal S (2004) Indirect interaction between and unspecialized endophytic fungus and a polyphagous moth. Basic Appl Ecol 5(2):183–191

Janarthine Rylo Sona S, Eganathan P (2012) Plant growth promoting endophytic *Sporosarcina aquimarina* SjAM 16103 isolated from the pneumatophores of *Avicennia marina L.* Int J Microbiol 12: doi:10.1155/2012/532060

Jankiewicz U, Kołtonowicz M (2012) The involvement of *Pseudomonas* bacteria in induced systemic resistance in plants. Prikl Biokhim Mikrobiol 48:276–281

Jerry B (1994) A role of endophytic fungi in regulating nutrients and energy in plants within a desert ecosystem. International symposium and workshop on desertification in developed countries. Accessed on 2011/10/25

Jha Y, Subramanian RB, Patel S (2011) Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Joseph B, Mini Priya R (2011) Bioactive compounds fromendophytes and their potential in pharmaceutical effect: a review. Am J Biochem Mol Biol 1(3):291–309

Kawaguchi M, Sydn K (1996) The excessive production of indole-3-acetic acid and its significance in studies of the biosynthesis of this regulator of plant growth and development. Plant Cell Physiol 37:1043–1048

Kawaide H (2006) Biochemical and molecular analysis of gibberellins biosynthesis in fungi. Biosci Biotech Biochem 70:583–590

Khan SA, Hamayun M, Yoon HJ, Kim H-Y, Suh SJ, Hwang SK, Kim JM, Lee I-J, Choo YS, Yoon UH, Kong WS, Lee BM, Kim JG (2008) Plant growth promotion and *Penicillium citrinum*. BMC Microbiol 8:231

Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M (2009a) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J Agric Biol Sci 1:48–58

Khan SA, Hamayun M, Kim HY, Yoon HJ, Lee IJ, Kim JG (2009b) Gibberellin production and plant growth promotion by a newly isolated strain of *Gliomastix murorum*. World J Microbiol Biotechnol 25:829–833

Khan SA, Hamayun M, Kim HY, Yoon HJ, Seo JC, Choo YS, Lee I-J, Kim SD, Rhee IK, Kim JG (2009c) A new strain of *Arthrinium phaeospermum* isolated from *Carex kobomugi* Ohwi is capable of gibberellin production. Biotechnol Lett 31:283–287

Khan AL, Hamayun M, Ahmad N, Waqas M, Kang SM, Kim YH, Lee IJ (2011a) *Exophiala* sp. LHL08 reprograms *Cucumis sativus* to higher growth under abiotic stresses. Physiol Plant 143(4):329–343

Khan AL, Hamayun M, Kim YH, Kang SM, Lee IJ (2011b) Ameliorative symbiosis of endophyte (*Penicillium funiculosum* sp. LHL06) under salt stress elevated plant growth of *Glycine max* L. Plant Physiol Biochem 49(8):852–862

Khan AL, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ (2011c) Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. Process Biochem 46:440–447

Kloepper JW, Ryu CM (2006) Bacterial endophytes as elicitors of induced systemic resistance. Soil Biol 9:33–52

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. Microbial Endophytes, pp 199–233

Kuklinsky-Sobral K, Araujo WL, Mendonca C, Geran LC, Piskala A, Azevedo JL (2004) Isolationand characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Ladha JK, Reddy PM (2000) Steps towards nitrogen fixation in Rice. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. International Rice Research Institute, Manila, Philippines, pp 33–46

Lana TG, Azevedo JL, Pomella AWV, Monteiro RTR, Silva CB, Araujo WL (2011) Endophytic and pathogenic isolates of the cacao fungal pathogen *Moniliophthora perniciosa* (Tricholomataceae) are indistinguishable based on genetic and physiological analysis. Genet Mol Res 10:326–334

Li J, Zhao G-Z, Huang H-Y et al (2012) Isolation and characterization of culturable endophytic actinobacteria associated with *Artemisia annua* L. Antony van Leeuwenhoek 101(3):515–527

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting Rhizobacteria. Ann Rev Microbiol 63:541–556

Luo S, Xu T, Chen L et al (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. Appl Microbiol Biotechnol 93(4):1745–1753

Machungo C, Losenge T, Kahangi E, Coyne D, Dubois T, Kimenju J (2009) Effect of endophytic *Fusarium oxysporum* on growth of tissue-cultured Banana plants. Afr J Hort Sci 2:160–167

MacMillan J (2002) Occurrence of gibberellins in vascular plants, fungi and bacteria. J Plant Growth Reg 20:387–442

Maheshwari DK (2013) Bacteria in Agrobiology: disease management. Springer Science & Business Media, Heidelberg, Germany, p 495

Maheshwari DK, Dheeman S, Agarwal M (2015) Phytohormone producing PGPR for sustainable agriculture. In: Maheshwari DK (ed) Bacterial metabolite in sustainable agroecosystem. Springer International Publishing, pp 159–182

Mahmoud RS, Narisawa K (2013) A new fungal endophyte, *Scolecobasidium humicola*, promotes tomato growth under organic nitrogen conditions. PLoS One 8(11):e78746. doi:10.1371/journal.pone.0078746

Malinowski DP, Belesky DP (1999) *Neotyphodium coenophialum*- endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. J Plant Nutr 22:835–853

Malinowski DP, Alloush GA, Belesky DP (2000) Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. Plant Soil 227(1–2):115–126

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar specific bacterial endophyte community in potato roots. Microb Ecol 60(1):157–166

Marella S (2014) Bacterial endophytes in sustainable crop production: applications, recent developments and challenges ahead. Int J Life Sci Res 2(2):46–56

Mariano RLR, Silveira EB, Assis SMP (2004) Importancia de bacterias promotoras de crescimento e de biocontrole de doencas de plantas para uma agricultura sustentavel. An Acad Pernamb Cien Agron Recife 1:89–111

Marina S, Angel M, Silva-Flores MA, Cervantes-Badillo MG, Rosales- Saavedra MT, Islas-Osuna MA, Casas-Flores S (2011) The plant growth-promoting fungus *Aspergillus ustus* promotes growth and induces resistance against different lifestyle pathogens in *Arabidopsis thaliana*. J Microbiol Biotechnol 21(7):686–696

Mastretta C, TaghaviS, van der Lelie D et al (2009) Endophytic bacteria from seeds of *Nicotiana tabacum* can reduce cadmium phytotoxicity. Int J Phytoremed 11(3):251–267

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26:267–268

Miche L, Balandreau J (2001) Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. Appl Environ Microbiol 67:3046–3052

Ming Q, Su C, Zheng C, Jia M, Zhang Q, Zhang H, Rahman K, Han T, Qin L (2013) Elicitors fromthe endophytic fungus *Trichoderma atroviride* promote *Salvia miltiorrhiza* hairy root growth and tanshinone biosynthesis. J Exp Bot (E-pub ahead of print)

Muller MM, Valjakka R, Suokko A, Hantula J (2001) Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Mol Ecol 10(7):1801–1810

Muńoz A, Gandía M, Harries E, Carmona L, Read ND, Marcos JF (2013) Understanding the mechanism of action of cell penetrating antifungal peptides using the rationally designed hexapeptide PAF26 as a model. Fungal Biol Rev 26:146–155

Muthukumarasamy R, Cleenwerck I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Park K, Son CY, Sa T, Caballero-Mellado J (2005) Natural association of *Gluconoacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. Syst Appl Microbiol 28:277–286

Nadeem A, Hamayun M, Khan SA, Khan AL, Lee IJ, Shin DH (2010) Gibberellin-producing endophytic fungi isolated from *Monochoria vaginalis*. J Microbiol Biotechnol 20(12):1744–1749

Nath R, Sharma GD, Barooah M (2012) Efficiency of tricalcium phosphate solubilization by two different endophytic *Penicillium* sp. isolated from tea (*Camelia sinensis* L.). Er. J Exp Biol 2 (4):1354–1358

Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat throughinoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73:121–131

Newman L, Reynolds C (2005) Bacteria and phyto-remediation: new uses for endophytic bacteria in plants. Trend Biotechnol 23:6–8

Ngamau CN, Matiru VN, Tani A, Muthuri CW (2014) Potential use of endophytic bacteria as biofertilizer for sustainable banana (*Musa* spp.) production. Afr J Hort Sci 8:1–11

Nigris S, Baldan E, Zottini M, Squartini A, Baldan B (2013) Is the bacterial endophyte community, living in Glera (*Vitis vinifera*) plants, active in biocontrol? In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art. Deutsche Phytomedizinische Gesellschaft, Braunschweig

Niu D, Liu H, Jiang C, Wang Y, Jin H, Guo J (2011) The plant growth–promoting rhi*zobacterium Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana,* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. Mol Plant Microb 24:533–542

Okon Y, Labandera-Gonzalez C (1994) Agronomic application of *Azospirillum*- An evaluation of 20 years worldwide field incubation. Soil Biol Biochem 26:1591–1601

Panka D, Piesik D, Jeske M, Musial N, Koczwara K (2013) Emission of volatile organic compounds by perennial ryegrass (*Lolium perenne*, L.)/*Neptyphodium lolii* association as a defense reaction towards infection by *Fusarium poae* and *Rhizoctonia solani*. In: Schneider C, Leifert C, Feldmann F (eds) Endophytes for plant protection: the state of the art. Braunschweig, Deutsche Phytomedizinische Gesellschaft, pp 121–122

Park KH, Lee O, Jung H, Jeong J, Jeon Y, Hwang D, Lee C, Son H (2010) Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. Appl Microbiol Biotechnol 86:947–955

Pennisi E (2001) The push to pit genomics against fungal pathogens. Sci 292:2273–2274

Peterson RL, Wagg C, Pautler M (2008) Associations between microfungal endophytes and roots: do structural features indicate function? Botany 86:445–456

Pineda A, Zheng SJ, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soilborne microbes. Trend Plant Sci 15:507–514

Posada F, Vega FE (2005) Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobromacacao*). Mycologia 97:1195–1200

Posada F, Vega FE (2006) Inoculation and colonization of coffee seedlings (*Coffea arabica* L.) with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycosci 47 (5):284–289

Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ, Riaz-Ul-Hassan S (2013) Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. SpringerPlus 2:8

Rabin LB, Pacovsky RS (1985) Rduced larva growth of two *Lepidoptera* (Noctuidae) on excised leaves of soybean infected with amycorrhizal fungus. J Econ Entom 78:1358–1363
Rademacher W (1994) Gibberellin formation in microorganisms. Plant Growth Reg 15:303–314
Rai M, Acharya D, Singh A (2001) Positive growth responses of the medicinal plants*Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza 11:123–128
Redecker D, Vonbereswordtwallrabe P, Beck DP (1997) Influence of inoculation with arbuscular mycorrhizal fungi on stable isotopes of nitrogen in *Phaseolus vulgaris*. Biol Fertil Soil 24:344–346
Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14(4):435–443
Reis VM, Baldani JI, Baldani VLD, Döbereiner J (2000) Biological nitrogen fixation in gramineae and palm trees. Crit Rev Plant Sci 19:227–247
Ren JH, Ye JR, LiuH XuXL, Wu XQ (2011) Isolation and characterization of a new *Burkholderia pyrrocinia* strain JK-SH007 as a potential biocontrol agent. World J Microbiol Biotechnol 27 (9):2203–2215
Richardson MD, Chapman GW, Hoveland CS, Bacon CW (1992) Sugar alkohols in endophyte-infected tall fescue. Crop Sci 32:1060–1061
Rodriguez RJ, Henson J, Volkenburgh EV, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerancein plants via habitat-adapted symbiosis. ISME J 2:404–416
Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330
Rosenblueth M, Martinez-Romero E (2006) Bacterialendophytes and their interactions with hosts. Mol Plant Microbe Interac J 19(8):827–837
Rothballer M, Eckert B, Schmid M, Fekete A, Schloter M, Lehner A, Pollmann S, Hartmann A (2008) Endophytic root colonization of gramineous plants by *Herbaspirillum frisingense*. FEMS Microbiol Ecol 66:85–95
Rowan DD, Hunt MB, Gaynor DL (1986) Peramine, a novel insect feeding deterrent from ryegrass infected with the endophyte Acremonium loliae. J Chem Soc, Chem Commun 1986:935–936
Rubini MR, Silva-Ribeiro R, Pomella AWV, Maki C, Araújo WL, Santos DR, Azevedo JL (2005) Diversity of endophytic fungal community of cacao (*Theobromacacao*) L. and biological control of *Crinipellis perniciosa* causal agent of Witches' broom disease. Int J Biol Sci 1:24–33
Rungin S, Indanand C, Suttiviriya P, Kruasuwan W, Jaemsaeng R, Thamchaipenet A (2012) Plant growth enhancing effects by a siderophore producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) Antony van Leeuwenhoek 102:463–472 doi:10.1007/s10482-012-9778-z
Rungjindamai N, Pinruan U, Choeyklin R, Hattori T, Jones EBG (2008) Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oilpalm, *Elaeis guineensis*, in Thailand. Fungal Divers 33:139–161
Russel JR, Huang J, Anand P et al (2011) Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 77(17):6076–6084
Rutschmann J, Stadler PA (1978) Chemical background. In: Berde B, Schild HO (eds) Ergot alkaloids and related compounds. Springer, Berlin, Germany
Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. Ann Rev Ecol Syst 29:319–343
Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686
Schulz B, Römmert AK, Dammann U, Aust HJ, Strack D (1999) The endophyte-host interaction: a balanced antagonism? Mycol Res 103:1275–1283
Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol Ecol 39:23–32
Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes-sustainable approach for managing phosphorous deficiency in agricultural soils. SpringerPlus 2:507

Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmuller R (2005) The endophyticfungus *Piriformospora* indica stimulates the expression of nitrate reductase and the starchdegrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeo domain transcription factor that binds to a conserved motif in their promoters. J Bot Chem 280:26241–26247

Shiomi HF, Silva HSAS, de Melo IS, Nunes FV, Bettiol W (2006) Bioprospecting endophytic bacteria for biological control of coffee leaf rust. Sci Agric (Piracicaba, Braz.) 63(1):32–39

Shweta S, Zuehlke S, Ramesha BT, Priti V, Mohana Kumar P, Ravikant G, Spiteller M, Vasudeva R, Uma SR (2010) Endophytic fungal strains of *Fusarium solani,* from *Apodytes dimidiata E.Mey. ex Arn* (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. Phytochem 71(1):117–122

Sieber TN (2002) Fungal root endophytes. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker, New York, Basel

Singh A, Sharma J, Rexer KH, Varma A (2000) Plant productivity determinants beyond minerals, water and light: *Piriformospora indica*—a revolutionary plant growth promoting fungus. Curr Sci 79:1548–1554

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainableagriculture and environmental development. Agric Ecosyst Environ 140(3–4):339–353

Sirrenberg A, Göbel C, Grond S, Czempinski N, Ratzinger A, Karlovsky P, Santos P, Feussner I, Pawlowski K (2007) *Piriformospora indica* affects plant growth by auxin production. Physiol Plant 131(4):581–589

Souza SA, Xavier AA, Costa MR, Cardoso AM, Pereira MC, Nietsche S (2013) Endophytic bacterialdiversity in banana 'Prata Ana' (*Musa* spp.) roots. Genetic. Mol Biol 36(2):252–264

Spaepen S, Vanderleyden J (2011) Auxin and plant microbe interaction. Perspect Biol 3:a001438 First published online November 17, 2010. doi:http://dx.doi.org/10. 1101/cshperspect.a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Srivastava R, Mehta CM, Sharma AK (2011) Fusarium pallidoroseum- A new biofertilizer responsible for enhancing plant growth in different crops. IntRes J Microbiol 2(6):192–199

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502

Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplartrees. Appl Environ Microbiol 75:748–757

Tan HM, Cao LX, He ZF, Su GJ, Lin B, Zhou SN (2006) Isolation of endophytic actinomycetes from different cultivars of tomato and their activities against *Ralstonia solanacearum* in vitro. World J Microbiol Biotechnol 22:1275–1280. doi:10.1007/s11274-006-9172-y

Tanaka A, Tapper BA, Popay A, Parker EJ, Scott B (2005) A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. Mol Microbiol 57:1036–1050

Tefera T, Vidal (2009) Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. Biocontrol 54 (5):663–669

Terekhova VA, Semenova TA (2005) The structure of micromycete communities and their synecologic interactions with basidiomycetes during plant debris decomposition. Microbiol 74 (1):91–96

Thormann MN, Currah RS, Bayley SE (2003) Succession of microfungal assemblages in decomposing peatland plants. Plant Soil 250(2):323–333

Usuki F, Narisawa K (2007) A mutualistic symbiosis between a dark, septate endophytic fungus, *Heteroconium chaetospira* and a nonmycorrhizal plant, Chinese cabbage. Mycology 99:175–184

Van Bael SA, Maynard Z, Rojas E, Mejia LC, Kyllo DA, Herre EA, Robbins N, Bischoff JF, Arnold AE (2005) Emerging perspectives on the ecological roles of endophytic fungi in tropical plants. In: Dighton J, White JF, Oudemans P (eds) The fungal community: its organization and role inthe ecosystem. CRC Press, Boca Raton

van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Vandenbussche F, Fierro AC, Wiedemann G, Reski R, van der Straeten D (2007) Evolutionary conservation of plant gibberellin signaling pathway components. BMC Plant Biol 7:65

Varma A, Verma S, Sudha Nirmal S, Bütehorn B, Franken P (1998) Piriformospora indica, a cultivable plant-growth-promoting root endophyte. ApplEnviron Microbiol 65(6):2741–2744

Varma A, Verma S, Sudha Sahay N, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivableplant-growth-promoting root endophyte. Appl Environ Microbiol 65:2741–2744

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA (2008) Entomopathogenic fungal endophytes. Biol Cont 46:72–82

Verma S, Varma A, Rexer KH, Hassel A, Kost G, SarbhoyA Bisen P, Butehorn B, Franken P (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycology 90:896–903

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K,Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel KH (2005) The endophytic fungus *Piriformis indica* reprograms barley to salt-stress tolerance, disease resistance andhigher yield. Proc Natl Acad Sci USA102:13386–13391

Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology 95:1368–1373

Wang S, Hu T, Jiao Y, Wei J, Cao K (2009) Isolation and characterizationof *Bacillus subtilis* EB-28, an endophytic bacterium strain displaying biocontrol activity against *Botrytis cinerea* Pers. Front Agric China 3(3):247–252

Waqas M, Khan AL, Hamayun M, Kamran M, Kang SM, Kim YH, Lee IJ (2012) Assessment of endophytic fungi cultural filtrate on soybean seed germination. Afr J Biotechnol 11(85):15135–15143

West CP, Gwinn KD (1993) Role of *Acremonium* in drought, pest and disease tolerance of grasses. In: Hume DE, Latch GCM, Easton HS (eds) Proceedings II International Symposium *Acremonium*/grass interactions: plenary papers. AgResearch, Grasslanda Research Centre, Palmerston North, NZ

Xin G, Zhang G, Kang JW, Staley JT, Doty SL (2009) Adiazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol Fert Soil 45(6):669–674

You YH, Yoon H, Kang SM, Shin JH, Choo YS, Lee IJ, Lee JM, Kim JG (2012) Fungal diversity and plant growth promotion of endophyticfungi from six halophytes in Suncheon Bay. J Microbiol Biotechnol 22(11):1549–1556

Zabalgogeazcoa I, Ciudad AG, Vázquez de Aldana BR, Criado BG (2006) Effects of the infection by the fungal endophyte *Epichloë festucae* in the growth and nutrient content of Festuca rubra. Eur J Agron 24:374–384

Zhang DX, Nagabhyru P, Schardl CL (2009) Regulation of a chemical defense against herbivory produced by symbiotic fungi in grass plants. Plant Physiol 150(2):1072–1082

Zhang X, Li J, Qi G, Wen K, Lu J, Zhao X (2011) Insecticidal effect of recombinant endophytic bacteriumcontaining *Pinelliaternata* agglutinin against white backed planthopper, Sogatellafurcifera. Crop Protec 30(11):1478–1484

Zhang X, Lin L, Zhu Z, Yang X, Wang Y, An Q (2013) Colonization and modulation of host growth and metal uptake by endophytic bacteria of *Sedum alfredii*. Int J Phytoremediation 15 (1):51–64

Zhao X, Qi G, Zhang X, LanN, Ma X (2010) Controlling sapsuckinginsect pests with recombinant endophytes expressing plant lectin. Nature Proc vol 21, article 21

Zou WX, Tan RX (1999) Advances in plant science, vol 2. China Higher Education Press, Beijing, pp 183–190

Chapter 8
Endophytic Actinobacteria: Beneficial Partners for Sustainable Agriculture

Ricardo Araujo, Onuma Kaewkla and Christopher M.M. Franco

Abstract Endophytic actinobacteria have been proven to be effective partners that have beneficial functions with a number of crop plants. A large number of studies have been carried out, showing these positive effects in laboratories and glasshouses, but with fewer reports of their effectiveness in the field. This chapter highlights the results of field trials of actinobacterial endophytes conducted with cereals, vegetables such as tomato, cucumber, or cabbage, legumes such as chickpea or pea, fruits such as melon or grapes, peanuts, and woody plants.

Keywords Actinobacteria · Endophyte · Biocontrol · Crop plants

8.1 Introduction

Actinobacteria are recognized for their propensity to produce secondary metabolites with a wide range of chemical structures and biological activity (Berdy 2005). Therefore, their presence within healthy plants indicates that they have evolved symbiotic functions of value to their hosts. They can be isolated easily from all parts of a plant, though are most abundant in roots and represent an important component of the plant microbiome (Bulgarelli et al. 2012; Edwards et al. 2015).

Endophytic actinobacteria which live inside plant tissues may produce antibiotics, inducers of plant systemic resistance or plant growth promoting substances to support plant growth (Conn and Franco 2004), and therefore, are a good choice for

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_8

a beneficial microorganism screening program. Isolation of endophytic actinobacteria typically involves a surface sterilization protocol applying 70% ethanol and 1–3% sodium hypochlorite to kill epiphytes. The choice and a number of isolation media and plates, as well as incubation time, is a crucial factor to yield relatively large numbers of rare genera (Kaewkla and Franco 2013).

Such benefits of actinobacteria and other bacteria have been often shown in vitro and in greenhouse experiments but these results can be difficult to translate to the field. A large number of strains that act in vitro as biocontrol agents fail to be effective in field trials due to the difficulty to adapt to a more complex environment that the field represents. Although protected within the plant, endophytic microorganisms remain conditioned by biotic and abiotic factors and have a pronounced association with the rhizosphere environment. When added to the seed or seedlings, endophytic actinobacteria can colonize seedlings and young plants efficiently and offer an advantage in terms of promoting healthier and higher-yielding crops. In fact, actinobacteria act inside the plant by promoting growth and facilitating the nutrient acquisition, phytohormone production, induction of defense responses, removal of contaminants, and competition with plant pathogens (Schrey and Tarkka 2008).

8.2 Wheat and Barley

Wheat (*Triticum* spp.) and barley (*Hordeum vulgare*) are some of the most important cereal grains worldwide (Pourkheirandish and Komatsuda 2007; Charmet 2011). Wheat is the most important source of carbohydrates in many parts of the world. Barley, besides its importance as a foodstuff for the human diet, is relevant animal forage and represents the fermentable feedstock for beer and other beverages. Every year the world production of wheat and barley is over 700 million tons and 100 million tons, respectively, making it the third and fourth most-produced cereals after maize and rice FAOSTAT (2014). Both cereal crops have the ability to grow at a range of climatic zones from temperate regions to the tropics. Presently, the major breeding objectives in these cereals are similar to other important crops and target a high grain yield and quality, disease resistance, and tolerance to heat stress, in order to increase the amount of cereals available to feed an increasing population (Barabaschi et al. 2016).

Nevertheless, some diseases can result in huge losses in wheat and barley crops annually. Among the most relevant diseases in both cereals are eyespot, powdery mildew caused by *Blumeria graminis* (f. sp. *tritici* associated with wheat, while f. sp. *hordei* affects barley), *Septoriatritici* blotch in wheat (caused by the ascomycete fungus *Mycosphaerella graminicola*, whose asexual stage is *Septoria tritici*; a close relative of *M. graminicola* is *Septoria passerinii* responsible for the speckled leaf blotch in barley), yellow or stripe rust associated to *Puccinia striiformis* (f. sp. *tritici* affects wheat, while f. sp. *hordei* infects barley), leaf rust (caused by *Puccinia triticina* in wheat and *Puccinia hordei* in barley), tan spot (caused by the fungus *Pyrenophora tritici-repentis* whose asexual stage is *Drechslera tritici-repentis*),

stem rust (caused by the fungus *Puccinia graminis*), crown rust caused by *Puccinia coronata*, *Fusarium* head blight associated with the plant pathogen *Fusarium graminearum* (the teleomorph is *Gibberellazeae*), and bacterial blight (caused *Xanthomonas campestris* pv. *translucens*) (McMullen et al. 1997; Hardwick et al. 2001; Turkington et al. 2002; Osborne and Stein 2007; Adhikari et al. 2012; Chen et al. 2014). In some areas, spot blotch (caused by *Cochliobolus sativus*) is also a relevant disease in both cereal crops, as well as *Stagono sporanodorum* blotch in wheat, and mild mosaic virus and leaf scald (caused by *Rhynchosporium secalis*), which are important diseases in barley (Duczek et al. 1985; Friesen et al. 2007; Zhan et al. 2008; Smith et al. 2014).

Root rots are another set of plant diseases (Take-all is one of the most relevant) particularly important in both wheat and barley that have been described in many countries resulting in huge losses (an average of 34% yield reduction on a range of cereals), particularly in Australia with losses over 26%, Brazil 15–38%, Canada 5–28%, France 15–75%, Italy, Morocco 4–6%, Turkey, and USA 40–50% (Orakçı et al. 2010). Several fungi are responsible for root rot disease on wheat and barley, including species of *Pythium, Rhizoctonia*, *Gaeumannomyces, Fusarium,* and *Bipolaris*. A variety of methods have been studied for use in control of root rots including crop rotation, tillage, stubble burning, and integrated control, however, some of these strategies may often not be economically feasible and/or result in soil erosion (Liu et al. 2011). It is a fact that biological control may complement previously described strategies and represent a more sustainable environmental alternative for reducing root rot and other plant diseases (Spadaro and Gullino 2005; Suprapta 2012).

Several studies have been conducted in order to determine if actinobacterial isolates could control root rot fungi in vitro and in field trials. Actinobacteria are quantitatively and qualitatively important in both, as plant endophytes and in the rhizosphere, where they may influence plant growth and protect the roots against invasion by pathogens. It was reported that rhizosphere-associated soils yielded almost twice as many actinobacteria as non-rhizosphere-associated soils (Intra et al. 2011). As a seed develops and the plant grows in the soil, the bacterial population tends to increase early, while actinobacteria and then fungi dominate at mid and later stages of growth (Chauhan et al. 2012). It is known that soil and plant microbiota can be altered by distinct agronomic practices coupled with crop rotations and result in an increase in the productivity of wheat (Yang et al. 2012). Furthermore, root endophytic bacteria in wheat, as well as final productivity, are sensitive to the climatic conditions and soil moisture. Microbial populations present in the soil are also relevant to completely understand the interaction between plant and rhizosphere, and it is well known that microbial communities differ according to the geographic location (Araujo 2010).

Streptomyces, Microbispora, Micromonospora, and *Nocardioides* represent the most abundant genera isolated from wheat plant samples (Coombs and Franco 2003). A number of these isolates can represent valid biocontrol agents as they were capable of suppressing wheat and barley fungal and oomycete pathogens, such as

Rhizoctonia solani, *Pythium* sp., and *Gaeumannomyces graminis*. In addition, it was proved that artificial addition of some actinobacteria to wheat seeds did not interfere with the indigenous endophytic populations, while the addition of mixed non-adapted microbes to the soil acted by reducing the endophytic diversity and level of colonization (Coombs and Franco 2003; Conn and Franco 2004). In fact, field trials may confirm the positive effect of the addition of actinobacterial inoculants to crops.

Field trials on wheat performance and growth after the addition of spores of endophytic actinobacteria as seed coatings were conducted in 2006 at a number of sites around Adelaide, South Australia (Franco et al. 2007). Wheat seeds coated with *Streptomyces* sp. were tested by professional agronomists from South Australian Research and Development Institute (SARDI) or Landmark Corporation. Trial sites were selected for testing distinct features: (a) growth promotion in the absence of disease and (b) disease suppression against the take-all fungus, *Rhizoctonia*, *Pythium,* and crown rot (contaminated soils were chosen according to the history of disease prevalence and soil DNA tests). Different soil types and climatic conditions were considered for each treatment, and control experiments and randomized block designs were conducted at each trial site. The field trials used custom farm practices in all sites (10–16) tested each year for four growing seasons. The values for grain yields of untreated controls and plants treated with the commercial fungicide Jockey® or streptomycetes were compared. The presence of GFP-tagged actinobacteria during development of the wheat roots (Fig. 8.1). The presence of streptomycetes in the plant during the early stages of root development was observed especially at the lateral root junctions which are a potential entry point from the soil (Fig. 8.2). Finally, it showed that in plots where wheat seeds coated with *Streptomyces* EN27 were added to soils with take-all, the grain yields were similar to those obtained with the commercial fungicide. In the absence of disease, wheat grain yield increased 5–15% compared with untreated plants. In general, the field trial described an improved wheat grain yield up to 60% in the presence of take-all, *Rhizoctonia* and crown rot diseases when endophytic actinobacteria (strain EN27) spores were added as a coating for wheat seeds, allowing the farmer to recover the cost associated with the application of the actinobacteria biocontrol agents (Franco et al. 2007).

However, not all field trials comply with the principle that treated seeds result in improved crops and plants growing faster. A set of selected seed treatments, including multiple chemical products and the fertilizer SuperBio® SoilBuilder (Advanced Microbial Solutions, LLC), were tested for barley growth promotion (Donald et al. 2009). Evaluation of early growth of the barley plots was assessed in terms of crop establishment and seedling vigor (height, dry mass). At the end of the trial, grain data included yield, test weight, and 1000-kernel weight. The trial showed that neither chemical products nor biological fertilizer showed an advantage for seedling vigor indices in comparison with the control, at any of the three tested sites. Similarly, at the end of the experimental trial, the same results were observed

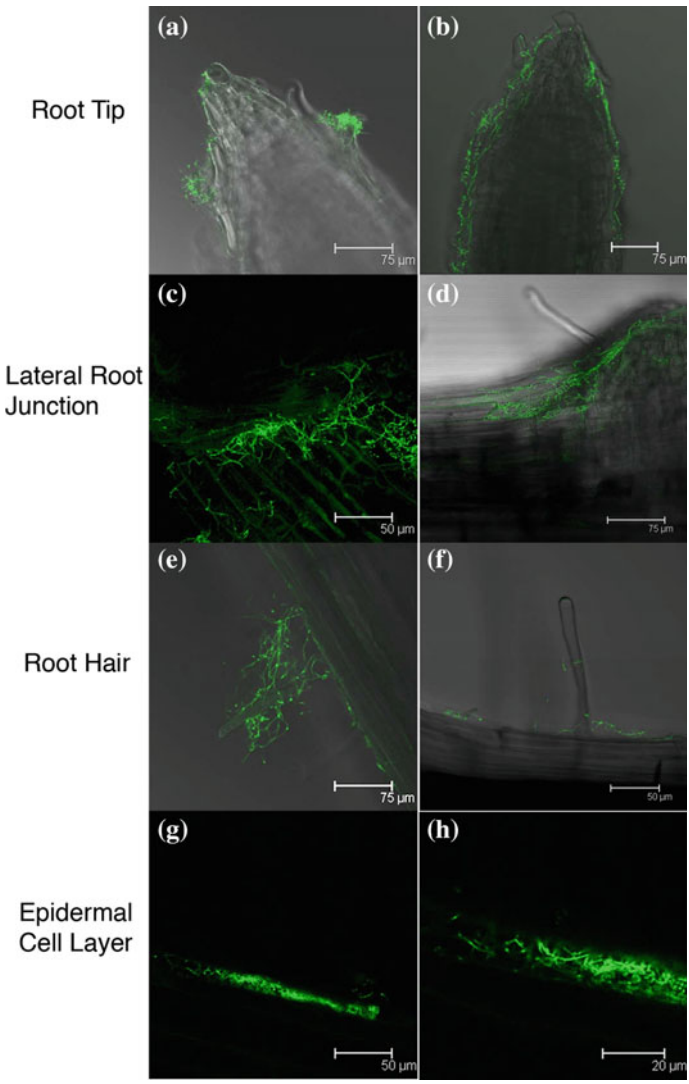

Fig. 8.1 Presence of GFP-tagged *Streptomyces* sp. EN27 in wheat roots

for harvested grain yield, test weight, and 1000-kernel weight for all seed treatments, confirming that none of these treatments had benefitted relative to the control. As stated by the authors, the negative results obtained in this trial for all tested products does not mean chemicals and biological fertilizers cannot provide beneficial effects in other crops or in other climatic conditions (Donald et al. 2009).

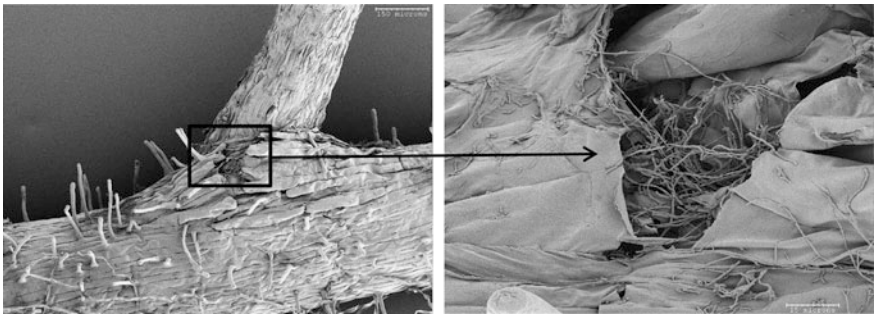

Fig. 8.2 Cryo-SEM micrograph showing the presence of actinobacteria at the lateral root junctions in wheat

8.3 Rice

Rice is a flowering plant in the family Poaceae which includes 20–24 species, distributed in tropical and subtropical regions of the world (Guo and Ge 2005). Rice (*Oryza sativa*) is the important economic crop in the world. In 2015, China was the country with the largest rice production in the world which produced more than 25% of the world production. However, critical problems are pest management, low yields, and the high cost of inorganic fertilizers and pesticides. The major rice pathogens are *Pyricularia grisea*, a fungus which causes blast disease and *Xanthomonas oryzae* pv. *oryzae,* a bacterial leaf blight disease (Priya and Kalaichelvan 2011). Most reports showed that genus *Streptomyces* was the dominant genus of endophytic actinobacteria discovered in the rice tissue (Tian et al. 2004; Gangwar et al. 2012; Kampapongsa and Kaewkla 2016).

There are many reports of endophytic actinobacteria that act as PGPB. There are direct PGPB benefits such as phytohormone and siderophore production, phosphate solubilization, nitrogen fixation, ACC deaminase production and indirect PGBP benefits such as antibiotic production and increase the plant immune system by systematic acquired resistance (SAR) or induced systematic resistance pathways. In a study to obtain biocontrol agents for rice bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Van Hop et al. 2014) 2690 actinobacterial isolates were screened from soil and leaf litter, among which 17 inhibited all 10 *Xoo* races in vitro. Field trials were carried out with two rice cultivars that were infected artificially with two races of *Xoo* and sprayed with a broth culture of *Streptomyces toxytricini.* This strain was able to suppress both the *Xoo* races significantly resulting in higher rice yields of 71–74% compared to untreated controls.

There are many rice pathogens amongst the bacteria, fungi, virus, as well as a phytoplasma. Blast disease caused by *Pyricularia grisea* or *Pyricularia oryzae* is the most dreaded disease of the rice plant. This disease can reduce up to 100% of production yield (Dean et al. 2005). Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is the second most severe disease which can

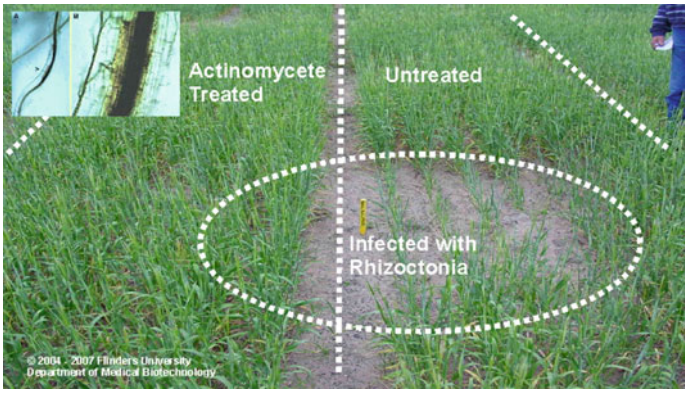

Fig. 8.3 Field trial at a site infected with *Rhizoctonia* bare patch

reduce the rice production yield by up to 50% (Lee et al. 2013). Several other diseases also exists such as Rice sheath blight caused by *Rhizoctonia solani* (Fig. 8.3). Sheath rots caused by *Sarocladium oryzae*, brown spot caused by *Cochliobolus miyabeanus*, bakanae disease caused by *Fusarium fujikuroi,* bacterial leaf streak and bacterial panicle blight caused by X. *oryzae* pv. *oryzicola,* and *Burkholderia glumae,* respectively (Liu et al. 2014). Some endophytic actinobacteria acted as a biocontrol agent to control these rice pathogens including the diseases both in vitro and in vivo.

Streptomyces sp. showed antimicrobial activity against *Rhizoctonia solani, Nigospora oryzae, Macrophomina phaseolina, Phoma sorghina,* and *Altenaria alternate* by dual culture method (Naik et al. 2009). Tian et al. (2004) reported that endophytic actinobacteria from rice roots and leaves belonging to the genus *Streptomyces* could inhibit *Pyricularia grisea*, *Rhizoctonia solani*, *Fusarium moniliforme,* and *Xanthomonas oryzae* pv. *oryzae*. About half of the population of all the isolates could inhibit at least one rice pathogen. Endophytic actinobacteria from rice showed activity against *Xanthomonas oryzae* pv. *oryzicola* and isolates belonging to *Streptomyces* showed strong inhibition (Hata et al. 2015). Endophytic actinobacteria from rice exhibited activity against many pathogenic fungi; *Aspergillus niger*, *Alternaria brassicicola*, *Botrytis cinerea*, *Chaetomium globosum*, *Fusarium oxysporum*, *Phytophthora drescle,* and *Rhizoctonia solani*. The result showed that *Saccharopolyspora* sp. R39 showed strong activity and *Streptomyces viridis* R3 exhibited good activity against all fungi tested (Gangwar et al. 2012).

Endophytic actinobacteria isolated from rice in Thailand showed activity against *Xanthomonas oryzae* pv. oryzae, *Curvularia lunata*, *Helminthosporium oryzae,* and *Pyricularia grisea* by using dual culture technique. The results showed that few isolates (18.8 and 3.4%) showed significant inhibition against *X. oryzae* pv. *oryzae,* and *P. grisea*, respectively. Most of the active isolates belonged to the genus *Streptomyces* (Kampapongsa and Kaewkla 2016). On the other hand, actinobacteria

isolated from rice tissues namely *Microbacterium* sp. SW521-L21 and SW521-37 observed high antagonistic activity against *Fusarium oxysporum* and *Rhizoctonia solani* in vitro. These isolates significantly reduced these fungal pathogens in rice plants compared to the untreated control (Ji et al. 2014).

8.4 Chickpeas

Streptomyces sp. isolated from vermicompost (Gopalakrishnan et al. 2011) were tested against *Fusarium oxysporum* f sp. *Cicero*, the causal agent of wilt in chickpeas in the field over two growth seasons (Gopalakrishnan et al. 2015a). The chickpea seeds were subjected to treatment with individual spore suspensions of the actinobacteria (10^8 CFU ml-1) for one hour before hand planting at a 26 plant m^{-2} density. All the five strains tested enhanced nodule number by 42–70%, nodule weight by 29–82% compared to untreated controls. The pod number increased from 31 to 51% and pod weight by 23–85% at 60 days after sowing compared to the untreated control. At the mature stage, the number and weight of seed were increased by 8–12% and 4–10%, respectively, showing the efficacy of having the actinobacterial partner. Later work by the same group (Gopalakrishnan et al. 2015b; Sreevidya et al. 2015) reinforced the efficacy of a number of actinobacteria applied in field trials.

8.5 Field Peas

Sweet peas are subjected to a wide variety of fungal diseases including powdery mildew caused by *Odium* sp. There are chemical controls such as alternate foliar sprays with Benlate and Caratan, but increased awareness of environmental problems has forced the search for sustainable alternatives. A *Streptomyces* strain, designated P4 (Thapanapongworakul 2003) obtained from the roots of a sweet pea has been found to be antagonistic to fungal pathogens, including powdery mildew (Akarapisan et al. 2008).

In a field trial setup with a nested split plot design, the inoculum was added as a fresh mycelial suspension to the surface sterilized seed in a peat moss mix prior to sowing (Sangmanee et al. 2009). The inoculum resulted in a statistically significant reduction in percentage leaf damaged by the powdery mildew. The upper leaves of the plants were more susceptible to the pathogenic fungi, had the highest reduction in disease symptoms measured at 45, 48, and 82% for snap pea, sugar pea, and top green pea, respectively. The conditions during spraying require a sticker as a coating agent to be added to the inoculum so as to prevent it from blowing off the plant. The P4 strain was found to have a synergistic effect on rhizobial nodulation to bring about higher nitrogen fixation.

A number of actinobacteria such as *Curtobacterium, Microbacterium, Micromonospora,* and *Streptomyces* enhance nodulation by Rhizobia in various legume plants (Martinez-Hidalgo et al. 2014). The synergistic interaction with *Rhizobium* has been shown to improve the plant biomass and the grain yield in soya plants (Bai et al. 2002). The combined inoculation of endophytic *Streptomyces* sp. with Rhizobia was observed to exert positive effects on the growth of legumes.

In another set of field trials with soybean *Streptomyces* sp. T4 was co-inoculated with *B. japonicum* USDA110 leading to increased nitrogen fixation, increased plant weight, and grain yield (Soe et al. 2012). Soe and Yamakawa (2013) examined whether low-density co-inoculation of *Bradyrhizobium yuanmingense* strain MAS34 and *Streptomyces griseoflavus* P4 would enhance nodulation, N_2 fixation, and seed yield in two soybean varieties. It was shown that there was a symbiotic interaction of the actinobacterium with selected indigenous Bradyrhizobial strains.

8.6 Tomato

The tomato plant (*Solanum lycopersicum*) originated from Central America and introduced to Europe during the sixteenth century, brought by the Spanish, rapidly spreading around the world; the word "tomato" derives from the Aztec language word "tomatl" (Bergougnoux 2014). The tomato plant is affected by several soil-borne pathogens, such as species of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia, Sclerotinia*, *Thielaviopsis,* and others (Lievens et al. 2006; Pane et al. 2013; Bergougnoux 2014). Bacterial spot caused by *Xanthomonas euvesicatoria, Xanthomonas vesicatoria, Xanthomonas perforans,* and *Xanthomonas gardneri*, early blight caused by *Alternaria solani*, corky root of tomato caused by *Pyrenochaeta lycopersici, Fusarium* wilt, and anthracnose caused by *Colletotrichum coccoides* are other serious diseases reported by tomato growers in several countries (Obradovic et al. 2004; Lievens et al. 2006; Pane et al. 2012; Raza et al. 2016). The benefits of actinobacteria to tomato could be observed in field trials conducted with actinobacteria through seed bacterization directly by adding specific strains of actinobacteria. Not much information is currently available, but there is one report mentioning the increased yield of tomato (over 10%) under garlic or wheat crop with actinobacteria treatment (Shi et al. 2013). The same manuscript reports that vitamin C content, protein, soluble sugar, and organic acid content of tomato were increased by 12, 14, 10, and 40%, respectively.

The addition of streptomycetes (or a mix of beneficial microbes including actinobacteria) directly to tomato plants was also evaluated. One of the field trials was carried out (at Valenzano, Bari, Italy) in a field naturally contaminated with *P. lycopersici* (Bubici et al. 2013). A set of four *Streptomyces* isolates and the isolate AtB-42 were evaluated for the biocontrol of corky root disease in tomato. Since AtB-42 had previously been tested in the field in a mixture with olive husk compost it proved to reduce tomato corky root disease by 30% and improved yield by 30%. The authors applied streptomycete inoculum (Day 7 before transplanting,

7 and 14 after transplanting) onto soil strips; 1 L spore suspension (1×10^6 spores mL^{-1}) was spread per linear meter. The severity of corky root was estimated after 120 days by assessing the percentage of the diseased root. The results showed that the streptomycete significantly controlled ($P < 0.05$) corky root of tomato, to the extent of 48% (reduction of disease severity ranged from 32 to 48% by testing five promising isolates of *Streptomyces* and StB-11 was the most effective isolate in greenhouse and field trials. However, a small difference could still be found compared with the results obtained from greenhouse tests (65% in a greenhouse *versus* 48% disease reduction in the field trial). This lack of correlation among effectiveness of biocontrol agents in greenhouse versus field trial is frequently observed which is mainly due to distinct climatic conditions (Spadaro and Gullino 2005; Alabouvette et al. 2006; Suprapta 2012). It was further suggested that plant protection was more difficult to achieve in the field due to the limited amount of soil receiving streptomycete inoculum (Bubici et al. 2013). Nevertheless, this constraint might be solved by formulations and improving the delivery system.

Another trial was conducted (in Canada) by testing six-week-old tomato transplant seedlings and streptomycete biocontrol treatments against bacterial spot, early blight, and anthracnose diseases (Cuppels et al. 2013). The plants received biocontrol treatments in the greenhouse at days 7 and 1 before being transferred to the field for four consecutive years (2005–2008). Biocontrol treatments of Mycostop® (Verdera Oy, Kurjenkellontie, Finland) and Actinovate® (Natural Industries, Houston, TX) were applied as aqueous foliar sprays. As described before, Mycostop® is a streptomycete-based (*Streptomyces griseoviridis* K61) biocontrol product registered for use in Canada and other countries against several root rots and wilt fungi (Lahdenperae et al. 1991). Another commercial disease control product registered in Canada using streptomycetes as the active ingredient is Actinovate® (*Streptomyces lydicus* WYEC108; Natural Industries, Houston, TX); strain WYEC108 not only suppresses phytopathogenic fungi but also promotes plant growth and root nodule formation on peas (Yuan and Crawford 1995; Tokala et al. 2002). The plants were inoculated with the bacterial spot pathogens (*X. gardneri* DC00T7A and *X. vesicatoria* DC93-1), the early blight pathogen (*A. solani* JAT2265), and the anthracnose pathogen (*C. coccoides* JAT2241). At the end of each growing season, the foliar disease severity and incidence of fruit lesion were estimated. Both *S. griseoviridis* K61 and *S. lydicus* WYEC108 treatments significantly suppressed ($P < 0.01$) foliar disease severity, but neither reduced bacterial spot disease during the entire growing season nor suppressed bacterial spot lesions on fruits. The combination of both streptomycetes with *Pseudomonas fluorescens* A506 exhibited that the *S. lydicus* WYEC108 + *P. fluorescens* A506 was most promising treatment resulted in significant reduction ($P < 0.01$) of both foliar disease severity and fruit lesions in two (2006 and 2007) out of four years, whereas *S. lydicus* WYEC108 + *P. fluorescens* A506 treatment was highly effective in controlling anthracnose disease. Nevertheless, in the year 2008, none of the treatments resulted in a significant reduction of fruit disease, proving once again inconsistency on the application of streptomycetes to field crops, possibly due to the consequence of different climatic conditions or inappropriate field application

methods (Cuppels et al. 2013). It is likely that these field trials may benefit from metagenomics studies capable of characterizing the complete group of microbiome interacting with the plant, as different geographic locations present distinct endemic microbial populations (Araujo et al. 2009; Araujo 2010). The authors suggested that tomato transplant seedlings may benefit from a streptomycete pre-treatment in the greenhouse before the plants were transferred to the field, in order to stabilize the streptomycete populations for 14 consecutive days following a single intervention (Cuppels et al. 2013).

Shilling and Lowell conducted field experiments on tomato plants subjected to irrigation, normal fertilization, and standard pesticide applications throughout the crop cycle. The results showed that the application of SC27 microbes, a solution containing 27 strains of soil fungi, bacteria, and actinobacteria, resulted in an increase in tomato plant biomass (no fruit) by 31%. The fruit weight increased from 44% after 55 days to 302% after 100 days when the set of beneficial microbes was added to the plants.

A fourth field test on tomato was conducted in the town of Los Alamos in Santa Barbara County, California, USA, in 2010. The effectiveness of Actinovate® in vegetable crops in field situations was tested to evaluate its value for the protection of fresh market tomatoes (cv. Better Boy, Early Girl, Beefmaster, Cherry Red, Celebrity, and Roma) (Quintana-Jones 2011). The treatments tested were: (i) initial Actinovate® treatment, (ii) initial RootShield® treatment (It contains active *Trichoderma harzianum* T-22 that protects roots from pathogens), (iii) initial Actinovate® application + drip applications, and (iv) initial Actinovate® application + drip applications + foliar applications. The effectiveness of the treatments against early blight (caused by *Alternaria solani*) was tested. It showed no significant differences in plant height among the four different treatments. The authors cautioned that predation by gophers and rabbits, climatic conditions, unidentified plant disease, and transplanting errors might have affected the final results (Quintana-Jones 2011). This last trial proved the results of field trials may differ among studies and how important it is to characterize the conditions under which crops are kept for further analyses.

8.7 Cucumber

Cucumber (*Cucumis sativus*) is now widely cultivated and affected by a range of diseases; particularly root rots caused by fungi and *Fusarium* wilt (El-Tarabily et al. 2009). A group of actinobacterial isolates proved their potential to improve cucumber fitness in pots under greenhouse conditions. The employment of these actinobacteria could help reduce the dependence of fungicides and increase the adoption of organic farming practices (El-Tarabily et al. 2009). A tunnel house under commercial production conditions was used to carry out two field trials and screen cucumber seedling resistance to damping-off, root and crown rots in presence of some isolates of actinobacteria (El-Tarabily et al. 2010). Millet seeds with

Pythium aphanidermatum were used to infect the soil. Seeds were germinated in vitro and when the roots were about 20 mm long, the root tips (3 mm) were in contact with an individual strain of actinobacterial suspension (10^8 cfu. mL^{-1}) for 3 h. The study evaluated the length, the dry and fresh weights of roots and shoots, disease severity, number, and yield of fruits. The reduction of damping-off of seedlings and the root and crown rots of mature cucumber plants were observed when a combination of multiple actinobacteria was added to the plants. In fact, all actinobacteria tested, individually or in combination, increased the lengths and weights of roots and shoots, the number and yield of fruits in comparison to the controls. Among the individual actinobacteria tested, an isolate of *Streptomyces spiralis* showed the best performance in promoting the growth of cucumber plants, followed by *Actinoplanes campanulatus* and *Micromonospora chalcea*. On the other hand, *S. spiralis* represents an endophyte capable of colonizing and persisting in cucumber roots for longer periods and at high concentrations in comparison to the other isolates (El-Tarabily et al. 2010). The ability to produce volatile metabolites as well as higher levels of β-1,3-glucanase β-1,4-glucanase and β-1,6-glucanases exhibited the advantageous to the strains (Valois et al. 1996; El-Tarabily et al. 2009).

8.8 Cabbage

Cabbage or headed cabbage (*Brassica oleracea*) is a leafy green or purple plant presently cultivated from the highest northern latitudes to the tropics. FAO reported global production of cabbage and other brassicas of around 70 million metric tons annually FAOSTAT (2014).

Cabbage is exposed to several diseases that may largely affect production. Fungal diseases include damping-off or wire stem (cause by *Pythium* spp., *Fusarium* sp. and *Rhizoctonia solani*), root rot or stunted growth due to *Rhizoctonia solani*, *Fusarium* yellows, blackleg (caused by *Leptosphaeria maculans*) dark leaf spots by *Alternaria brassicae* and *A. brassicicola* (Valkonen and Kopone 1990). *Plasmodiophora brassicae* causes clubroot characterized by swollen roots, while the oomycete *Peronospora parasitica* causes downy mildew (similar to powdery mildew) (Dias et al. 1993; Murakami et al. 2000). A relevant bacterial disease is black rot caused by *Xanthomonas campestris* (Gay and Tuzun 2000). Cabbage is also susceptible to attacks on the roots by root-knot nematodes and cabbage root maggots, and on the leaves by several insects, mainly aphids, harlequin cabbage bugs, thrips, striped flea beetles, moths, and caterpillars, e.g., the caterpillar stage of the butterfly *Pierisrapae* is a major cabbage pest in many countries (Ratnadass et al. 2012).

Two field experiments were conducted in 2002 and 2003 at the Seed Improvement and Propagation Station, Taichung, Taiwan with special references to root rot. *Streptomyces padanus* alone and in combination with a granulated product named PBGG (*Pseudomonas Brassica* Glycerine Granule) were tested against *Rhizoctonia* damping-off under field conditions (Chung et al. 2005). Treatments of

S. padanus and 1% PBGG (w/w) are combined with *S. padanus*. The components in each treatment were incorporated 15–20 cm into soil employing a rototiller. The incidence of damping-off, fresh weight, and number of plants were recorded for each treatment. The results showed that the treatment of *S. padanus* with 1% PBGG effectively reduced the incidence of *Rhizoctonia* damping-off in comparison to other combinations not so effective. Furthermore, *S. padanus* +1% PBGG resulted in a significant growth and development ($P < 0.05$). *R. solani* could also be suppressed by the addition of *Streptomyces* sp. in soil with PBGG possibly due to the production of compounds toxic for the mold. Interestingly performance of inoculants in greenhouse trial proved similar to that observed in field trials (Chung et al. 2005).

8.9 Pepper

Chili pepper (*Capsicum annuum*) is affected by several diseases such as root and stem rot caused by *Sclerotium rolfsii*, bacterial wilt caused by *Ralstonia solanacearum*, anthracnose caused by *Colletotrichum capsici*, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici,* and root knot caused by *Meloidogyne incognita* (Thomas et al. 1995; Boukaew et al. 2011; Raza et al. 2016).

It is clearly exhibited that the yield of pepper increased by 9.4% under actinobacteria treatment of garlic and wheat crops (Shi et al. 2013). In fact, the pepper nutritional quality index was also higher in plants with actinobacterial treatment.

Another field trial was conducted to evaluate the activity of *Streptomyces mycarofaciens* SS-2-243 and *Streptomyces philanthi* RL-1-178 for biocontrol of *S. rolfsii* root and stem rot, and *R. solanacearum* wilt of chili pepper (Boukaew et al. 2011). Thirty-day-old chili pepper seedlings were placed under field conditions in a randomized design. Both *S. rolfsii* and *R. solanacearum* were inoculated on the soil, about 5 cm away from the 15 days old seedling. *Streptomyces* sp. was applied near the chili plants at an interval of seven days. The disease incidence and a number of infected plants were measured every week till maturity of the crop (2 months). The results showed that *S. rolfsii* and *R. solanacearum* caused a mortality rate of 92.5% in the control treatment just after four weeks. *S. philanthi* RL-1-178 demonstrated high efficacy for controlling root and stem rot, as well as wilt of chili pepper, showing a survival rate of 59% (against 2.5% in the control plot). The final yield obtained from the plot with *S. philanthi* RL-1-178 treatment was approximately 239.50 kg ha^{-1} and represented five times increase in yield to that of control plot. Although not as efficient as *S. philanthi* RL-1-178, *S. mycarofaciens* SS-2-243 was also capable of controlling both diseases and showed a survival rate of 32.5% and a final yield 3.5 times more compared to the control. In fact, the disease inhibition rates and final yield observed in the presence of streptomycetes were similar to the values showed by the combination of the chemical treatments carboxin and streptomycin sulfate. Curiously, the authors of this study disagreed with the strategy of coating the seeds with *Streptomyces* sp. as the streptomycetes filtrate was

observed to inhibit in vitro chili pepper seed germination (Boukaew et al. 2011). Nevertheless, the addition of *Streptomyces* isolates to germinated seedlings and appropriate cultural practices could effectively improve field crops, especially chili pepper yields.

8.10 Eggplant

Eggplant (*Solanum melongena*) is affected by fungal disease *Verticillium* wilt causing serious damage to the crops (Bubici et al. 2013). A field trial was carried out at Valenzano, Bari, Italy in a field contaminated with *Verticillium dahlia* (Bubici et al. 2013). A group of five *Streptomyces* isolates was evaluated for the biocontrol of *Verticillium* wilt of eggplant. Streptomycete inoculum was applied three times (7 days before transplanting, 7 and 14 days after transplanting) onto soil strips; 1 L spore suspension (1×10^6 spores mL^{-1}) spread per linear meter. The severity of foliar symptoms of *Verticillium* wilt was evaluated 30, 50, 70, and 90 while the severity of vascular browning monitoring at regular intervals. The soil applications of tested streptomycetes could not control *Verticillium* wilt. As evidence by the values obtained on disease severity similar to the control plants (Bubici et al. 2013).

8.11 Potato

Potato (*Solanum tuberosum*) suffered due to relevant diseases include *Rhizoctonia*, *Sclerotinia*, *Verticillium dahlia*, black leg, powdery mildew, powdery scab, late blight, and leafroll virus (Shepardson et al. 1980; Atidrivon 1995; Gao et al. 2000; Beauséjour et al. 2003). Insects transmit potato diseases or damage the plants. Some nematodes also damage the crop, causing potato wilt (Ratnadass et al. 2012).

In order to test the inhibitory effect of *Streptomyces melanosporofaciens* EF-76 and chitosan, individually and in combination, a field trial was conducted on common scab of potato (Beauséjour et al. 2003). The formulation powder (talc or chitosan, with or without *S. melanosporofaciens* EF-76) was added on the top of each plant (*Solanum tuberosum*). The plots (each with 26 seed tubers) were arranged in a randomized trial with four replicates. Common scab symptoms, disease severity, and yield were evaluated from each plot. After harvesting, both chitosan and *S. melanosporofaciens* spores (talc) protected and reduced the disease severity to a similar level of around 20% in the year 2000 and 2001. Nevertheless, the best efficacy for protection against the potato scab was achieved by chitosan with *S. melanosporofaciens* spores, where 35% disease reduction achieved in 2000 (in 2001 the reduction was 23% for this combination). In fact, none of the seed treatments affected the yield at harvest and each year the yield values were similar for all the treatments. The development of products based on chitosan microbeads

with the inclusion of actinobacteria spores may represent an interesting method. This formulation developed to facilitate the application of chitosan oligomers and streptomycetes in order to potentiate the antagonistic activity against few important diseases (Beauséjour et al. 2003).

The diversity of bacterial communities of soil and potato was studied by the Biolog system following the addition of *S. melanosporofaciens* EF-76 and chitosan to the soil (Prévost et al. 2006). The formulations were prepared as described by Beausejour et al. (Beauséjour et al. 2003). Interestingly chitosan supplemented with *S. melanosporofaciens* EF-76 spores reduced the incidence of common scab potato disease. In fact, the treatment with chitosan supplemented with *S. melanosporofaciens* EF-76 was the only treatment that reduced common scab incidence. Beausejour et al. (2003) and Prevost et al. (2006) carried out testing in the same field, with the same potato cultivar and the same inoculum applied on tubers, nevertheless the individual effectiveness of chitosan and EF-76 spores to control common scab was not observed. This study indicates that the combination treatment largely increased the percentage of marketable tubers. The impact of the combined application of chitosan and EF-76 on microbial communities was low in the field, with only geldanamycin-resistant actinobacteria (*S. melanosporofaciens* EF-76) being increased slightly on progeny tubers (Prevost et al. 2006).

8.12 Lettuce

Lettuce drop caused by *Sclerotinia sclerotiorum* is a serious disease of lettuce and its biocontrol strategies are increasing due to harmful non-target effects of chemicals. Chen et al. (2016) isolated two *Streptomyces* isolates, *S. exfoliates* FT05 W and S. cyaneus ZEA17I inhibiting the growth of *Sclerotinia sclerotiorum* in vitro. These strains and *Streptomyces lydicus* WYEC 108 (from Actinovate®) were tested in a field experiment to evaluate the biocontrol of *S. sclerotiorum.* The *Streptomyces* sp. was applied to seed (5×10^3 CFU/seed) initially and after two weeks. This was followed by inoculation with *S. sclerotiorum* a week later and the following day was transplanted in the field under a plastic tunnel. The dead plants were enumerated until 142 days after transplanting against an untreated control in which [50%] mortality was observed. The potential biocontrol strains *S. exfoliatus* FT05 W and *S. cyaneus* ZEA17I were protective by 40 and 10%, respectively, whereas *S. lydicus* WYEC 108 showed no significant protection. Subsequent experiments to observe the colonization employed GFP-labeled *S. exfoliatus* FT05 W and *S. cyaneus* showed that both strains were able to colonize the host at the time of seed germination and root development. The GFP-tagged strains were persistent in the plants up to 3 weeks.

8.13 Commercial Bio-Fungicides

Presently there are two actinobacterial-based bio-fungicides in the market available to apply to multiple crops

(a) Actinovate bio-fungicide (Monsanto) is described as adding extra protection against multiple common foliar and soil-borne diseases found in crops. The product is based on the activity of *Streptomyces lydicus* WYEC 108.
(b) Biological fungicide Mycostop® (AGBio) is designated for vegetables, herbs, ornamentals, peanuts, and seedling production. According to the manufacturer, it controls damping-off, wilt, and root diseases caused by *Fusarium, Phytophthora, Alternaria, Pythium, Rhizoctonia* sp., and *Botrytis* sp., and promotes growth and increases yield in healthy crops. The product contains *Streptomyces* sp. isolated from Finnish sphagnum peat and its activity is dependent on the target pathogen and environmental conditions (Lahdenperae et al. 1991).

8.14 Conclusions

The lack of consistency of the results found in some field trials compared to the results observed in vitro and in the greenhouse tests reflects the variability and sometimes unpredictability of climatic conditions for optimal expression of the suppressive activities of actinobacteria biocontrol agents (Alabouvette et al. 2006; Xu and Jeger 2013). There is no doubt that the evaluation of climatic conditions is essential for a complete understanding of biocontrol activity in field crops. Furthermore, it remains critical to characterize microbial populations present in and around the host plants, especially endophytic communities and at the rhizosphere, in field conditions. The geographic location of the field is relevant as it is well known that microbial communities might change from place to place and over time (Araujo et al. 2009; Araujo 2010). In fact, some endemic microbes may severely limit the activity of the selected biocontrol agents and out compete it from reaching the infection court of the pathogen (Cuppels et al. 2013).

References

Adhikari TB, Gurung S, Hansen JM, Bonman JM (2012) Pathogenic and genetic diversity of *Xanthomonas translucens* pv. undulosa in North Dakota. Phytopathology 102(4):390–402

Akarapisan A, Bhromsiri A, Sangmanee P (2008) Selection of suitable isolates of endophytic actinomycetes and rhizobia for improvement of N_2 fixation and disease control of various *Pisum sativum* on the highland area. Asian J Food Agro-Ind 5:799–806

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: the european situation. Eur J Plant Pathol 114(3):329–341

Araujo R (2010) Endemism versus dispersion: contribution of microbial genetics for forensic evidences. Open Foren Sci J 3:14–21

Araujo R, Amorim A, Gusmão L (2009) Microbial forensics: Do *Aspergillus fumigatus* strains present local or regional differentiation? Foren Sci Int: Genet Suppl Ser 2(1):297–299

Atidrivon D (1995) Biology, ecology, and epidemiology of the potato late blight pathogen *Phytophthora infestans* in soil. Phytopathology 85:1053–1056

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48(3):230–238

Barabaschi D, Tondelli A, Desiderio F, Volante A, Vaccino P, Vale G, Cattivelli L (2016) Next generation breeding. Plant Sci 242:3–13

Beauséjour J, Clermont N, Beaulieu C (2003) Effect of *Streptomyces melanosporofaciens* strain EF-76 and of chitosan on common scab of potato. Plant Soil 256(2):463–468

Berdy J (2005) Bioactive microbial metabolites. J Antibiotics (Tokyo) 58(1):1–26

Bergougnoux V (2014) The history of tomato: From domestication to biopharming. Biotechnol Adv 32(1):170–189

Boukaew S, Chuenchit S, Petcharat V (2011) Evaluation of *Streptomyces* spp. for biological control of *Sclerotium* root and stem rot and *Ralstonia* wilt of chilli pepper. Biocontrol 56 (3):365–374

Bubici G, Marsico AD, D'Amico M, Amenduni M, Cirulli M (2013) Evaluation of *Streptomyces* spp. for the biological control of corky root of tomato and *Verticillium* wilt of eggplant. Appl Soil Ecol 72:128–134

Bulgarelli D, Rott M, Schlaeppi K, Loren Ver, van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488(7409):91–95

Charmet G (2011) Wheat domestication: lessons for the future. C R Biol 334(3):212–220

Chauhan PS, Puri N, Sharma P, Gupta N (2012) Mannanases: microbial sources, production, properties and potential biotechnological applications. Appl Microbiol Biotechnol 93(5):1817–1830

Chen W, Wellings C, Chen X, Kang Z, Liu T (2014) Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici*. Mol. Plant Pathol 15(5):433–446

Chen X, Pizzatti C, Bonaldi M, Saracchi M, Erlacher A, Kunova A, Berg G, Cortesi P (2016) Biological control of lettuce drop and host plant colonization by rhizospheric and endophytic *Streptomycetes*. Front Microbiol 7:714

Chung WC, Huang JW, Huang HC (2005) Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. Biol Control 32(2):287–294

Conn VM, Franco CM (2004) Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism. Appl Environ Microbiol 70(11):6407–6413

Coombs JT, Franco CM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69(9):5603–5608

Cuppels DA, Higham J, Traquair JA (2013) Efficacy of selected streptomycetes and a streptomycete + pseudomonad combination in the management of selected bacterial and fungal diseases of field tomatoes. Biol Control 67(3):361–372

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H, Read ND, Lee YH, Carbone I, Brown D, Oh YY, Donofrio N, Jeong JS, Soanes DM, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun MH, Bohnert H, Coughlan S, Butler J, Calvo S, Ma LJ, Nicol R, Purcell S, Nusbaum C, Galagan JE, Birren BW (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434(7036):980–986

Dias JS, Ferreira ME, Williams PH (1993) Screening of *Portuguese cole* landraces (*Brassica oleracea* L.) with *Peronospora parasitica* and *Plasmodiophora brassicae*. Euphytica 67 (1):135–141

Donald K, Andy H, Sina A, Margaret S, Kristin F (2009) Organic seed treatments tested in barley production. http://www.organicagcentre.ca/Docs/TechnicalBulletins09/E2009-33OrganicBarley.pdf

Duczek LJ, Verma PR, Spurr DT (1985) Effect of inoculum density of *Cochliobolus sativus* on common root rot of wheat and barley. Can J Plant Pathol 7(4):382–386

Edwards J, Johnson C, Santos-Medellin C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci USA 112(8):E911–E920

El-Tarabily KA, Nassar AH, Hardy GE, Sivasithamparam K (2009) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J Appl Microbiol 106(1):13–26

El-Tarabily KA, Hardy GE, Giles E, Sivasithamparam K (2010) Performance of three endophytic actinomycetes in relation to plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber under commercial field production conditions in the United Arab Emirates. Eur J Plant Pathol 128(4):527–539

FAOSTAT (2014) http://faostat.fao.org/site/567/DesktopDefault.aspx - ancor, accessed, from http://faostat.fao.org/site/567/DesktopDefault.aspx - ancor

Franco C, Michelsen P, Percy N, Conn V, Listiana E, Moll S, Loria R, Coombs J (2007) Actinobacterial endophytes for improved crop performance. Australas Plant Pathol 36(6):524–531

Friesen TL, Meinhardt SW, Faris JD (2007) The Stagonospora nodorum-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. Plant J 51(4):681–692

Gangwar M, Rani S, Sharma N (2012) Investigating endophytic actinomycetes diversity from rice for plant growth promoting and antifungal activity. J Adv Life Sci 1:10–21

Gao AG, Hakimi SM, Mittanck CA, Wu Y, Woerner BM, Stark DM, Shah DM, Liang J, Rommens CM (2000) Fungal pathogen protection in potato by expression of a plant defensin peptide. Nat Biotechnol 18(12):1307–1310

Gay PA, Tuzun S (2000) Temporal and spatial assessment of defense responses in resistant and susceptible cabbage varieties during infection with *Xanthomonas campestris* pv. campestris. Physiol Mol Plant Pathol 57(5):201–210

Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O (2011) Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. Crop Protec 30(8):1070–1078

Gopalakrishnan S, Srinivas V, Alekhya G, Prakash B (2015a) Effect of plant growth-promoting *Streptomyces* sp. on growth promotion and grain yield in chickpea (*Cicer arietinum L*). Biotech 5(5):799–806

Gopalakrishnan S, Srinivas V, Alekhya G, Prakash B, Kudapa H, Varshney RK (2015b) Evaluation of *Streptomyces* sp. obtained from herbal vermicompost for broad spectrum of plant growth-promoting activities in chickpea. Org Agric 5(2):123–133

Guo YL, Ge S (2005) Molecular phylogeny of Oryzeae (Poaceae) based on DNA sequences from chloroplast, mitochondrial, and nuclear genomes. Am J Bot 92(9):1548–1558

Hardwick NV, Jones DR, Slough JE (2001) Factors affecting diseases of winter wheat in England and Wales, 1989–98. Plant Pathol 50(5):650–652

Hata EM, Sijam K, Ahmad ZAM, Yusof MT, Azman NA (2015) In vitro antimicrobial assay of actinomycetes in rice against *Xanthomonas oryzae* pv. *oryzicola* and as potential plant growth promoter. Brazilian Arch Biol Technol 58:821–832

Intra B, Mungsuntisuk I, Nihira T, Igarashi Y, Panbangred W (2011) Identification of actinomycetes from plant rhizospheric soils with inhibitory activity against *Colletotrichum* spp., the causative agent of anthracnose disease. BMC Res Notes 4:98

Ji SH, Gururani MA, Chun SC (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiol Res 169(1):83–98

Kaewkla O, Franco CM (2013) Rational approaches to improving the isolation of endophytic actinobacteria from Australian native trees. Microb Ecol 65(2):384–393

Kampapongsa D, Kaewkla O (2016) Biodiversity of endophytic actinobacteria from jasmine rice (*Oryza sativa* L. KDML 105) grown in Roi-Et Province, Thailand and their antimicrobial activity against rice pathogens. Ann Microbiol 66(2):587–595

Lahdenperae ML, Simon E, Uoti J (1991) Mycostop - a novel biofungicide based on *Streptomyces* bacteria. Biotic interactions and soil-borne diseases: proceedings of the first conference on the European Foundation for Plant Pathology, pp 258–263

Lee SW, Han SW, Sririyanum M, Park CJ, Seo YS, Ronald PC (2013) Retraction. A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity. Science 342(6155):191

Lievens B, Brouwer M, Vanachter ACRC, Cammue BPA, Thomma BPHJ (2006) Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples. Plant Sci 171(1):155–165

Liu B, Huang L, Kang Z, Buchenauer H (2011) Evaluation of endophytic bacterial strains as antagonists of take-all in wheat caused by *Gaeumannomyces graminis* var. *tritici* in greenhouse and field. J Pest Sci 84(3):257–264

Liu W, Liu J, Triplett L, Leach JE, Wang GL (2014) Novel insights into rice innate immunity against bacterial and fungal pathogens. Ann Rev Phytopathol 52:213–241

Martinez-Hidalgo P, Galindo-Villardon P, Trujillo ME, Igual JM, Martinez-Molina E (2014) *Micromonospora* from nitrogen-fixing nodules of alfalfa (*Medicago sativa* L.). A new promising Plant Probiotic Bacteria. Sci Rep 4(6389)

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81(12):1340–1348

Murakami H, Tsushima S, Shishido Y (2000) Soil suppressiveness to clubroot disease of Chinese cabbage caused by *Plasmodiophora brassicae*. Soil Biol Biochem 32(11–12):1637–1642

Naik BS, Shashikala J, Krishnamurthy YL (2009) Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. Microbiol Res 164(3):290–296

Obradovic A, Jones JB, Momol MT, Balogh B, Olson SM (2004) Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. Plant Dis 88 (7):736–740

Orakçı GE, Yamaç M, Amoroso MJ, Cuozzo SA (2010) Selection of antagonistic actinomycete isolates as biocontrol agents against root-rot fungi. Fresenius Environ Bull 19(3):417–424

Osborne LE, Stein JM (2007) Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119(1–2):103–108

Pane C, Celano G, Villecco D, Zaccardelli M (2012) Control of *Botrytis cinerea*, *Alternaria alternata* and *Pyrenochaeta lycopersici* on tomato with whey compost-tea applications. Crop Protec 38:80–86

Pane C, Piccolo A, Spaccini R, Celano G, Villecco D, Zaccardelli M (2013) Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. Appl Soil Ecol 65:43–51

Pourkheirandish M, Komatsuda T (2007) The importance of barley genetics and domestication in a global perspective. Ann Bot 100(5):999–1008

Prévost K, Couture G, Shipley B, Brzezinski R, Beaulieu C (2006) Effect of chitosan and a biocontrol streptomycete on field and potato tuber bacterial communities. Biocontrol 51:533–546

Priya CS, Kalaichelvan PT (2011) Strategies for antagonistic activity of local actinomycete isolates against rice fungal pathogens. Asian J Exo Biol Sci 2:648–653

Quintana-Jones TA (2011) Evaluation of drip applications and foliar sprays of the biocontrol product Actinovate on powdery mildew and other fungal diseases of tomato. Faculty of California Polytechnic State University, San Luis Obispo

Ratnadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron Sustain Dev 32 (1):273–303

Raza W, Ling N, Zhang R, Huang Q, Xu Y, Shen Q (2016) Success evaluation of the biological control of *Fusarium* wilts of cucumber, banana, and tomato since 2000 and future research strategies. Crit Rev Biotechnol 1–11

Sangmanee P, Bhromsiri A, Akarapisan A (2009) The potential of endophytic actinomycetes, (*Streptomyces* sp.) for the biocontrol of powdery mildew disease in sweet pea (*Pisum sativum*). Asian J Food Agro-Ind (Special Issue):93–98

Schrey SD, Tarkka MT (2008) Friends and foes: streptomycetes as modulators of plant disease and symbiosis. Antonie Van Leeuwenhoek 94(1):11–19

Shepardson S, Esau K, McCrum R (1980) Ultrastructure of potato leaf phloem infected with potato leafroll virus. Virology 105(2):379–392

Shi G, Liang Y, Yao X, Zeng R, Mu L (2013) Effects of actinomycetes on yields and qualities of tomato and pepper under different for crops. Bull Soil Water Cons 1:055

Shilling D, Lowell C Tomato University trials, University of Florida, US, accessed, from https://sepixa.com/wp-content/pdf/tomato.pdf

Smith O, Clapham A, Rose P, Liu Y, Wang J, Allaby RG (2014) A complete ancient RNA genome: identification, reconstruction and evolutionary history of archaeological barley stripe mosaic virus. Sci Rep 4:4003

Soe KM, Yamakawa T (2013) Low-density co-inoculation of Myanmar *Bradyrhizobium yuanmingense* MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. Am J Plant Sci 4:1879–1892

Soe KM, Bhromsiri A, Karladee D, Yamakawa T (2012) Effects of endophytic actinomycetes and *Bradyrhizobium japonicum* strains on growth, nodulation, nitrogen fixation and seed weight of different soybean varieties. Soil Sci Plant Nutr Soil Science and Plant Nutrition 58:319–325

Spadaro D, Gullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Protec 24(7):601–613

Sreevidya M, Gopalakrishnan S, Melø TM, Simic N, Bruheim P, Sharma M, Srinivas V, Alekhya G (2015) Biological control of *Botrytis cinerea* and plant growth promotion potential by *Penicillium citrinum* in chickpea (*Cicer arietinum* L.). Biocontr. Sci Technol 25(7):739–755

Suprapta DN (2012) Potential of microbial antagonists as biocontrol agents against plant pathogens. J ISSAAS 18(2):1–8

Thapanapongworakul P (2003) Characterization of endophytic actinomycetes capable of controlling sweet pea root rot diseases and effects on root nodule bacteria. Master'' dissertation. Chiang Mai University, Thailand

Thomas SH, Murray LW, Cardenas M (1995) Relationship of pre-plant population densities of *Meloidogyne incognita* to damage in three chile pepper cultivars. Plant Dis 79:557–559

Tian XL, Cao LX, Tan HM, Zeng QG, Jia YY, Han WQ, Zhou SN (2004) Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. World J Microbiol Biotechnol 20(3):303–309

Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68(5):2161–2171

Turkington TK, Orr DD, Clear RM, Patrick SK, Burnett PA, Xi K (2002) Fungal plant pathogens infecting barley and wheat seed from Alberta, 1995-1997. Can J Plant Pathol 24(3):302–308

Valkonen JPT, Koponen H (1990) The seed-borne fungi of Chinese cabbage (*Brassica pekinensis*), their pathogenicity and control. Plant Pathol 39(3):510–516

Valois D, Fayad K, Barasubiye T, Garon M, Dery C, Brzezinski R, Beaulieu C (1996) Glucanolytic actinomycetes antagonistic to *Phytophthora fragariae* var. rubi, the causal agent of raspberry root rot. Appl Env Microbiol 62(5):1630–1635

Van Hop D, Phuong Hoa PT, Quang ND, Ton PH, Ha TH, Van Thi N, Van Hai T, Kim Quy NT, Anh Dao NT, Thom VT (2014) Biocontrol Sci 19:103–111

Xu XM, Jeger MJ (2013) Combined use of two biocontrol agents with different biocontrol mechanisms most likely results in less than expected efficacy in controlling foliar pathogens under fluctuating conditions: a modeling study. Phytopathology 103(2):108–116

Yang C, Hamel C, Gan Y, Vujanovic V (2012) Bacterial endophytes mediate positive feedback effects of early legume termination times on the yield of subsequent durum wheat crops. Can J Microbiol 58(12):1368–1377

Yuan WM, Crawford DL (1995) Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. Appl Environ Microbiol 61 (8):3119–3128

Zhan J, Fitt BDL, Pinnschmidt HO, Oxley SJP, Newton AC (2008) Resistance, epidemiology and sustainable management of *Rhynchosporium secalis* populations on barley. Plant Pathol 57 (1):1–14

Chapter 9
Bacterial Endophytes for Ecological Intensification of Agriculture

Shrivardhan Dheeman, Dinesh K. Maheshwari and Nitin Baliyan

Abstract Intensification in modern agriculture using endophytic bacteria employs to neglect hurdles of sustainable agriculture. Endophytes are contributing in current and future progresses of ecological intensification. Such microorganisms are the key driver to establish equilibrium between growing demand of food for ever-increasing population and agricultural production. Intensification and extensification to feed human population by applying beneficial soil microorganisms, either alone or in combination, have major contribution for achieving sustainable agriculture. Exploitation of interactions' process between endophytic organisms and plants contributes to plant growth promotion for crop productivity enhancement and overall ecological intensification. Studying ecology of bacterial endophyte (both above- and below-ground bacteria including other associative beneficial bacteria) offers potential for plant growth and health promotion so as to increase nutrient values in plant by fortifying nutrient or phytoremediation of citrant and recalcitrant pollutant in soil ecology. The consequences of endophytism including invasion, colonization, niche stabilization, and acquisition provide feasible approach for ecological intensification through stimulated plant growth by their phytohormone production and managing nutrient by facilitating mineralization of essential nutrients like P, K, and Zn. Nitrogen fixation by azotrophic endophyte is another beneficial aspect to contribute in ecological intensification of agriculture. Disease management credit productivity enhancement via indirect way and thus corroborate in intensification of agriculture.

Keywords Endophyte · Agricultural intensification · Nutrient management Disease control

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_9

9.1 Introduction

Intensification in modern agriculture is dedicated to raise crop productivity through systemic irrigation and copious use of inorganic nutrients and agrochemicals on the one hand and exploitation of endo-rhizospheric bacteria for growth and health promotion of plant with their mechanisms of phytohormone production, mineral solubilization, and indirectly controlling disease on the other hand. The introduction of mechanical reform of soil allows better root penetration and growth of plant, which also alter community of soil beneficial bacteria. In parallel, there has been an extensive conversion of land use over the past decades, with loss of natural elements (Tscharntke et al. 2005). The climate change, pollution, and biotic invasions have degraded biodiversity to such an extent that many soil ecosystem services hurdle to contribute in sustainable agriculture (Millennium Ecosystem Assessment 2005; Watson et al. 2011). Exploration and exploitation of beneficial soil bacteria inhabiting in plant tissues in the form of endophytes are important strategies to augment ecological intensification to boost crop yield, minimize negative impacts and ensure agricultural productivity enhancement. As a matter of fact, ecological intensification of agriculture (EIA) is meant for producing more food per unit resource use while minimizing the impact of food production on the environment. Therefore, it is necessary to investigate the deep existence of classical interactions between plants, antagonists, and mutualistic bacterial symbionts, both below ground and above ground. These endophytic bacteria are significant and required to manage plant growth and health. Endo-symbionts or endophytes open new avenues of both abiotic and biotic plant stress management (Sziderics et al. 2007; Lee and Luan 2012). Endophytes were defined as non-pathogenic bacteria isolated from or within plants, including rhizobia and other microbes (Hallmann et al. 1997; Hardoim et al. 2008). These are very likely to interact with their host plant, due to their ability to provide easily accessible nutrient sink; thus, they secure their tenancy inside apoplastic intercellular spaces of plants (Rosenblueth and Martínez-Romero 2006; Weyens et al. 2009). The current and future progresses of ecological intensification have been substantiated by endophytism, a phenomenon of mutualistic plant–microbe association in which microbe invades plant tissue and secures symptomless tenancy (Wani et al. 2015). Recruitment of core rhizomicrobes from seeds also suggests different modes of transmission of specific microorganisms from one generation to another. To meet future climatic, economic, and social challenges, agriculture needs to be made more productive, stable, and resilient while minimizing environmental impacts. In this article, we described ecology of bacterial endophytes, endophytism to overcome the challenges and their role to achieve effective ecological intensification.

9.2 Ecological Intensification for Agriculture (EIA)

The generalization mode is contrary to the context-specific, which relies on ecosystem-based principles of ecological intensification (Tscharntke et al. 2005). Therefore, models of ecological intensification involved agroecology, organic agriculture, and conservation agriculture (e.g., evergreen agriculture). Indeed, the term 'Intensification ecologique' was first used by Dugué et al. (2011). Such systems differ especially with respect to their impact on environment and agriculture, as well as the surrounding natural environment. Thus, it is important to shift ecological intensification to that of interdisciplinary agriculture. It is achieved by studying the ecology of monospecific populations (crops) or autoecology, i.e., individual species in relation to their environment.

9.3 Global Need for Ecological Intensification

The population growth, growing affluence of diets of populous country, deficiency of cultivable land, and competition from urbanization in combination led to drive the process of agriculturing intensification. Due to rapid deforestation and industrialization, the shrinkage of cultivable land is a hurdle in the way of agricultural intensification. Intensification especially depends on excessive use of fertilizer and pesticides, copious irrigation, more intensive cropping, and soil mechanization (Matson et al. 1997). However, these are having negative and adverse consequences on soil nutritional balance and microbial dynamics in soil. Further, demands for sustainable agroproduction lie on the application of non-renewable resources. The fertility and biodiversity can be maintained by use of organic compost and bio-products alternative to chemicals and pesticides, which focus on emerging technologies and production systems with potential to increase agricultural output per unit and minimize ecological harm to soil and thus transform in 'ecological intensification'. To feed the increasingly growing human population (i) intensification (i.e., use of off-farm inputs to achieve higher yields) and (ii) extensification (i.e., increase in the cultivated area to increase yield) are the two basic requirement in agriculture (Matson and Vitousek 2006). The new challenges that agronomist may face in twenty-first century are adoption of strategies which are able to increase food production without further increasing the area of arable land and with low environmental impact. To achieve ecological intensification, soil microorganisms can be exploited majorly to maintain natural fertility of soils. Also, the biological activities of soil microorganisms in the rhizosphere are mediating the nutrient solubility. Therefore, the availability of micronutrients is meant to meet out the nutritional requirements by enhancing plant nutrition availability under limited or

deficient conditions. Further, it also reduces detrimental effects of excess of micronutrients. In this context, increasing food production without further increasing the area of arable land may require a rational exploitation of soil biology and fertility to achieve sustainable management. The major contribution of seed/soil bacterization includes improvement in the establishment of symbiotic or associative interaction and exhibition of their beneficial functions such as nitrogen fixation, degradation of compounds polluting soil, promotion of plant growth, and biological control (van Elsas and Heijnen 1990; Whipps 2001). Therefore, such rhizobacteria showed beneficial traits for the development, growth promotion of plant by means of direct and indirect ways and referred as plant growth-promoting rhizobacteria (PGPR) with due their holistic association with plants. Endodermis and root cortex community of beneficial bacteria have been used to invade and to be colonized in the root tissues (Quadt-Hallman et al. 1997a, b). The endophytic bacteria used to induct in the crew of native resident of rhizosphere are the entities to be pronounced as beneficial bacteria (Darbyshire and Greaves 1973; Old and Nicolson 1978). Thus, endophytes as beneficial bacteria or PGPR have paramount place to come across to the need of 'ecological intensification of agriculture.'

During the late 1960s, the green revolution allowed food production to keep pace with the world population growth. The crop productivity was boosted by improvements in technology and changes in farming systems (Khush 2001). Application of synthetic agrochemicals (i.e., nitrogenous fertilizers and pesticides) was made for increasing grain yield (Saikia and Barman 2013), but it also led to a widely evident decline in soil quality/multifunctionality. The beneficial soil microorganisms either alone or in combination with mineral or organic fertilizers utilized to boost crop productivity and preserve the soil fertility without threatening the crop ecosystem and its environment (Maheshwari et al. 2010). For many decades, beneficial bacteria are the denizens to be introduced into soil or on seeds, roots, bulbs, or other planting material so as to increase plant growth and health promotion.

9.4 Microbial Endophytes for EIA

In quench of conserving biodiversity, potential worth of microbial endophytes is largely conjectural. By the definition, endophytes living interior of plants without inflicting negative effects (Bacon and White 2000). In fact, nearly 300,000 species of land plant on earth is likely to host one or more endophyte species (Senthilkumar et al. 2011). Despite this anticipated diversity, relatively few of these organisms have been characterized. Many endophytes are bioactive metabolite producers that antagonize the growth of other microorganisms. In some cases, they acquire ability to synthesize the similar defensive natural products produced by the plant. They

also produce phytohormone identical metabolite, to provide plant health and growth support. For example, endophytic bacteria isolated from micropropagated *Echinacea* plants were able to produce IAA like phytohormones (Lata et al. 2006). Further, Patil et al. (2011) isolated *Azetobacter diazotrophicus* L1 from sugarcane (*Officinarum saccharum*) and optimized the production of IAA. On the other hand, earlier, Nassar et al. (2005) observed significant growth promotion bought by IAA-producing root endophyte *Williopsis saturnus* in *Zea mays* L.

Plant rhizosphere contains microbiome in a similar way to humans and other animals. There is a diverse range of microbes that live around, on, and within plant's organs and tissues, which mimic to help plants in multifarious ways. Exploitation of interactions between endophytic organisms and plants results in plant health and growth promotion and thus plays a substantial part to cut the input cost for sustainable agriculture. The knowledge upgradation on the mechanisms enabling these endophytic bacteria to be associated with plants became essential to achieve the goal of advancement in biotechnological potential plant–soil–microbe interaction (Senthilkumar et al. 2011). A successful establishment of the invading bacteria depends on its selection that must personalize the soil and crop association. Germida et al. (1998) reported that bacterial endophytes live in plant roots as a subset of the communities found in the rhizosphere. Earlier, Sturz et al. (1997) studied endophytic population range about 10^4 viable bacteria per gram nodule. Thus, it is inculcating that endophytic bacteria sink similar metabolic and taxonomic features with PGPR (Misko and Germida 2002). Similar to PGPR, endophytic bacteria enhance plant growth also by phosphate solubilization (Kuklinsky-Sobral et al. 2004), siderophore production, nitrogen fixation (Knoth et al. 2014), quorum sensing (QS) signal interference (Hartmann et al. 2015), phytohormone production (Hoffman et al. 2013) and exhibiting antifungal activity (Doley and Jha 2016), interference with pathogen toxin production, etc. (Rosenblueth and Martínez-Romero 2006). Endophytic organisms produce essential vitamins for uptake by plants (Pirttilä et al. 2004) that facilitate further uptake of minerals (Gilroy and Jones 2000) and nitrogen metabolism and assimilation (Compant et al. 2005). There is a need to enhance knowledge on the precise traits of endophytic bacteria, aimed to quantifying their contribution in plant growth promotion. Endophytic bacteria ubiquitously inhabit interior of various plants and are observed as an unexplored reservoir of plant growth-promoting bacteria (Lodewyckx et al. 2002; Rosenblueth and Martínez-Romero 2006). The ambiance determinants influence their interactional processes that contribute in plant growth promotion and ecological intensification.

9.5 Ecology of Bacterial Endophyte

Together with above- and below-ground bacteria, beneficial bacteria form an enormous group of functional bacteria known as 'plant growth-promoting bacteria' (PGPB). Gray and Smith (2005) considered on below-ground bacteria and separated PGPR into two major classes, i.e., extracellular PGPR (ePGPR), existing in the rhizosphere, on the rhizoplane and intracellular PGPR (iPGPR), which exist inside root cells with asymptomatic infection. Here, the term ePGPR and iPGPR represented only below-ground community. Thus, ePGPB is suggested for those reside in the rhizosphere/phyllosphere and on the rhizoplane/phylloplane. The iPGPB exist inside plant cells/ and tissues with asymptomatic infection above and below ground. In influence of overlap, the following definition of endophytic bacteria fits best: 'bacteria that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and do not visibly harm the plant' (Hallmann et al. 1997). Therefore, in the present article, the term 'endophyte' is used for iPGPB. Endophytes came into existence 120 years ago, when bacteria were observed to exist inside the plants without causing any apparent disease. The usage of the term itself reflects its definition and spectrum that includes bacteria (Kobayashi and Palumbo 2000), ectomycorrhizal helper bacteria (Founoune et al. 2002), in pathogenic and commensalistic symbioses (Sturz and Nowak 2000). Endophytic bacteria, form intimate associations with plants and fix N_2 (Ladha and Reddy 2003). Such group of endophytic bacteria was isolated from crops such as sugar beet (Dent et al. 2004) and other agronomic crops such as potato (Sessitsch et al. 2002), paddy (Sun et al. 2008), and wheat (Germida and Siciliano 2001).

9.5.1 Above-Ground Endophytes

A diverse array of bacteria inhabits interior of various plant organs and tissues, including the phyllosphere and the rhizosphere. Understanding of the diversity, distribution and function of above-ground endophytic bacteria is important from the ecological and agroeconomical developments. It is attentive to discover how habitants of different plant parts have the potential to influence the structure of bacterial communities. Most studies on endophytic bacteria has been explored it as plant growth promoting bacteria due to their biological control traits, plant growth-promoting effects, endophytic nitrogen-fixing activity, and other physiological actions. Thus, it is crucial to understand the beneficial consequences of endophytes of aerial plant parts. Various workers have isolated, identified, and characterized above-ground endophytic bacteria from different parts of the plants. For example, *Bacillus subtilis* FB17 isolated from *Arabbodopsis thaliana* roots' plants after infection by bacterial pathogen *Pseudomonas syringae* pv. Tomato DC3000 (Rudrappa et al. 2008; Lakshmanan et al. 2012) Bacteria associated with the phylloplane as observed by Beattie and Lindow (1995) reflect the growth

patterns of leaf bacteria and study proved an active exchange occurs between the internal and external populations of bacteria. While focused on arial plant–endophyte interactions in this section, we considered endophytic bacteria fluctuate between endophytic and epiphytic colonization. *Pantoea agglomerans* has often been isolated from disinfected plant tissues (Sturz et al. 1998; Wilson et al. 1999) and the rhizosphere (Lottmann et al. 1999). The legumes comprise endophytic bacteria *Bacillus*, *Delftia*, *Methylobacterium*, *Microbacterium*, *Pseudomonas, Paenibacillus,* and *Stenotrophomonas* in the leaf tissue. Struz et al. (1997) studied diverse endophytic bacteria recovered from red clover nodule, root stem, and foliage and observed their tremendous effect on health and growth promotion of host plants. A large number of them are observed for their potential in plant growth promotion and biological control (De Oliveira Costa et al. 2012). Bacterial endophytes *Alcaligenes* sp. and *Pseudomonas aeruginosa* isolated from the rubber plant offer antagonism against *Phytophthora meadii* causing dreaded disease in *Hevea brasiliensis* Abraham et al. (2013). Araújo et al. (2001) studied fungi and bacteria (*Alcaligenes sp*., *Bacillus spp*., *Burkholderia cepacia, Curtobacterium flaccumfaciens, Enterobacter cloacae, Methylobacterium extorquens*, and *Pantoea agglomerans*) isolated from leaf tissues of citrus rootstocks and principally in vitro interaction studies of *G. citricarpa* and endophytic bacteria showed metabolite-mediated inhibition and a stimulatory growth effect on *P. agglomerans*. Various endophytic genera such as *Sphingomonas*, *Pseudomonas*, *Sphingobium*, *Methylobacterium*, *Petrobacter*, *Devosia*, *Cetobacterium*, and *Brevundimonas* were detected in stems and roots of rice. There bacterial genera are indigenous bacteriome which might have vertically transmitted in the plant tissues (Wang et al. 2016). The majority of endophytic bacteria may move to aerial parts of plant, with a decrease in bacterial density (Compant et al. 2010).

9.5.2 Below-Ground Endophytes

Bacteria uphold tremendous diversity and community composition in the endosphere is influenced with deterministic processes of colonization. Accounting the heterogeneity of soil in general and the microhabitat level in particular, the distribution of plant roots in soil, plant root–bacterium interaction occurs. Soil bacteria has ability to approach plant roots via their chemotaxis-induced or flagella mediated motility. The aggregations of microcolonies or biofilm in microniche is strongest determining factors to develop below-ground niche or microbiome that confer competence and livelihood in the rhizosphere. A wide range of other functional properties of endophytes are future to make competent endophytes successful colonizers in the plant endosphere (Hallmann et al. 2009).

9.5.2.1 Root Nodulating Endophytes

In natural ecosystems, bacteria associated with plants affect its health and growth. Their potential to affect plant health is brought by efficient colonization in plant interior or rhizosphere, entices utmost important. Rhizobia are putative endophytes of legume plant (Aeron et al. 2014). De Meyer et al. (2015a, b), including *Devosia* (Rivas et al. 2003), *Ochrobactrum* (Trujillo et al. 2005), *Microvirga* (Radl et al. 2014), *Methylobacterium* (Sy et al. 2001), and *Phyllobacterium* (Zakhia et al. 2006) belong to alphaproteobacteria; some other genera of Betaproteobacteria include *Burkholderia* and *Cupriavidus* have been described (De Meyer et al. 2014). On the other hand, few non-rhizobial endophytes (NRE) include in α-proteobacteria *Aminobacter* (Estrella et al. 2009), and β-proteobacteria *Herbaspirillum* (Valverde et al. 2003) and *Shinella* (Lin et al. 2008) also observed. Further γ-proteobacteria such as *Pantoea, Enterobacter,* and *Pseudomonas* have also been reported (Aserse et al. 2013). *Methylobacterium nodulans* has been originally isolated from root nodules of *Crotalaria podocarpa* (Sy et al. 2001), Kumar et al. (2009) reported decisive aim of establishing the intimate interaction among diazotrophic bacteria and non-legumes became important to fix nitrogen for plants. In fact, *Azorhizobium caulinodans* and *Methylobacterium* spp. are also capable for N_2 fixing in free-living condition. Diverse root nodulating endophytes in different host are summarized in Table 9.1.

Table 9.1 Diversity of root nodulating endophytes

Host	Microorganisms	References
Robinia pseudoacacia	*Mesorhizobium robiniae* sp. Nov.	Zhou et al. (2010)
Lotus arabicus *Lotus creticus,* *Argyrolobium uniflorum* and *Medicago sativa* (Tunisia)	*Ensifer numidicus* sp. nov. and *Ensifer garamanticus* sp. nov	Merabet et al. (2010)
Lupinus angustifolius	*Micromonospora*	Trujillo et al. (2010)
Pueraria lobata (Willd.) Ohwi	*Devosia yakushimensis* sp. nov.	Bautista et al. (2010)
Cytisus villosus	*Bradyrhizobium cytisi* sp. nov.	Chahboune et al. (2011)
Multiple legume species	*Rhizobium vignae* sp. nov.,	Chen et al. (2011)
Lablab purpureus and *Arachis hypogaea*	*Bradyrhizobium lablabi* sp. nov.	Chang et al. (2011)
Various wild legumes growing in China	*Rhizobium herbae* sp. nov. and *Rhizobium giardinii*-related bacteria and minor microsymbionts	Wang et al. (2011a, b)

(continued)

Table 9.1 (continued)

Host	Microorganisms	References
Oxytropis glabra	*Rhizobium tubonense* sp. nov.	Zhang et al. (2011)
Leguminous species	*Rhizobium vallis* sp. nov.,	Wang et al. (2011a, b)
Sphaerophysa salsula	*Rhizobium sphaerophysae* sp. nov.	Xu et al. (2011)
Sphaerophysa salsula	*Paracoccus sphaerophysae* sp. nov	Deng et al. (2011)
Dalea leporina, *Leucaena leucocephala* and *Clitoria ternatea*	*Rhizobium grahamii* sp. nov.	López-López et al. (2012)
Phaseolus vulgaris, *siratro*, *cowpea* and *Mimosa pudica*	*Rhizobium mesoamericanum* sp. nov	López-López et al. (2012)
Mimosa spp.	*Burkholderia symbiotica* sp. nov	Sheu et al. (2012)
from soybean (*Glycine max* L.) nodules	*Bradyrhizobium huanghuaihaiense* sp. nov	Zhang et al. (2012)
Cytisus villosus	*Bradyrhizobium rifense* sp. nov.	Chahboune et al. (2012)
Kummerowia stipulacea	*Rhizobium cauense* sp. nov.	Liu et al. (2012)
Lebeckia ambigua	*Burkholderia sprentiae* sp. nov	De Meyer et al. (2013a,b)
Mimosa spp.	*Burkholderia diazotrophica* sp. nov	Sheu et al. (2013)
Retama sphaerocarpa and *Retama monosperma*	*Bradyrhizobium retamae* sp. nov.	Guerrouj et al. (2013)
Rhynchosia ferulifolia	*Burkholderia rhynchosiae* sp. nov.	De Meyer et al. (2013a,b)
Cicer arietinum	*Paenibacillus endophyticus* sp. nov	Carro et al. (2013)
Soybean nodules	*Bradyrhizobium daqingense* sp. nov	Wang et al. (2013a, b)
Calliandra grandiflora	*Rhizobiumcalliandrae* sp. nov., *Rhizobiummayense* sp. nov. and *Rhizobiumjaguaris* sp. Nov	Rincón-Rosales et al. (2013)
Alfalfa nodules	*Endobacter medicaginis* gen. nov., sp. Nov	Ramírez-Bahena et al. (2013)
Lebeckia ambigua	Burkholderia sp. nov.	Howieson et al. (2013)
Astragalus sinicus	*Mesorhizobium qingshengii* sp. nov.,	Zheng et al. (2013)
Phaseolus vulgaris	*Phyllobacterium endophyticum* sp. Nov	Flores-Félix et al. (2013)
Phaseolus vulgaris	*Rhizobiumfreirei* sp. Nov	Dall'Agnol et al. (2013)
Astragalus luteolus and *Astragalus ernestii*	*Mesorhizobium sangaii* sp. nov.	Zhou et al. (2013)
Phaseolus vulgaris L	*Rhizobium* sp. nov.	Ribeiro et al. (2013)

(continued)

Table 9.1 (continued)

Host	Microorganisms	References
Oxytropis ochrocephala	*Rhizobium qilianshanense* sp. Nov	Xu et al. (2013)
Pongamia pinnata	*Rhizobium pongamiae* sp. Nov	Kesari et al. (2013)
Lemna aequinoctialis	*Rhizobium paknamense* sp. Nov	Kittiwongwattana and Thawai (2013)
Arachis hypogaea	*Bradyrhizobium arachidis* sp. Nov	Wang et al. (2013a, b)
Psoralea corylifolia, *Sesbania cannabina* and *Medicago lupulina*	of *Ensifer psoraleae* sp. nov., *Ensifer sesbaniae* sp. nov., *Ensifer morelense* comb. nov. and *Ensifer americanum* comb. Nov	Wang et al. (2013a, b)
Pea legume	*Bacillus simplex*	Schwartz et al. (2013)
Lebeckia ambigua	*Burkholderia dilworthii* sp. nov	De Meyer et al. (2014)
Cowpea	*Microvirga vignae* sp. nov	Radl et al. (2014)
Lupinus albus	*Paenibacillus lupini* sp. nov	Carro et al. (2014)
Lupinus albus	*Cohnella lupini* sp. nov.,	Flores-Félix et al. (2014a, b)
Dipogon lignosus	*Burkholderia* sp.	Liu et al. (2014)
Acacia melanoxylon R. Br.	*Bradyrhizobium ganzhouense* sp. nov.,	Lu et al. (2014)
Phaseolus vulgaris	*Fontibacillus phaseoli* sp. nov	Flores-Félix et al. (2014a, b)
Aspalathus abietina Thunb.	*Burkholderia aspalathi* sp. nov	Mavengere et al. (2014)
Vicia faba	*Rhizobium laguerreae* sp. nov.	Saïdi et al. (2014)
Phaseolus vulgaris	*Rhizobium azibense* sp. nov	Mnasri et al. (2014)
Vigna unguiculata (Genisteae legumes)	*Bradyrhizobium* sp. sver. vignae	Bejarano et al. (2014)
Vigna unguiculata	*Bradyrhizobium manausense* sp. nov	Silva et al. (2014)
Centrolobium paraense	*Bradyrhizobium neotropicale* sp. nov	Zilli et al. (2014)
Phaseolus vulgaris L.	*Rhizobiumparanaense* sp. nov	Dall'Agnol et al. (2014)
Sophora flavescens	*Rhizobium sophorae* sp. nov. and *Rhizobium sophoriradicis* sp. nov.	Jiao et al. (2015a, b)
Vicia faba and *Pisum sativum*	*Rhizobium anhuiense* sp. nov	Zhang et al. (2015)
from nodules of the relict species Vavilovia formosa (Stev.) Fed	*Bosea vaviloviae* sp. nov.,	Safronova et al. (2015)
Lens culinaris	*Rhizobium lentis* sp. nov., *Rhizobium bangladeshense* sp. nov. and *Rhizobium binae* sp. nov.	Rashid et al. (2015)

(continued)

Table 9.1 (continued)

Host	Microorganisms	References
Dipogon lignosus	*Burkholderia dipogonis* sp. nov	Sheu et al. (2015)
Neptunia oleracea Lour.	*Rhizobium undicola*	Ghosh et al. (2015a, b)
Capsicum annuum var. *grossum*	*Rhizobium capsici* sp. nov	Lin et al. (2015)
Sophora flavescens	*Phyllobacterium sophorae* sp. nov.,	Jiao et al. (2015a, b)
Arachis hypogaea	*Rhizobium pakistanensis* sp. nov.,	Khalid et al. (2015)
Soybean	*Diaphorobacter ruginosibacter* sp. nov.,	Wei et al. (2015)
Phaseolus vulgaris L.	*Rhizobium ecuadorense* sp. nov	Ribeiro et al. (2015)
Medicago sativa L.	*Bacillus megaterium* BMN1	Khalifa and Almalki (2015)
Abrus precatorius L. *Biocatalysis*	*Enterobacter* spp.	Ghosh et al. (2015a, b)
Sophora longicarinata and Sophora microphylla	*Mesorhizobium waimense* sp. Nov *Mesorhizobium cantuariense* sp. nov	De Meyer et al. (2015a, b)
Pisum sativum	*Micromonospora luteifusca* sp. nov	Carro et al. (2016)
Pueraria candollei var.	*Rhizobium puerariae* sp. nov.	Boonsnongcheep et al. (2016)
Periandra mediterranea	*Paenibacillus periandrae* sp. nov	Menéndez et al. (2016)
Phaseolus vulgaris	*Rhizobium acidisoli* sp. nov	Román-Ponce et al. (2016)
Sophora	*Mesorhizobium calcicola* sp. nov., *Mesorhizobium waitakense* sp. nov., *Mesorhizobium sophorae* sp. nov., *Mesorhizobium newzealandense* sp. nov. and *Mesorhizobium kowhaii* sp. nov.	De Meyer et al. (2016)
Centrosema sp.	*Bradyrhizobium centrosemae* (symbiovar *centrosemae*) sp. nov., *Bradyrhizobium americanum* (symbiovar *phaseolarum*) sp. nov. and a new Bradyrhizobium viridifuturi (symbiovar *tropici*)	Ramírez-Bahena et al. (2016)
Arachis hypogaea	Endophytic occupation of legume root nodules by nifH-positive non-rhizobial bacteria	Dhole et al. (2016)
Trifolium alexandrinum	*Rhizobium bangladeshense* symbiovar trifolii and *Rhizobium aegyptiacum* sp. nov	Shamseldin et al. (2016)
Vigna and *Arachis*	*Bradyrhizobium vignae* sp. nov.	Grönemeyer et al. (2016)

9.5.2.2 Non-root Nodulating Endophytes

An endophytic bacterium offers a vast potential for agronomic performance of plants. The diversity of bacterial endophytes thus promises compatible and fruitful association with all agronomically and agricultural important crops, including monocots and dicots. Non-root nodulating endophytes exist in diverse plant species as part of their root microbiome and to influence plant growth positively. Beside, symbiotic endophytes such as rhizobia, a majority of non-root nodulating endophytes summarized in Table 9.2. Legumes and rhizobia develop symbiotic

Table 9.2 Diversity of non-root nodulating endophytes

Bacteria	Plant	References
Bacillus subtilis	Holy Basil (Ocimum sanctum)	Tiwari et al. (2010)
Achromobacter xylosoxidans and *Bacillus pumilus*	Sunflower (*Helianthus annuus*)	Forchetti et al. (2010)
Paenibacillus polymyxa	Switchgrass (Panicum virgatum L.)	Ker et al. (2012)
Bacillus subtilis, *Bacillus* sp., *Enterobacter* sp., *Enterobacter ludwigii*, *Lactobacillus plantarum*, *Pseudomonas* sp., *Pantoea punctata*, and *Curtobacterium citreum*	Strawberry (*Fragaria ananassa*)	de Melo Pereira et al. (2012)
Bacillus sp. SLS18	Sweet sorghum (*Sorghum bicolor*)	Luo et al. (2012)
Escherichia fergusonii, *Acinetobacter calcoaceticus*, *Salmonella Enterica*, *Brevibacillus choshinensis*, *Pectobacterium Carotovorum*, *Bacillus megaterium*, *Microbacterium testaceum*, *Cedecea Davisae*	Coffee (*Coffea* sp.)	Silva et al. (2012)
Pseudomonas spp.	Eggplant; Brinjal (*Solanum melongena*)	Ramesh and Phadke (2012)
Paenibacillus	Orchid (Orchidaceae)	Faria et al. (2013)
Sphingomonas sp. LK11	Tomato (*Lycopersicon esculentum*)	Khan et al. (2014)
Enterobacter sp. FD17	Maize (*Zea mays*)	Naveed et al. (2014)
Phomopsis liquidambari	Rice (*Oryza sativa* L.)	Yang et al. (2014)
Neofusicoccum australe	Myrtle (*Myrtus communis*)	Nicoletti et al. (2014)
Bacillus spp.	Maize (*Zea mays*)	Gond et al. (2015)

(continued)

Table 9.2 (continued)

Bacteria	Plant	References
Microbacterium, *Agrobacterium*, *Sphingobacterium*, *Herbaspirillum*, *Erwinia*, *Pseudomonas* and *Stenotrophomonas*	*Sorghum bicolor*	Maropola et al. (2015)
Bacillus sp.	Poplar and willow	Kandel et al. (2015)
Bacillus sp.	Rice (*Oryza sativa* L.)	Rangjaroen et al. (2015)

relationships mediated by a complex give-and-take of signals in molecular dialogues. Despite the highly specific signaling to recruit actual partner for nodulation, the presence of non-rhizobial bacteria in the root nodules has also been reported (Pandya et al. 2013). As evidence of healthy nodule endophytes not necessarily contains only the symbiotic bacteria, other diazotrophic bacteria have been documented, i.e., *Bacillus* in soybean (Bai et al. 2002), *Klebsiella* in groundnut, clover, bean, etc. (Ozawa et al. 2003) and *Pseudomonas* in acacia and soybean (Kuklinsky-Sobral et al. 2004; Hoque et al. 2011a, b). Some human pathogens, such as *Salmonella* spp., and *Pseudomonas* spp., have also been found as endophytes that cannot be eliminated by application of disinfection and surface sterilization procedures that eliminate superficially occurring bacteria (Rosenblueth and Martínez-Romero 2006). Inoculation of endophytic bacteria in the rhizospheric ecology must be carefully evaluated to avoid the chance of pathogen inoculation. Health of plant infected with endophytes increased both during inter- and intraspecific competition. These bacteria sequestered within plants tissues, but as the plant grows under favorable environment, bacteria within tissues continue its growth and offer protection to the plant throughout plant's growth cycle. Thus, bacteria are deemed as endo-symbionts and behave in mutualistic relationships. Intracellular spaces interconnected with large area spaces that contain high levels of carbohydrates, amino acids, and inorganic nutrients are the microniche of these endophytic bacteria This microniche serves to support bacterial growth in intercellular spaces (Bacon and Hinton 2007). The intercellular spaces as novel microniche can be protected, offering numerous advantages over rhizospheric niche. Endophyte as voracious colonizer colonizes in microniche of phytopathogens and thus competes out the pathogens and acts as potent biocontrol agents. Soares et al. (2016) isolated and identified *B. amyloliquefaciens* from *Hedera helix* L. and proved biocontrol by reporting systemic colonization in leaves, petioles, and seeds, hormones synthesis and production of different antifungal lipopeptides, eventual inhibition of *Alternaria tenuissima* along with plant growth promotion. It is meritorious to use endophytic bacteria as biocontrol agent those offers potential contribution for surrogate transformation of plants which results increased nutritional qualities or holistic and eco-safe pesticides to be utilized for phytoremediation of soil and water pollutants.

9.6 Endophytism

The variations in the endophytic bacterial communities can be attributed to plant age, plant source, type of tissue, sampling time, and environmental condition (Kobayashi and Palumbo 2000). The endophytic diversity is a function of different maturation stages specific to each plant, which might influence the different types and amounts of root exudates (Ferreira et al. 2008). The competition among endophytes understood us that few are too aggressive to be colonize and displace the others from the rhizosphere as observed with *Pantoea sp*. which outcompeted *Ochrobactrum sp*. in rice rhizosphere (Verma et al. 2004) and *Rhizobium etli* strains in maize rhizosphere (Rosenblueth and Martínez-Romero 2004). It was interesting to note that the type of soil acts as detrimental factor for endophytic population in wheat significantly (Conn and Franco 2004). The process of adoption and to become resident in plant's interior tissues has various phases and establishes them under the phenomenon called 'endophytism.'

9.6.1 Bacterial Interaction and Adhesion

The intimate association between plant and microbe specific to seeds, roots, stems, leaves, flowers and fruits (Compant et al. 2010) dynamically raise quest how microbe invade, colonize and harbor interior of the plants. Further, exploration of interplay amid soil, plants, and endophytes needs in-depth analysis and review of researched made to understand role of endophytes in ecosystems. The bacterial adhesion to the surfaces was studied based on physicochemical approaches. Adhesion of bacteria on the negatively charged polystyrene is reversible and quantitatively estimated using the DLVO theory for the stability of colloid. Adhesion increased with increasing electrolyte strength. Adhesion of bacteria affects with the high or low value of DLVO and also determines the adhesion potential during primary and secondary adhesion. The magnitudes of adhesion in the natural environment by several soil bacteria such as *Pseudomonas* and other bacterial genera have been discussed by van Loosdrecht et al. (1989). These form microcolonies or biofilms to be colonized on roots surface or interior later by, but prior to colonization, the production of signal by bacterial surface components in combination with bacterial functional plays significant role in the process of biofilm formation. Bacterial aggregates in the form of microcolonies adhere at a solid–liquid interface followed by adsorption on a thin film of organic molecules that constitutes the adhesion site exhibited. Extracellular polymers encourage or provide immobilization efficiency in bacterial cells and mechanical stability in the biofilm structure, ligand interaction with the substratum, and encased them in architectural and functional microbial community (Bogino et al. 2013). Cell communication system in terms of quorum sensing (QS) helps bacterial species to communicate and coordinate the behavior at community level first maintaining their quorum and later

by regulation of gene expression. The QS process is governed by *N*-acylhomoserine lactones (AHLs) mostly in Gram-negative bacteria. There are very few reports shown in the communication by QS signaling molecules in diverse bacteria regardless of bacterial endophytes. Nievas et al. (2012) investigated on decipherization, characterization, and biological effects of quorum sensing on *Bradyrhizobia* symbiotic bacteria of peanut.

9.6.2 Invasion

The discharge of cellulolytic enzymes mainly cellulases, pectinases, etc. is involved in cellulolysis of cell wall that allowed penetration, localization, and dissemination of bacteria in plant tissues (Lodewyckx et al. 2002). The consequences of penetration not necessarily involve in a much defined way to active mechanisms beside, and entire microbiome of rhizosphere expectedly becomes endophytic at one or any stage of plant's life cycle (Hardoim et al. 2008). Bacteria can enter in plant tissue via wounds (including broken trichomes), stomata, lenticels, lateral roots, and radicles depending upon the host plants. However, the wounds in root hairs and at epidermal conjunctions are thought to be main entry portal of endophytic bacteria (Reinhold-Hurek and Hurek 1998). Although entry of bacteria in the host induces plant defense mechanisms, but it is confined only in case of pathogens but not reported to that of entry accomplished by endophytic bacteria.

Endophytic bacteria are potential producer of cell wall degrading enzymes' activity as common features (Elbeltagy et al. 2000). The discharge of cellulolytic enzymes acts on cell wall material such as cellulose and pectin to dissolve and allow the process of bacteria to invade inside the plant tissues and tenancies in the plant part. The enzymatic activities by endophytes have been explored as central and efficient methods to be entered into the host plant and resulting successful colonization. Endo-glucanase is major determinant for the endophytic colonization in endo-rhizosphere which was evidently studied in *Azoarcus* strain and contrasted with those strain has lack endo-glucanse unable to be colonized in the rice plants (Reinhold-Hurek et al. 2006). These bacteria invade through root hairs and spaces between damaged epidermal cells or fissures at the cortical and intercellular cracks (Chaintreuil et al. 2000; James et al. 2002). Further, to invade, colonized and for survival, bacteria must overcome plant immune responses activated to attempt defence against forgien microbial invasion. Bacteria involve several mechanisms to accomplish such phenomenon such as surface molecules which included polysaccharides and few other mechanism antioxidant activity, ethylene biosynthesis inhibition, and activation of virulence genes also detrimental factors (Soto et al. 2006).

Endophytic invasion inside the root comprises production of multiple signaling and reciprocal signaling interplayed amid endophytes and plants (Rudrappa et al. 2008). Quorum sensing (QS) mechanisms for microbe signaling approach involve the production and perception of low molecular weight molecules. These molecules

called autoinducers are able to diffuse out from individual bacteria to the environment (Chernin 2011). Thus, individual bacteria act in concerted model to increase the fitness and survival of their communities (Elasri et al. 2001). Production and signaling by *N*-acyl homoserine lactones (AHLs) is the most common QS signaling communication in Gram-negative bacteria (Elasri et al. 2001). In addition, extracellular molecules signify their immense role in communication of bacteria and plants, where plants release host-specific compounds such as flavonoids that induce allelopathy for endophytic colonization (Balachandar et al. 2006). The release of specific flavonoids helps bacteria to be colonized in interior of plant tissues via activation and expression of certain gene (Bais et al. 2004).

Besides, endophytic bacteria has been developed and strategized the ways in which they use plant hormone as signaling molecule to activate pathways for pheromone- or phytohormone-mediated signaling accomplished by two-component system. Endophytes thus receive signals to produce various metabolites such as ACC-deaminase and indole acetic acid (IAA) to direct signaling pathways (Spaepen et al. 2007) and to communicate with the host plants, e.g., induce IAA and abscisic acid biosynthesis in *Arabidopsis thaliana* due to *Pseudomonas syringae* as mentioned by Schmelz and Engelberth (2003) and de Torres-zabala et al. (2007).

9.6.3 Colonization

The endophytic tissue colonization by bacteria in host plant reflects their aptitude to adapt thyself in specified ecological niches. These integration results intimate association without causing any adverse effect to the plant (Sturz and Nowak 2000; Compant et al. 2005). In case of below-ground system, tissue colonization bought by the bacterial ability to be established on or in the rhizosphere and endo-rhizosphere, to grow, thrive, and disseminate in the entire plant system (Whipps 2001; Lugtenberg et al. 2002; Babalola and Glick 2012). The colonization events for endophytic bacteria include various steps, i.e., entry in root interior, microcolonies formation, and microbial aggregation either inter- or intracellularly. The chemical substances secreted as the root exudates strongly influence to the primary colonizers of the bacterial population to drive nutrients in their microniche (Bais et al. 2001; Dakora and Phillips 2002; Walker et al. 2003). Attractive behavior of bacteria to the rhizosphere is response to rhizodeposits due to compositional richness with several amino acids, sugars, organic acids, purines/pyrimidines, vitamins and other metabolic products. Further, to provide nutritional substances, plants start producing molecular dialogue for crosstalking by cell to cell communication system which becomes detrimental factor of colonization by endophytic bacteria (Bais et al. 2006; Compant et al. 2011). Endophytes happen to be sufficient to receive signals molecules produced by plants, and become able to invade in plant tissue via wound or disturbed cells of roots from different parts such as root junctions and root caps. As the bacteria complete successful entry, it appears to form microcolonies within the vascular tissue or in the spaces between plant cells

(James et al. 1997). The primary colonization of endophyte in plants by the chance showed that a competent endophyte can also exist within plant tissues (Hardoim et al. 2008).

9.6.4 Niche Stabilization and Acquisition

The xylem components particularly vessels have been signified as ideal niches for endophytic bacteria to provide quick and consistent delivery of water and solutes across plant parts. Myriad researchers have been documented bacterial endophytes, including diazotrophic not only to provide splendid transport route but also to supply substrate (in continuous). Input endophytic bacteria aroused in the vessels or in sap exuded. It has been observed that xylem vessel invaded by bacteria is a non-functioning vessel (Zimmermann 1983).

The intracellular locations are major spaces of bacteria inhabited by true endophytes (i.e., established within living tissues). The plant has anatomical spaces, i.e., apoplast, which extends to the entire length of plant, except in the vascular tissues, sometimes distinct with cell walls (wall apoplast) and the lumens of the xylem (xylem apoplast) (Canny 1995). The dynamics of filling and emptying with bacterial communities is yet to be investigated. However, very limited information is known about colonization and niche tenancy in intercellular spaces, but as a matter of fact there, moderate pH provides nutrition supplements for niche stabilization for diazotrophs such as *Gluconacetobacter diazotrophicus* (Welbaum and Meinzer 1990).

More knowledge on intercellular niche stabilization of endophytes associated with crop plants under various biotic and abiotic influences and in field conditions remains to be explored. It is evidenced that different genotypes of crops are expressible to produce a variety of metabolites able to attract bacteria for succesful colonization (Elvira-Recuenco and van Vuurde 2000). This is due to feasibility of plants to secrete appropriate substances into the intercellular spaces which also serves as growth factor and promotes bacterial colonization.

9.7 Approaches for Ecological Intensification

Below-ground interactions amid plant, bacteria, soil, and rhizosphere create an environment drive important ecosystemic processes, i.e., productivity, biogeochemical cycles, and resistance to biotic and abiotic stresses. The root is preliminary designed to access below-ground resources of microbiome and anchorage them to contribute in nutrient acquisition and nutrient cycling (Ryan et al. 2016). The knowledge on plant nutrition in the rhizosphere is obtained in hydroponics or microcosm system confined to single crop or cultivar, and there is a need to move forward toward more biodiversity-based agriculture for achieving sustainable

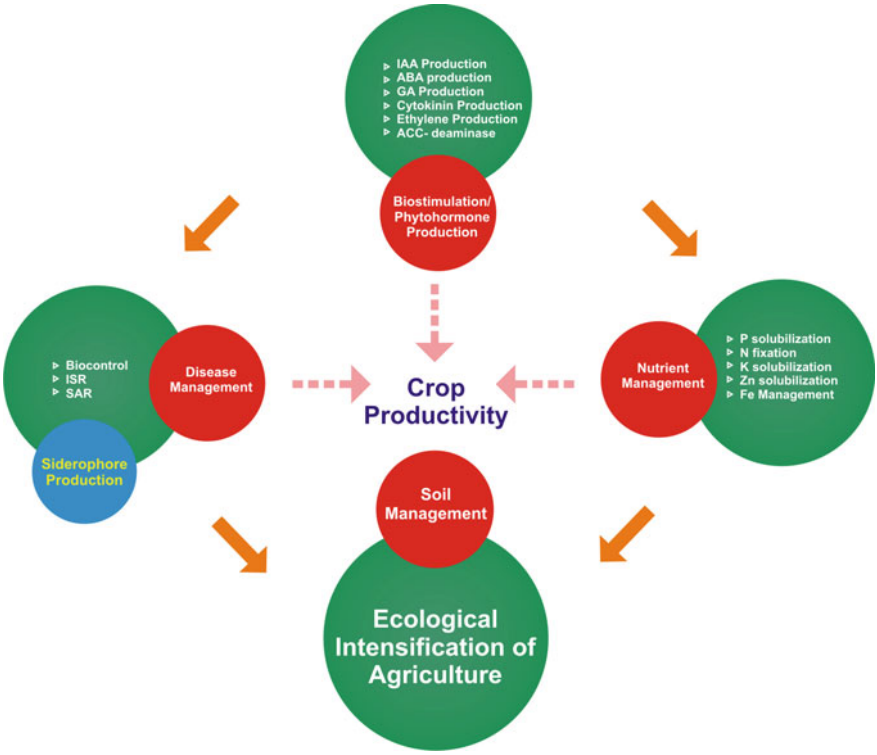

Fig. 9.1 Overall schematic representation of ecological intensification of agriculture (EIA) by endophytes and their mechanistic roles

intensification of agroecosystem. Positive plant–microbe interaction plays a significant role for P acquisition. P fertility had a major effect on rhizospheric microbial communities. Higher nitrogen often linked to increase crop yield as well as to reduce nitrate leachings. Delayed nitrogen fertilization improves root biomass as reviewed by Mommer et al. (2016). Bacterial endophytes enhance plant growth by facilitating mobilization and uptake of both macronutrients and micronutrients. Shakeel et al. (2015) studied solubilization of zinc (Zn) from different Zn ores like zinc phosphate, zinc carbonate, and zinc oxide carried out by *Bacillus* sp. SH-10 and SH-17 which further enhanced Zn translocation toward the rice rhizosphere. Biostimulation by phytohormones enhances plant growth and impart immunity (Compant et al. 2010). Plant hormones have pivotal roles in the regulation of via cellular signal for immune responses to microbial phytopathogens Pieterse et al. (2012). Overall, various modes contribute in crop productivity enhancement which is solely an outcome of ecological intensification. Agroecological intensification is a practical and knowledge-based approach to compensate and saturate the requirements of marginal farmers so as to increase production using more efficient tools and technique for environmental sustainability. This is a biological mechanisms centric approach to suppress pests and diseases and enhance total crop

photosynthesis for yield enhancement. The management of soil nutrient cycles for a healthier and more productive crop also is substantiated with these beneficial endophytes (Côte et al. 2008). All mechanisms together that compensate and contribute to raise ecological intensification of agriculture have been illustrated in Fig. 9.1

9.7.1 Biostimulation

Endophytic bacteria stimulate plant growth via phytohormone like metabolite production including plant growth regulators (PGRs) such as lipochito-oligosaccharides and lumichrome (Chi et al. 2005; Mehboob et al. 2009). IAA is most common and necessary metabolite to induce plant growth and anatomical development in terms of cell elongation, apical dominance maintenance, vascular tissues formation, senescence prevention of cell. On the other hand, it is also functional to counteract root apical dominance by promoting production of cytokinins help in formation of lateral roots and the root system (Chang et al. 2013). Further, IAA also prevents the ethylene evolution and depletion response to its concentration (Woodward and Bartel 2005). IAA-producing endophytes represent a vast range of bacterial phyla/classes associated with variety of plants. IAA-producing bacterial endophytes are common inhabitants of both the rhizo- as well as the endosphere (Mohite 2013). Tsavkelova et al. (2007) isolated and analyzed IAA-producing endophytic and epiphytic bacteria included the genera *Erwinia, Bacillus, Pseudomonas,* and *Flavobacterium* from terrestrial orchids. Further, supernatants from endophyte cultures stimulated root formation and increased root length as well as the number of developing roots, indicating the potential role of endobacterial auxins in root development (Tsavkelova et al. 2007). Dawwam et al. (2013) observed the enhanced ability of bacterial endophytes of potato roots for IAA synthesis; further, a high frequency of IAA-producing rhizobacteria associated with plants growing under saline conditions (96 and 74% of total bacterial strains) was observed by Mapelli et al. (2012). Earlier, Yanni et al. (2001) studied the effect of IAA-producing rhizobacteria on inoculation with rice seedlings and observed increased seedling vigor, root length, shoot length, and yield of rice plants, and the effect on plant growth includes initiation of early flowering, improvement of crop yield, and bigger fruit size (Albermann et al. 2013). Khan et al. (2014) observed plant growth promotion by GA and IAA Sphingomonas sp. LK11 producing endophytic bacteria in plant growth promotion and productivity enhancement. Endophytic bacteria have been identified as potential crop growth regulators. Plant growth regulators (PGRs) of endophytic origin not only induce growth and development but also alleviate environmental stresses. Endophytic bacteria also produce gibberellins (GAs) which stimulate and

initiate the process of cell elongation, cell division, and morphological differentiation in host plants (Gray and Smith 2005).

The rhizobacteria have been proved beneficial in enhancing productivity of many agricultural important crops such as wheat, soybean, mustered, tomato, bell pepper, mung bean, and rice (Ahemad and Kibret 2014). Phytohormone production affects plant growth promotion in general and cell division, cell elongation, and differentiation of cells and tissues in particular (Duca et al. 2014; Tivendale et al. 2014). The influence of IAA varies with respect to the plant organ and developmental stages, e.g., below ground, it advances xylem and phloem formation in roots, and initiates formation of adventitious and lateral roots (Duca et al. 2014; Tivendale et al. 2014). In above ground, it increases photosynthesis mechanism, pigments biosynthesis, metabolites production, initiation and late development of seed, flower, fruit, and leaves (Duca et al. 2014). However, IAA production in certain amount is also a property of pathogenic microbes; implicate adverse effect (Spaepen et al. 2007). The optimum amount of IAA then increases surface area and length of roots, looses cell wall, and helps in producing root exudates. It also raises two-way traffic for nutrient uptake and transport across membrane down to promote host plant associated microbial growth (Ahemad and Kibret 2014). Few other phytohormones such as Gibberellins are also considered as the most pragmatic phytohormone to enhance the agriculture and horticulture productivity in eco-safe manner. The effect on plant growth includes initiation of early flowering, improvement of crop yield, and bigger fruit size (Albermann et al. 2013). Khan et al. (2014) studied on tomato plant growth promotion by GA and IAA-producing *Sphingomonas* sp. LK11 understand us the consistent significance of phytohormone producing endophytic bacteria in plant growth and health promotion and productivity enhancement.

9.7.2 Nutrient Management

From the pool of essential nutrients, plant requires nitrogen (N), phosphorus (P), and potassium (K) are found in soil in relatively high quantities. Most often, these elements are available in plenty amount in soil due to inaccessibility in soluble or immobilized form, as these are found in complex compound form. Thus, endophytes trigger few metabolic weapons to solubilize the complex compound form of phosphates and thus make available for their host plants to obtain in converted, mobilized, and soluble form. Primarily, nitrogen fixing (diazotrophic) symbionts, such as nodule-forming rhizobia and actinobacteria, are potential contender and often exhibit to replenish ammonia or derived compound into available nitrogen (elemental) and symbolize highly important N input to their respective host plant particularly in nitrogen-deficient soil (Fabra et al. 2010). A vast array of studies reveled the untold story of the biodiversity and microbial community dynamics of associative N-fixing bacteria (Xie et al. 2003; Wakelin et al. 2010; Mao et al. 2011) Endophytic diazotrophic bacteria, particularly G. *diazotrophicus*, *Bacillus* spp.,

Burkholderia spp., and H. *seropedicae*, have been widely found in variety of crop (Estrada et al. 2013).

Phosphorus is another major nutrient with regulatory behavior for plant. Inorganic phosphates available in soils are rapidly immobilized and rendered inaccessible for plants. Due to this rapid immobilization, agricultural soil holds giant reservoirs of inaccessible phosphates (Rodríguez and Fraga 1999). Thus, bacterial endophytes can radially solubilize inorganic phosphates into organic one by production of organic acids or enzymes and making them available for host plant accumulation (Bulgarelli et al. 2013). The solubilization of insoluble phosphates is direct mode of action facilitated by plant growth-promoting bacteria (PGPB) for enhancement of nutrient availability in the rhizosphere. Plant growth-promoting bacteria exhibit in soil and need to outcompete with other bacterial or fungal species commonly residing in the rhizosphere. On the other hand, endophytic bacteria secure their specified ecological niche wherein they function as PGPR such as nutrient immobilization (Rodríguez and Fraga 1999). Phosphate solubilization by these endophytes is prime mechanism to help the plant to accumulate mobilized nutrient. Phosphate-solubilizing endophytes of peanut helped plant for growth and health promotion were reported by Taurian et al. (2010). Phosphate-solubilizing bacteria are playing several other functions in terms of plant beneficial properties, which include the ability to grow on nitrogen-free medium and the production of phytohormones like metabolites. On the other hand, enzyme or acid mediated phosphate solubilization also defends plant against few pathogenic entities in rhizosphere. The active growth of plant is also favored by phosphate-solubilizing endophytes with their mechanistic behavior to restore environmental pressure. Kuklinsky-Sobral et al. (2004) analyzed both the epi- and endophytic bacteria isolated from several growth stages and different cultivars of soybean and found that from the early stages of plant growth, phosphate solubilizers were less than 50 and about 60% endobacteria represented Pseudomonadaceae, Burkholderiaceae, and Enterobacteriaceae. Puente et al. (2009) studied endophytic bacteria isolated from cardon cactus, grow in desert, and able to establish on solid rock. The majorities of these endophytes were capable of solubilizing Fe/Ca-phosphates and pulverizing rocks present in cactus rhizosphere and get colonized for development of seedlings. The endophytes were grown in pot to determine their potential to solubilize mineral and rock phosphate, where bacterized plants grew well in the absence of nutrients and on the other hand endophyte-free cacti failed to develop. This was suggested that the endophytes promote plant growth by providing mineralized nutrient sink in rhizosphere (Puente et al. 2009). Palaniappan et al. (2010) studied on *Lespedeza* root nodule inhabiting endophytes and found them able to solubilize mineral phosphates along with other plant growth-promoting attribute. Earlier, Dias et al. (2009) also suggested that endophytes of strawberry represent *Bacillus subtilis* and *B. megaterium* as dominant genera solubilize calcium phosphate in vitro and in vivo. The efficiency of phosphate solubilization markedly differs among the rhizospheric microbial population and with different genera. Further, it could be established that endophytes having plant growth promotion ability bear phosphate solubilization traits with other abilities such as IAA production, enzyme production (Gusain et al. 2015).

Acid-producing endophytes are able to enhance the solubilization of phosphatic rock (Gyaneshwar et al. 2002). Iron chelation accomplished by siderophores is a common function that exhibited among the more than half of rhizospheric bacterial communities (Sayyed et al. 2013). For example, metagenome of rice bacterial endophyte (non-cultivable) has been explored with a high number of genes that are expressible and encode several proteins which potentially employed in synthesis of iron-chelating agent (siderophore). Thus, after chelation, ferric-siderophore membrane receptors uptake iron via protein transporters in expense of energy currency (active transport) (Sessitsch et al. 2012). Iron-chelating bacteria can deprive putative pathogens for available iron, therefore exerting antagonistic activity (Sánchez-Contreras et al. 2013).

9.7.3 Disease Management

In the endophytic relationship, bacteria provide a unique opportunity for plant protection and biological control of deleterious phytopathogens infecting plants. Antifungal activity of endophytes in relation to biosynthesis of diverse allelochemicals has been studied by Lodewyckx et al. (2002). There are certain endophytic bacteria exist to contribute significant plant defense against soilborne fungal pathogens (Hallmann et al. 1997; Sturz and Nowak 2000). The extensively recognized strategies of biological control employed by endophyte are antibiosis, antagonism, and competition for an ecological (trophic) niche (Blumenstein et al. 2015), production of inhibitory allelochemicals (Singh et al. 2015), and immunogenic response by induced systemic resistance (ISR) (Gómez-Lama et al. 2014) in host plants against pathogens and/or abiotic stresses. More prominently, ISR mediated by free-living rhizobacterial as well as endophytic PGPB, but iPGPB (intracellular PGPB) has also been accounted to have ISR activity. For example, *P. fluorescens* provide induced systemic resistance as defense against *F. oxysporum* f. sp. *radicislycopersici* on tomato (M'piga et al. 1997), *B. pumilus SE34* against *F. oxysporum* f. sp. *pisi* on pea roots (Benhamou et al. 1996), *P. fluorescens* EP1 triggered ISR in tomato and sugarcane against *Colletotrichum falcatum* and *Verticllium dahliae* respectively (Sharma and Nowak 1998), and *F. oxysporum* f. sp. *vasinfectum* on cotton roots (Conn and Day 1996). So far, mechanism of disease suppression has not been come out clearly (French et al. 2016). More research is needed to fill the lacuna of understanding mechanism of endophyte mediated biocontrol system.

Antagonism of phytopathogens by endophytes can be broken down due to production of lytic enzymes or antimicrobials to make the shared environment inhospitable for pathogens (McSpadden-Gardener and Fravel 2002). Increased knowledge of the multiple modes of action used by BCAs has reduced the use of this method as a primary selection step since an in vitro screen on agar does little to

mimic the natural environment and readily eliminates potential BCAs that utilize other modes of action. Similarly, *Bacillus* spp. found to secrete several commercial products like antibiotics shared with plant pathogens (Gupta and Utkhede 1986; Toharisman et al. 2005). An advantage of *Bacillus* produced antibiotics is that they are often effective against a diversity of plant pathogens (Kloepper et al. 2004). Some bacteria produce phytohormones and nutrient solubilizing enzymes that produce PGP effects. These traits coupled with the biocontrol ability produce deleterious effect on phytopathogens. Abraham et al. (2013) isolated leaf, petiole, and root tissues bacterial endophytes from endophytic *Hevea brasiliensis* capable to arrest the growth of *Phytophthora meadii* causing leaf fall disease. The bioassay was evaluated in two clones of *H. brasiliensis* with *Alcaligenes* sp. Thus, study suggests biocontrol ability of bacterial endophytes was specific to crop specific plant variety.

Several commercial BCAs operate primarily through the mechanism of niche displacement. *Pseudomonas fluorescens* A506 (BlightBan A506) colonizes apple and pear blossoms and prevents *Erwinia. amylovora* from reaching adequate populations for quorum sensing by excluding resources required for the pathogen (Wilson and Lindow 1993). The key to the success of mycoparasitism in biological control is that the biological control agent (BCA) must come in direct contact with the targeted pathogen and must persist in the same environment as the pathogen (Card et al. 2016). The last and most recently recognized mode of action is induction of host defenses commonly known as induced resistance. There are several advantages to induced resistance that is often effective again a broad range of pathogens (van Wees et al. 1999) evolved for broad-spectrum activity. Overall, the key to understand the modes of action utilized by BCAs is to evaluate multiple modes of action require for disease incidence reduction.

9.7.4 Productivity Enhancement

Sustainable agriculture needs the exploitations of different strategies to increase or maintain the current scenario and fate of food production to make available and enough food to every people, without damaging the agricultural ecosystem, environment and human health. Thus, endophytes are thought to be ideal and perfect contender to cope these problems by providing the internal plant homeostasis, plant growth and health promotion and resisting biotic and abiotic stresses (Sherameti et al. 2008). These are excel to promote the growth of primary as well as secondary yield parameter of plant by managing nutrient sink in the rhizosphere, protecting plants from deleterious infections mediated by fungal and bacterial pathogens and ultimately production of plant growth regulators (Hallmann et al. 1997; Sturz and Nowak 2000; Lodewyckx et al. 2002; Hardoim et al. 2008). Thus, endophytes are known to enhance the yield and their bioactive content (Tiwari et al. 2010, 2013).

These endophytes help plant to uptake solubilized phosphate (Wakelin et al. 2004), enhancing hyphal growth and mycorrhizal colonization (Will and Sylvia 1990) and by producing siderophores (iron-chelating molecules which increase its availability to plants) (Costa and Loper 1994). Endophytic bacteria found responsible for the allelopathic effects observed with these plants over maize, causing reduced plant emergence and plant height (Sturz et al. 1997). Dutta et al. (2008) reported improvement of plant growth and disease suppression in pea plant co-inoculated with fluorescent pseudomonads and *Rhizobium*. Hung et al. (2007) studied the effect of endophytes on soybean growth and development and proved influence positively on root weights. PGP endophytic bacteria influence seed germination, root and hypocotyl growth and increased seedling vigor. Presence of root endophyte in the cortical parenchymatous tissue of *Vetiver* used for enhancement of essential oil metabolism (del Giudice et al. 2008). Harish et al. (2009) studied the effect of bio-formulations of consortial combinations of rhizobacteria *Pseudomonas fluorescens* (Pf1) and endophytic *Bacillus* sp. (EPB22) enhanced yield of banana. Populations of endophytic bacteria also exhibited in high and stable number throughout the growing period. Stajković et al. (2009) assessed productivity enhancement *Medicago sativa* L by non-rhizobial endophytes from the root nodules. One of the bacterial endophyte, *Bacillus subtilis* HC8, isolated from hogweed *Heracleum sosnowskyi*, found potential to promote plant growth and biological control of foot and root rot diseases in tomato (Malfanova et al. 2011). In field experiment inoculated with endophytic bacteria exhibited sugarcane plants more superior in terms of plant height and shoot counts. *Bacillus* spp. and *Pseudomonas* spp. have been observed to promote plant growth in grape wine, tomato, maize, rice, and sugar beet through various mechanisms (Wang et al. 2009). Conventional manipulation of soil microorganisms has been practised since immemorial decades. For example, sewage and manure applications for enhancement of soil fertility dramatically affect autochthonous communities of soil biota. The practice of monoculture is in itself instrumental in altering soil microbial populations at the field level. Thus, maybe it is possible to influence plant endophytic populations by seed bacterization, soil inoculation and by identifying the genetic (bacterial) component responsible for their beneficial effects. Endophytic microbes have merit over rhizospheric bacteria as they deliver fixed nitrogen straight to host plant tissue and able to fix nitrogen more competently then the free-living bacteria due to less oxygen pressure in the interior of plants than that of soil. Ji et al. (2014) studied 576 endophytes as substitute of chemical fertilizers and decrease production costs as well as a substantial increase in crops production. Mercado-Blanco et al. (2016) reported *Pseudomonas fluorescens* PICF7, an indigenous olive roots inhabitant, displays endophytic lifestyle in this woody crop and exerts biocontrol against the fungal phytopathogen *Verticillium dahlia* due their PGP behavior showed enhanced vegetative growth significantly increases in term of number of grains (up to 19.5%) and grain weight (up to 20.5%) per plant. Govindarajan et al. (2008) proved the significant effect of *Burkholderia vietnamensis* as an endophyte increase grain yield

in paddy crop. Jha et al. (2013) explored the potential of endophytic association with plant in agricultural sustainability in particular and yield enhancement in general. Potential of biofertilizers formulated using endophytic bacteria for enhanced production of banana in sustained way (Tani et al. 2015). *Pseudomonas fluorescens* PICF7, an indigenous olive roots inhabitant, displays endophytic lifestyle in this woody crop and exerts biocontrol against the fungal phytopathogen *Verticillium dahlia* and displayed effective role in enhancement of barley yield (Marcado-Balnco et al. 2016).

9.8 Conclusion

In modern agriculture, endophytes are contributing equilibrium between growing demand and agricultural production. Intensification and extensification augment ecological intensification to boost crop yield and minimize negative impacts and ensure agricultural productivity enhancement. To reduce ecological harm to soil and thus transform in 'ecological intensification' beneficial endophytes can spoor up the need of agricultural sustainability and intensification. Microbiome residing in plant roots can be subset and reasonable to enhance crop production and ecological intensification. Concerning on endophytic more precisely and plant growth-promoting nature of endophyte in particular drawn attention for their bio-formulations and use in strengthen the future of green agriculture. The aim has to be transparent to harness the reservoir of beneficial endophytic bacterial populations capable to restore soil sources and stabilize them at optimum levels. The challenge to the research community will be to develop systems to optimize beneficial plant–endophyte bacterial relationships. More concerned research must be carried out on how such relationships can be employed in productively enhancement so as to sustain agricultural ecology.

References

Abraham A, Philip S, Jacob CK, Jayachandran K (2013) Novel bacterial endophytes from *Hevea brasiliensis* as biocontrol agent against *Phytophthora* leaf fall disease. Biocontrol 58(5):675–684

Aeron A, Chauhan PS, Dubey RC, Maheshwari DK, Bajpai VK (2014) Root nodule bacteria from *Clitoria ternatea* L. are putative invasive nonrhizobial endophytes. Can J Microbiol 61(2):131–142

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J King Saud Univer Sci 26(1):1–20

Albermann S, Linnemannstöns P, Tudzynski B (2013) Strategies for strain improvement in *Fusarium fujikuroi*: overexpression and localization of key enzymes of the isoprenoid pathway and their impact on gibberellin biosynthesis. App Microbiol Biotechnol 97(7):2979–2995

Araújo WL, Maccheroni W Jr, Aguilar-Vildoso CI, Barroso PA, Saridakis HO, Azevedo JL (2001) Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. Can J Microbiol 47(3):229–236

Aserse AA, Rasanen LA, Aseffa F, Hailemariam A, Lindstrom K (2013) Diversity of sporadic symbionts and nonsymbiotic endophytic bacteria isolated from nodules of woody, shrub, and food legumes in Ethiopia. App Microbiol Biotechnol 97:10117e10134

Assessment ME (2005) Millennium ecosystem assessment findings. Millennium Ecosystem Assessment

Babalola OO, Glick BR (2012) Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. Sci Res Essay 7(28):2431–2439

Bacon CW, Hinton DM (2007) Bacterial endophytes: the endophytic niche, its occupants, and its utility. In: Gnanamanickam SS (ed) Plant-associated Bacteria. Springer, Netherlands, pp 155–194

Bacon CW, White J (2000) Microbial endophytes. Marcel Dekker Inc., New York, 10016 p 500

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48:230–238

Bais HP, Loyola-Vargas VM, Flores HE, Vivanco JM (2001) Root-specific metabolism: the biology and biochemistry of underground organs. Vitro Cell Dev Biol Plant 37(6):730–741

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Ann Rev Plant Biol 57:233–266

Balachandar D, Sandhiya GS, Sugitha TC, Kumar K (2006) Flavonoids and growth hormones influence endophytic colonization and in planta nitrogen fixation by a diazotrophic *Serratia* sp. in rice. World J Microbiol Biotechnol 22:707–712

Bautista VV, Monsalud RG, Yokota A (2010) *Devosia yakushimensis* sp. nov., isolated from root nodules of *Pueraria lobata* (Willd.) Ohwi. Int J Syt Evol Microbiol 60(3):627–632

Beattie GA, Lindow SE (1995) The secret life of foliar bacterial pathogens on leaves. Ann Rev Phytopathol 33(1):145–172

Bejarano A, Ramírez-Bahena MH, Velázquez E, Peix A (2014) *Vigna unguiculata* is nodulated in Spain by endosymbionts of Genisteae legumes and by a new symbiovar (*vignae*):of the genus *Bradyrhizobium*. Syst Appl Microbiol 37(7):533–540

Benhamou N, Kloepper JW, Quadt-Hallman A, Tuzun S (1996) Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol 112(3):919–929

Blumenstein K, Albrectsen BR, Martín JA, Hultberg M, Sieber TN, Helander M, Witzell J (2015) Nutritional niche overlap potentiates the use of endophytes in biocontrol of a tree disease. Biocontrol 60(5):655–667

Bogino PC, Oliva MDLM, Sorroche FG, Giordano W (2013) The role of bacterial biofilms and surface components in plant-bacterial associations. Int J Mol Sci 14(8):15838–15859

Boonsnongcheep P, Prathanturarug S, Takahashi Y, Matsumoto A (2016) *Rhizobium puerariae* sp. nov., an endophytic bacterium from the root nodules of the medicinal plant *Pueraria candollei* var. *candollei*. Int J Syt Evol Microbiol 66(3):1236–1241

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Ann Rev Plant Biol 64:807–838

Canny MJ (1995) Apoplastic water and solute movement: new rules for an old space. Ann Rev Plant Biol 46(1):215–236

Card S, Johnson L, Teasdale S, Caradus J (2016) Deciphering endophyte behaviour-the link between endophyte biology and efficacious biological control agents. FEMS Microbiol Ecol fiw114

Carro L, Flores-Félix JD, Cerda-Castillo E, Ramírez-Bahena MH, Igual JM, Tejedor C, Velázquez E, Peix A (2013) *Paenibacillus endophyticus* sp. nov., isolated from nodules of *Cicer arietinum*. Int J Syt Evol Microbiol 63(12):4433–4438

Carro L, Flores-Félix JD, Ramírez-Bahena MH, García-Fraile P, Martínez-Hidalgo P, Igual JM, Tejedor C, Peix A, Velázquez E. (2014) *Paenibacillus lupini* sp. nov., isolated from nodules of *Lupinus albus*. Int J Syt Evol Microbiol 64(9):3028–3033

Carro L, Riesco R, Spröer C, Trujillo ME (2016) *Micromonospora luteifusca* sp. nov. isolated from cultivated *Pisum sativum*. Syst Appl Microbiol 39(4):237–242

Chahboune R, Carro L, Peix A, Barrijal S, Velázquez E, Bedmar EJ (2011) *Bradyrhizobium cytisi* sp. nov., isolated from effective nodules of *Cytisus villosus*. Int J Syt Evol Microbiol 61 (12):2922–2927

Chahboune R, Carro L, Peix A, Ramírez-Bahena MH, Barrijal S, Velázquez E, Bedmar EJ (2012) *Bradyrhizobium rifense* sp. nov. isolated from effective nodules of *Cytisus villosus* grown in the Moroccan Rif. Syst Appl Microbiol 35(5):302–305

Chaintreuil C, Giraud E, Prin Y, Lorquin J, Bâ A, Gillis M et al (2000) Photosynthetic bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. Appl Environ Microbiol 66(12):5437–5447

Chang YL, Wang JY, Wang ET, Liu HC, Sui XH, Chen WX (2011) *Bradyrhizobium lablabi* sp. nov., isolated from effective nodules of *Lablab purpureus* and *Arachis hypogaea*. Int J Syt Evol Microbiol 61(10):2496–2502

Chang L, Ramireddy E, Schmülling T (2013) Lateral root formation and growth of *Arabidopsis* is redundantly regulated by cytokinin metabolism and signalling genes. J Exp Bot 64(16):5021–5032

Chen WF, Sui XH, Wang ET, Chen WX (2011) *Rhizobium vignae* sp. nov., a symbiotic bacterium isolated from multiple legume species. Int J Syt Evol Microbiol 61(3):580–586

Chernin LS (2011) Quorum-sensing signals as mediators of PGPRs' beneficial traits. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, Germany, pp 209–236

Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71(11):7271–7278

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42(5):669–678

Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A (2011) Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol 62(1):188–197

Conn CE, Day FP (1996) Response of root and cotton strip decay to nitrogen amendment along a barrier island dune chronosequence. Can J Microbiol 74(2):276–284

Conn VM, Franco CM (2004) Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism. Appl Environ Microbiol 70(11):6407–6413

Costa JM, Loper JE (1994) Characterization of siderophore production by the biological control agent *Enterobacter cloacae*. Mol Plant Microbe Interac 7(4):440–448

Côte F, Tomekpe K, Staver C, Depigny S, Lescot T, Markham R (2008) Agro-ecological intensification in banana and plantain (*Musa* spp.): an approach to develop more sustainable cropping systems for both smallholder farmers and large-scale commercial producers. In: IV International symposium on Banana: International conference on banana and plantain in Africa: Harnessing International, pp 457–463

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245(1):35–47

Dall'Agnol RF, Ribeiro RA, Ormeño-Orrillo E, Rogel MA, Delamuta JRM, Andrade DS et al. (2013) *Rhizobium freirei* sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. Int J Syst Evol Microbiol 63(11):4167–4173

Dall'Agnol RF, Ribeiro RA, Delamuta JRM, Ormeño-Orrillo E, Rogel MA, Andrade DS et al. (2014) *Rhizobium paranaense* sp. nov., an effective N2-fixing symbiont of common bean (*Phaseolus vulgaris* L.): with broad geographical distribution in Brazil. Int J Syst Evol Microbiol 64(9):3222–3229

Darbyshire JF, Greaves MP (1973) Bacteria and protozoa in the rhizosphere. Pestic Sci 4:349–360

Dawwam GE, Elbeltagy A, Emara HM, Abbas IH, Hassan MM (2013) Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. Ann Agric Sci 58(2):195–201

de Melo Pereira GV, Magalhães KT, Lorenzetii ER, Souza TP, Schwan RF (2012) A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. Microb Ecol 63(2):405–417

De Meyer SE, Cnockaert M, Ardley JK, Maker G, Yates R, Howieson JG, Vandamme P (2013a) *Burkholderia sprentiae* sp. nov. isolated from Lebeckia ambigua root nodules. Int J Sys Evol Microbiol 63(11):3950–3957

De Meyer SE, Cnockaert M, Ardley JK, Trengove RD, Garau G, Howieson JG, Vandamme P (2013b) *Burkholderia rhynchosiae* sp. nov. isolated from *Rhynchosia ferulifolia* root nodules. Int J Sys Evol Microbiol 63(11):3944–3949

De Meyer SE, Cnockaert M, Ardley JK, Van Wyk BE, Vandamme PA, Howieson JG (2014) *Burkholderia dilworthii* sp. nov. isolated from *Lebeckia ambigua* root nodules. Int J Sys Evol Microbiol 64(4):1090–1095

De Meyer SE, Tan HW, Heenan PB, Andrews M, Willems A (2015a) *Mesorhizobium waimense* sp. nov. isolated from *Sophora longicarinata* root nodules and *Mesorhizobium cantuariense* sp. nov. isolated from *Sophora microphylla* root nodules. Int J Sys Evol Microbiol 65 (10):3419–3426

De Meyer SE, De Beuf K, Vekeman B, Willems A (2015b) A large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium). Soil Biol Biochem 83:1–11

De Meyer SE, Tan HW, Andrews M, Heenan PB, Willems A (2016) *Mesorhizobium calcicola* sp. nov. *Mesorhizobium waitakense* sp. nov. *Mesorhizobium sophorae* sp. nov. *Mesorhizobium newzealandense* sp. nov. and *Mesorhizobium kowhaii* sp. nov. isolated from Sophora root nodules. Int J Sys Evol Microbiol 66(2):786–795

De Oliveira Costa LE, de Queiroz MV, Borges AC, de Moraes CA, de Araújo EF (2012) Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). Braz J Microbiol 43(4):1562–1575. doi:10.1590/S1517-838220120004000041

de Torres-zabala M, Bennett MH, Mansfield JW, R. Egea, L. Bo, and M. Grant (2007) *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. EMBO J 26

Del Giudice L, Massardo DR, Pontieri P, Bertea CM, Mombello D, Carata E, De Stefano M (2008) The microbial community of Vetiver root and its involvement into essential oil biogenesis. Env Microbiol 10(10):2824–2841

Deng ZS, Zhao LF, Xu L, Kong ZY, Zhao P, Qin W, Wei GH (2011) *Paracoccus sphaerophysae* sp. nov., a siderophore-producing, endophytic bacterium isolated from root nodules of *Sphaerophysa salsula*. Int J Sys Evol Microbiol 61(3):665–669

Dent KC, Stephen JR, Finch-Savage WE (2004) Molecular profiling of microbial communities associated with seeds of *Beta vulgaris* subsp. vulgaris (sugar beet). J Microbiol Methods 56 (1):17–26

Dhole A, Shelat H, Vyas R, Jhala Y, Bhange M (2016) Endophytic occupation of legume root nodules by nifH-positive non-rhizobial bacteria, and their efficacy in the groundnut (Arachis hypogaea). Ann Microbiol 1–11

Dias AC, Costa FE, Andreote FD, Lacava PT, Teixeira MA, Assumpção LC, Melo IS (2009) Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. World J Microbiol Biotechnol 25(2):189–195

Doley P, Jha DK (2016) Antimicrobial activity of bacterial endophytes from medicinal endemic plant Garcinia lancifolia Roxb. Ann Plant Sci 4(12):1243–1247

Duca D, Lorv J, Patten CL, Rose D, Glick BR (2014) Indole-3-acetic acid in plant–microbe interactions. Antonie Van Leeuwenhoek 106(1):85–125

Dugué P, Vayssieres J, Chia E, Ouedraogo S, Havard M, Coulibaly D, Vall E (2011) L'intensification écologique: réflexions pour la mise en pratique de ce concept dans les zones de savane d'Afrique de l'Ouest. In: Partenariat, modélisation, expérimentations: quelles leçons pour la conception de l'innovation et l'intensification écologique? p 15

Dutta S, Mishra AK, Kumar BD (2008) Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol Biochem 40(2):452–461

Elasri M, Delorme S, Lemanceau P, Stewart G, Laue B, Glickmann E, Oger PM, Dessaux Y (2001) Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. Appl Environ Microbiol 67:1198–1209

Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato YI, Morisaki H, Minamisawa K (2000) Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Sci Plant Nutr 46(3):617–629

Elvira-Recuenco M, Van Vuurde JWL (2000) Natural incidence of endophytic bacteria in pea cultivars under field conditions. Can J Microbiol 46(11):1036–1041

Estrada GA, Baldani VLD, de Oliveira DM, Urquiaga S, Baldani JI (2013) Selection of phosphate-solubilizing diazotrophic *Herbaspirillum* and *Burkholderia* strains and their effect on rice crop yield and nutrient uptake. Plant Soil 369(1–2):115–129

Estrella MJ, Munoz S, Soto MJ, Ruiz O, Sanjuan J (2009) Genetic diversity and host range of rhizobia nodulating *Lotus tenuis* in typical soils of the Salado river basin (Argentina). Appl Environ Microbiol 75:1088e1098

Fabra A, Castro S, Taurian T, Angelini J, Ibañez F, Dardanelli M, Valetti L (2010) Interaction among *Arachis hypogaea* L. (peanut) and beneficial soil microorganisms: how much is it known? Crit Rev Microbiol

Faria DC, Dias ACF, Melo IS, de Carvalho Costa FE (2013) Endophytic bacteria isolated from orchid and their potential to promote plant growth. World J Microbiol Biotechnol 29(2):217–221

Ferreira A, Quecine MC, Lacava PT, Oda S, Azevedo JL, Araújo WL (2008) Diversity of endophytic bacteria from Eucalyptus species seeds and colonization of seedlings by *Pantoea agglomerans*. FEMS Microbiol Lett 287(1):8–14

Flores-Félix JD, Carro L, Velázquez E, Valverde Á, Cerda-Castillo E, García-Fraile P, Rivas R (2013) *Phyllobacterium endophyticum* sp. nov., isolated from nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 63(3):821–826

Flores-Félix JD, Carro L, Ramírez-Bahena MH, Tejedor C, Igual JM, Peix A, Velázquez E (2014a) *Cohnella lupini* sp. nov., an endophytic bacterium isolated from root nodules of Lupinus albus. Int J Syst Evol Microbiol 64(1):83–87

Flores-Félix JD, Mulas R, Ramírez-Bahena MH, Cuesta MJ, Rivas R, Brañas J, Velázquez E (2014b) *Fontibacillus phaseoli* sp. nov. isolated from *Phaseolus vulgaris* nodules. Antonie Van Leeuwenhoek 105(1):23–28

Forchetti G, Masciarelli O, Izaguirre MJ, Alemano S, Alvarez D, Abdala G (2010) Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. Curr Microbiol 61(6):485–493

Founoune H, Duponnois R, Ba AM, Sall S, Branget I, Lorquin J, Chotte JL (2002) Mycorrhiza helper bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. New Phytol 153(1):81–89

French E, Kim BS, Iyer-Pascuzzi AS (2016) Mechanisms of quantitative disease resistance in plants. In: Seminars in cell developmental biology May 19 Academic Press

Gardener BBM, Fravel DR (2002) Biological control of plant pathogens: research, commercialization, and application in the USA. Plant Health Progress 10

Germida J, Siciliano S (2001) Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol Fertil Soil 33(5):410–415

Germida JJ, Siciliano SD, de Freitas JR, Seib AM (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26(1):43–50

Ghosh PK, De TK, Maiti TK (2015a) Ascorbic acid production in root, nodule and *Enterobacter* spp. (Gammaproteobacteria) isolated from root nodule of the legume *Abrus precatorius* L. Biocatal Agric Biotechnol 4(2):127–134

Ghosh PK, Kumar De T, Maiti TK (2015b) Production and metabolism of indole acetic acid in root nodules and symbiont (*Rhizobium undicola*): isolated from root nodule of aquatic medicinal legume *Neptunia oleracea* Lour. J Bot 2015

Gilroy S, Jones DL (2000) Through form to function: root hair development and nutrient uptake. Trend Plant Sci 5(2):56–60

Gómez-Lama Cabanás C, Schilirò E, Valverde-Corredor A, Mercado-Blanco J (2014) The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. Front Microbiol 5:427

Gond SK, Bergen MS, Torres MS, White JF Jr (2015) Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiol Res 172:79–87

Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C (2008) Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microbial Ecol 55(1):21–37

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37(3):395–412

Grönemeyer JL, Hurek T, Bünger W, Reinhold-Hurek B (2016) *Bradyrhizobium vignae* sp. nov., a nitrogen-fixing symbiont isolated from effective nodules of *Vigna* and *Arachis*. Int J Syst Evol Microbiol 66(1):62–69

Guerrouj K, Ruíz-Díez B, Chahboune R, Ramírez-Bahena MH, Abdelmoumen H, Quinones MA, Peix A (2013) Definition of a novel symbiovar (sv. retamae): within *Bradyrhizobium retamae* sp. nov., nodulating *Retama sphaerocarpa* and *Retama monosperma*. Syst Appl Microbiol 36 (4):218–223

Gupta VK, Utkhede RS (1986) Factors affecting the production of antifungal compounds by *Enterobacter aerogenes* and *Bacillus subtilis*, antagonists of *Phytophthora cactorum*. J Phytopathol 117(1):9–16

Gusain YS, Kamal R, Mehta CM, Singh US, Sharma AK (2015) Phosphate solubilizing and indole-3-acetic acid producing bacteria from the soil of Garhwal Himalaya aimed to improve the growth of rice. J Environ Biol 36(1):301

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Food security in nutrient-stressed environments: exploiting plants' genetic capabilities. Springer, Netherlands, pp 133–143

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43(10):895–914

Hallmann J, Davies KG, Sikora R (2009) 17 Biological control using microbial pathogens, Endophytes and Antagonists. Root-knot Nematodes, 380

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trend Microbiol 16(10):463–471

Harish S, Kavino M, Kumar N, Balasubramanian P, Samiyappan R (2009) Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against Banana bunchy top virus. Biol Cont 51(1):16–25

Hartmann A, Rothballer M, Hense BA, Schröder P (2015) Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. The plant microbiome and its importance for plant and human health, 41

Hoffman MT, Gunatilaka MK, Wijeratne K, Gunatilaka L, Arnold AE (2013) Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. PLoS One 8(9):e73132

Hoque MS, Broadhurst LM, Thrall PH (2011a) Genetic characterization of root-nodule bacteria associated with *Acacia salicina* and *A. stenophylla* (*Mimosaceae*):across south-eastern Australia. Int J Syst Evol Microbiol 61:299–309

Hoque MS, Broadhurst LM, Thrall PH (2011b) Genetic characterization of root-nodule bacteria associated with *Acacia salicina* and *A. stenophylla* (*Mimosaceae*):across south-eastern Australia. Int J Syst Evol Microbiol 61:299–309

Howieson JG, De Meyer SE, Vivas-Marfisi A, Ratnayake S, Ardley JK, Yates RJ (2013) Novel *Burkholderia* bacteria isolated from Lebeckia ambigua–a perennial suffrutescent legume of the fynbos. Soil Biol Biochem 60:55–64

Hung PQ, Kumar SM, Govindsamy V, Annapurna K (2007) Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. Biol Fertil Soils 44(1):155–162

James EK, Olivares FL, Baldani JI, Döbereiner J (1997) *Herbaspirillum*, an endophytic diazotroph colonizing vascular tissue *Sorghum bicolor* L. Moench J Exp Bot 48(3):785–798

James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PP, Ladha JK (2002) Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Mol Plant Microb Interact 15(9):894–906

Jha PN, Gupta G, Jha P, Mehrotra R (2013) Association of rhizospheric/endophytic bacteria with plants: a potential gateway to sustainable agriculture. Green J Agric Sci 3(2):73–84

Ji SH, Gururani MA, Chun SC (2014) Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiol Res 169(1):83–98

Jiao YS, Yan H, Ji ZJ, Liu YH, Sui XH, Wang ET, Chen WF (2015) *Rhizobium sophorae* sp. nov. and *Rhizobium sophoriradicis* sp. nov., nitrogen-fixing rhizobial symbionts of the medicinal legume *Sophora flavescens*. Int J Syst Evol Mcrobiol 65(2):497–503

Jiao YS, Yan H, Ji ZJ, Liu YH, Sui XH, Zhang XX, Chen WF (2015b) *Phyllobacterium sophorae* sp. nov., a symbiotic bacterium isolated from root nodules of *Sophora flavescens*. Int J Syst Evol Microbiol 65(2):399–406

Kandel SL, Herschberger N, Kim SH, Doty SL (2015) Diazotrophic endophytes of poplar and willow for growth promotion of rice plants in nitrogen-limited conditions. Crop Sci 55 (4):1765–1772

Ker K, Seguin P, Driscoll BT, Fyles JW, Smith DL (2012) Switchgrass establishment and seeding year production can be improved by inoculation with rhizosphere endophytes. Biomass Bioenergy 47:295–301

Kesari V, Ramesh AM, Rangan L (2013) *Rhizobium pongamiae* sp. nov. from root nodules of Pongamia pinnata. BioMed Res Int, 2013

Khalid R, Zhang YJ, Ali S, Sui XH, Zhang XX, Amara U, Hayat R (2015) *Rhizobium pakistanensis* sp. nov., isolated from groundnut (*Arachis hypogaea*) nodules grown in rainfed Pothwar, Pakistan. Antonie Van Leeuwenhoek 107(1):281–290

Khalifa AY, Almalki MA (2015) Isolation and characterization of an endophytic bacterium, *Bacillus megaterium* BMN1, associated with root-nodules of *Medicago sativa* L. growing in Al-Ahsaa region, Saudi Arabia. Annal Microbiol 65(2):1017–1026

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Lee IJ (2014) Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. J Microbiol 52(8):689–695

Khush GS (2001) Green revolution: the way forward. Nature Rev Genet 2(10):815–822

Kittiwongwattana C, Thawai C (2013) *Rhizobium paknamense* sp. nov., isolated from lesser duckweeds (*Lemna aequinoctialis*). Int J Syst Evol Microbiol 63(10):3823–3828

Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathol 94(11):1259–1266

Knoth JL, Kim SH, Ettl GJ, Doty SL (2014) Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia. New Phytol 201(2):599–609

Kobayashi DY, Palumbo JD (2000) Bacterial endophytes and their effects on plants and uses in agriculture. Microb Endophyt 19:199–233

Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Env Microbiol 6:1244–1251

Kumar S, Pandey P, Maheshwari DK (2009) Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. Eur J Soil Biol 45:334–340

Ladha JK, Reddy PM (2003) Nitrogen fixation in rice systems: state of knowledge and future prospects. Plant Soil 252(1):151–167

Lakshmanan V, Kitto SL, Caplan JL, Hsueh YH, Kearns DB, Wu YS, Bais HP (2012) Microbe-associated molecular patterns-triggered root responses mediate beneficial rhizobacterial recruitment in *Arabidopsis*. Plant Physiol 160(3):1642–1661

Lata H, Li XC, Silva B, Moraes RM, Halda-Alija L (2006) Identification of IAA-producing endophytic bacteria from micropropagated *Echinacea* plants using 16S rRNA sequencing. Plant Cell Tiss Org Cul 185(3):353–359

Lee SC, Luan S (2012) ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant, Cell Environ 135(1):53–60

Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, Chen WX, (2008) *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. Int J Syst Evol Microbiol 58:1409e1413

Lin SY, Hung MH, Hameed A, Liu YC, Hsu YH, Wen CZ, Young CC (2015) *Rhizobium capsici* sp. nov., isolated from root tumor of a green bell pepper (*Capsicum annuum* var. grossum) plant. Antonie Van Leeuwenhoek 107(3):773–784

Liu TY, Li Y, Liu XX, Sui XH, Zhang XX, Wang ET, Puławska J (2012) *Rhizobium cauense* sp. nov., isolated from root nodules of the herbaceous legume *Kummerowia stipulacea* grown in campus lawn soil. Syst Appl Microbiol 35(7):415–420

Liu WY, Ridgway HJ, James TK, James EK, Chen WM, Sprent JI, Andrews M (2014) *Burkholderia* sp. induces functional nodules on the South African invasive legume Dipogon lignosus (Phaseoleae):in New Zealand soils. Microbial Ecol 68(3):542–555

Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie DV (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21(6):583–606

López-López A, Rogel-Hernández MA, Barois I, Ceballos AIO, Martínez J, Ormeño-Orrillo E, Martínez-Romero E (2012) *Rhizobium grahamii* sp. nov., from nodules of *Dalea leporina*, *Leucaena leucocephala*, *Clitoria ternatea*, and *Rhizobium mesoamericanum* sp. nov., from nodules of *Phaseolus vulgaris*, siratro, cowpea and *Mimosa pudica*. Int J Syst Evol Microbiol 62(9):2264–2271

Lottmann J, Heuer H, Smalla K, Berg G (1999) Influence of transgenic T4-Lysozime-producing potato plants on potentially beneficial plant-associated bacteria. FEMS Microbiol Ecol 29:365–377

Lu JK, Dou YJ, Zhu YJ, Wang SK, Sui XH, Kang LH (2014) *Bradyrhizobium ganzhouense* sp. nov., an effective symbiotic bacterium isolated from *Acacia melanoxylon* R. Br. nodules. Int J Syst Evol Microbiol 64(6):1900–1905

Lugtenberg BJ, Chin-A-Woeng TF, Bloemberg GV (2002) Microbe–plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek 81(1–4):373–383

Luo S, Xu T, Chen L, Chen J, Rao C, Xiao X, Liu Y (2012) Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. Appl Microbiol Biotechnol 93 (4):1745–1753

Maheshwari DK, Kumar S, Kumar B, Pandey P (2010) Co-inoculation of Urea and DAP tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as integrated approach for growth enhancement of *Brassica juncea*. Ind J Microbiol 50(4):425–431

Malfanova N, Kamilova F, Validov S, Shcherbakov A, Chebotar V, Tikhonovich I, Lugtenberg B (2011) Characterization of Bacillus subtilis HC8, a novel plant-beneficial endophytic strain from giant hogweed. Microb Biotech 4(4):523–553

Mao Y, Yannarell AC, Mackie RI (2011) Changes in N-transforming archaea and bacteria in soil during the establishment of bioenergy crops. PLoS One 6(9):e24750

Mapelli F, Borin S, Daffonchio D (2012) Microbial diversity in deep hypersaline anoxic basins. In: Adaption of microbial life to environmental extremes, Springer, Vienna, pp 21–36

Maropola MKA, Ramond JB, Trindade M (2015) Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum bicolor* (l. Moench). J Microbiol Method 112:104–117

Matson PA, Vitousek PM (2006) Agricultural intensification: will land spared from farming be land spared for nature? Conserv Biol 20(3):709–710

Matson PA, Parton WJ, Power AG, Swift MJ (1997) Agricultural intensification and ecosystem properties. Science 277(5325):504–509

Mavengere NR, Ellis AG, Le Roux JJ (2014) *Burkholderia aspalathi* sp. nov., isolated from root nodules of the south African legume *Aspalathus abietina* Thunb. Int J Syst Evol Microbiol 64 (6):1906–1912

Mehboob I, Naveed M, Zahir ZA (2009) Rhizobial association with non-legumes: mechanisms and applications. Crit Rev Plant Sci 28(6):432–456

Menéndez E, Ramírez-Bahena MH, Carro L, Fernández-Pascual M, Klenk HP, Velázquez E, Scotti MR (2016) *Paenibacillus periandrae* sp. nov., isolated from nodules of *Periandra mediterranea*. Int J Syst Evol Microbiol 66(4):1838–1843

Merabet C, Martens M, Mahdhi M, Zakhia F, Sy A, Le Roux C, Willems A (2010) Multilocus sequence analysis of root nodule isolates from *Lotus arabicus* (Senegal), *Lotus creticus*, *Argyrolobium uniflorum* and *Medicago sativa* (Tunisia):and description of *Ensifer numidicus* sp. nov. and *Ensifer garamanticus* sp. nov. Int J Syst Evol Microbiol 60(3):664–674

Mercado-Blanco J, Alós E, Rey MD, Prieto P (2016) *Pseudomonas fluorescens* PICF7 displays endophytic lifestyle in cultivated cereals and enhances yield in barley. FEMS Microbiol Ecol fiw 092

Misko AL, Germida JJ (2002) Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola. FEMS Microbiol Ecol 42(3):399–407

Mnasri B, Liu TY, Saidi S, Chen WF, Chen WX, Zhang XX, Mhamdi R (2014) *Rhizobium azibense* sp. nov., a nitrogen fixing bacterium isolated from root-nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 64(5):1501–1506

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA): producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nut 13(3):638–649

Mommer L, Hinsinger P, Prigent-Combaret C, Visser EJ (2016) Advances in the rhizosphere: stretching the interface of life. Plant Soil (2016) 407:1

M'piga P, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporumf* spp. radicis-lycopersiciin tomato plants treated with the endophytic bacterium *Pseudomonas fluorescensstrain* 63-28. Physiol Mol Plant Pathol 50(5):301–320

Nassar AH, El-Tarabily KA, Sivasithamparam K (2005) Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.): roots. Biol Fert Soil 42(2):97–108

Naveed M, Mitter B, Yousaf S, Pastar M, Afzal M, Sessitsch A (2014) The endophyte *Enterobacter* sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. Biol Fertil Soil 50(2):249–262

Nicoletti R, Ferranti P, Caira S, Misso G, Castellano M, Di Lorenzo G, Caraglia M (2014) Myrtucommulone production by a strain of Neofusicoccum australe endophytic in myrtle (Myrtus communis). World J Microbiol Biotechnol 30(3):1047–1052

Nievas F, Bogino P, Sorroche F, Giordano W (2012) Detection, characterization, and biological effect of quorum-sensing signaling molecules in peanut-nodulating bradyrhizobia. Sensors 12 (3):2851–2873

Old KM, Nicolson TH (1978) The root cortex as part of a microbial continuum. In: Loutit MV, Miles JAR (eds) Microbial ecol. Springer, Berlin, pp 291–294

Ozawa T, Ohwaki A, Okumura K (2003) Isolation and characterization of diazotrophic bacteria from the surface-sterilized roots of some legumes. Sci Rep Grad Sch Agric Biol Sci 55:29–36

Palaniappan P, Chauhan PS, Saravanan VS, Anandham R, Sa T (2010) Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. Biol Fert Soil 46(8):807–816

Pandya M, Kumar GN, Rajkumar S (2013) Invasion of rhizobial infection thread by non-rhizobia for colonization of Vigna radiata root nodules. FEMS Microbiol Lett 348(1):58–65

Patil NB, Gajbhiye M, Ahiwale SS, Gunjal AB, Kapadnis BP (2011) Optimization of Indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. Int J Environ Sci 2(1):295

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Ann Rev Cell Devel Biol 28:489–521

Pirttilä AM, Joensuu P, Pospiech H, Jalonen J, Hohtola A (2004) Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures. Physiol Planta 121(2):305–312

Puente ME, Li CY, Bashan Y (2009) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. Environ Exp Bot 66(3):402–408

Quadt-Hallman A, Benhamou N, Kloepper JW (1997a) Bacterial endophytes in cotton: mechanisms of entering the plant. Can J Microbiol 43:577–582

Quadt-Hallman A, Hallman J, Kloepper JW (1997b) Bacterial endophytes in cotton: location and interaction with other plant associated bacteria. Can J Microbiol 43:254–259

Radl V, Simoes-Araujo JL, Leite J, Passos SR, Martins LMV, Xavier GR, Rumjanek NG, Baldani JI, Zilli JE, (2014) *Microvirga vignae* sp. nov., a root nodule symbiotic bacterium isolated from cowpea grown in semi-arid Brazil. Int J Syst Evol Microbiol 64:725e730

Ramesh R, Phadke GS (2012) Rhizosphere and endophytic bacteria for the suppression of eggplant wilt caused by Ralstonia solanacearum. Crop Protec 37:35–41

Ramírez-Bahena MH, Flores-Félix JD, Chahboune R, Toro M, Velázquez E, Peix A (2016) *Bradyrhizobium centrosemae* (symbiovar centrosemae) sp. nov., *Bradyrhizobium americanum* (symbiovar phaseolarum) sp. nov. and a new symbiovar (tropici) of *Bradyrhizobium viridifuturi* establish symbiosis with *Centrosema* species native to America. Syst Appl Microbiol 39(6):378–383

RamírezBahenaMH, Tejedor C, Martín I, Velázquez E, Peix A (2013) *Endobacter medicaginis* gen. nov., sp. nov., isolated from alfalfa nodules in an acidic soil. Int J Syst Evol Microbiol 63 (5):1760–1765

Rangjaroen C, Rerkasem B, Teaumroong N, Noisangiam R, Lumyong S (2015) Promoting plant growth in a commercial rice cultivar by endophytic diazotrophic bacteria isolated from rice landraces. Ann Microbiol 65(1):253–266

Rashid MHO, Young JPW, Everall I, Clercx P, Willems A, Braun MS, Wink M (2015) Average nucleotide identity of genome sequences supports the description of *Rhizobium lentis* sp. nov., *Rhizobium bangladeshense* sp. nov. and *Rhizobium binae* sp. nov. from lentil (*Lens culinaris*) nodules. Int J Syst Evol Microbiol 65(9):3037–3045

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. Trend Microbiol 6 (4):139–144

Reinhold-Hurek B, Maes T, Gemmer S, Van Montagu M, Hurek T (2006) An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* sp. strain BH72. Mol Plant Microb Interact 19(2):181–188

Ribeiro RA, Ormeno-Orrillo E, Dall'Agnol RF, Graham PH, Martinez-Romero E, Hungria M (2013) Novel *Rhizobium* lineages isolated from root nodules of the common bean (*Phaseolus vulgaris* L.) in Andean and Mesoamerican areas. Res Microbiol 164(7):740–748

Ribeiro RA, Martins TB, Ormeño-Orrillo E, Delamuta JRM, Rogel MA, Martínez-Romero E, Hungria M (2015) *Rhizobium ecuadorense* sp. nov., an indigenous N2-fixing symbiont of the Ecuadorian common bean (*Phaseolus vulgaris* L.) genetic pool. Int J Syst Evol Microbiol 65 (9):3162–3169

Rincón-Rosales R, Villalobos-Escobedo JM, Rogel MA, Martinez J, Ormeño-Orrillo E, Martínez-Romero E (2013) *Rhizobium calliandrae* sp. nov., *Rhizobium mayense* sp. nov.

and *Rhizobium jaguaris* sp. nov., rhizobial species nodulating the medicinal legume Calliandra grandiflora. Int J Syst Evol Microbiol 63(9):3423–3429

Rivas R, Willems A, Subba-Rao NS, Mateos PF, Dazzo FB, Kroppenstedt RM, Martínez-Molina E, Gillis M, Velazquez E (2003) Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with Neptunia natans, an aquatic legume from India. Syst Appl Microbiol 26:47e53

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17(4) 319–339

Román-Ponce B, Zhang YJ, Vásquez-Murrieta MS, Sui XH, Chen WF, Padilla JCA, Wang ET (2016) *Rhizobium acidisoli* sp. nov., isolated from root nodules of *Phaseolus vulgaris* in acid soils. Int J Syst Evol Microbiol 66(1):398–406

Rosenblueth M, Martínez-Romero E (2004) *Rhizobium etli* maize populations and their competitiveness for root colonization. Arch Microbiol 181(5):337–344

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microb Interact 19(8):827–837

Rudrappa TK, Paré Czymmek PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556

Ryan PR, Delhaize E, Watt M, Richardson AE (2016) Plant roots: understanding structure and function in an ocean of complexity. Ann Bot 118(4):555–559

Safronova VI, Kuznetsova IG, Sazanova AL, Kimeklis AK, Belimov AA, Andronov EE, Willems A (2015) *Bosea vaviloviae* sp. nov., a new species of slow-growing rhizobia isolated from nodules of the relict species Vavilovia formosa (Stev.) Fed. Antonie Van Leeuwenhoek 107(4):911–920

Saïdi S, Ramírez-Bahena MH, Santillana N, Zúñiga D, Álvarez-Martínez E, Peix A, Velázquez E (2014) *Rhizobium laguerreae* sp. nov. nodulates *Vicia faba* on several continents. Int J Syst Evol Microbiol 64(1):242–247

Saikia S, Barman U (2013) Increasing rate of adoption of rice technologies in Assam: a need of the hour for food security

Sánchez-Contreras M, Martinez-Granero E, Redondo-Nieto M, Rivilla R, Martín M (2013). Biocontrol of fungal root pathogens by fluorescent Pseudomonas. Beneficial plant-microbial Interactions. Ecol Applicat 270

Sayyed RZ, Chincholkar SB, Reddy MS, Gangurde NS, Patel PR (2013) Siderophore producing PGPR for crop nutrition and phytopathogen suppression. Bacteria in agrobiology: disease management. Springer, Berlin, Heidelberg, pp 449–471

Schmelz E, Engelberth J (2003) Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. Proc Nat Acad Sci USA 100:10552–10557

Schwartz AR, Ortiz I, Maymon M, Herbold CW, Fujishige NA, Vijanderan JA, DeMason DA (2013) *Bacillus* simplex—a little known PGPB with anti-fungal activity—alters pea legume root architecture and nodule morphology when coinoculated with *Rhizobium leguminosarum* bv. viciae. Agronomy 3(4):595–620

Senthilkumar M, Anandham R, Madhaiyan M, Venkateswaran V, Sa T (2011) Endophytic bacteria: perspectives and applications in agricultural crop production. Bacteria in agrobiology: crop ecosystems. Springer, Berlin, Heidelberg, pp 449–471

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol Ecol 39(1):23–32

Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Hurek T (2012) Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol Plant Microb Inter 25(1):28–36

Shakeel M, Rais A, Hassan MN, Hafeez FY (2015) Root associated *Bacillus* sp. improves growth, yield and zinc translocation for basmati rice (*Oryza sativa*) varieties. Front Microbiol 6

Shamseldin A, Carro L, Peix A, Velázquez E, Moawad H, Sadowsky MJ (2016) The symbiovar trifolii of *Rhizobium bangladeshense* and *Rhizobium aegyptiacum* sp. nov. nodulate Trifolium alexandrinum in Egypt. Syst Appl Microbiol 39(4):275–279

Sharma VK, Nowak J (1998) Enhancement of verticillium wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). Can J Microbiol 44(6):528–536

Sherameti I, Tripathi S, Varma A, Oelmüller R (2008) The root-colonizing endophyte Pirifomospora indica confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Mol Plant Microb Interect 21(6):799–807

Sheu SY, Chou JH, Bontemps C, Elliott GN, Gross E, James EK, Chen WM (2012) *Burkholderia symbiotica* sp. nov., isolated from root nodules of *Mimosa* spp. native to north-east Brazil. Int J Syst Evol Microbiol 62(9):2272–2278

Sheu SY, Chou JH, Bontemps C, Elliott GN, Gross E, dos Reis Junior FB, Young JPW (2013) *Burkholderia diazotrophica* sp. nov., isolated from root nodules of *Mimosa* spp. Int J Syst Evol Microbiol 63(2):435–441

Sheu SY, Chen MH, Liu WY, Andrews M, James EK, Ardley JK, Chen WM (2015) *Burkholderia dipogonis* sp. nov., isolated from root nodules of *Dipogon lignosus* in New Zealand and Western Australia. Int J Syst Evol Microbiol 65(12):4716–4723

Silva HS, Tozzi JP, Terrasan CR, Bettiol W (2012) Endophytic microorganisms from coffee tissues as plant growth promoters and biocontrol agents of coffee leaf rust. Biol Cont 63(1):62–67

Silva FV, De Meyer SE, Simões-Araújo JL, da Costa Barbé T, Xavier GR, O'Hara G, Zilli JE (2014) *Bradyrhizobium manausense* sp. nov., isolated from effective nodules of *Vigna unguiculata* grown in Brazilian Amazonian rain forest soils. Int J Syst Evol Microbiol 64 (7):2358–2363

Singh B, Kaur T, Kaur S, Manhas RK, Kaur A (2015) An alpha-glucosidase inhibitor from an endophytic *Cladosporium* sp. with potential as a biocontrol agent. Appl Biochem Biotechnol 175(4):2020–2034

Soares MA, Li HY, Bergen M, da Silva JM, Kowalski KP, White JF (2016) Functional role of an endophytic *Bacillus amyloliquefaciens* in enhancing growth and disease protection of invasive English ivy (*Hedera helix* L.). Plant Soil doi:10.1007/s11104-015-2638-7

Soto MJ, Sanjuan J, Olivares J (2006) Rhizobia and plant-pathogenic bacteria: common infection weapons. Microbiology 152(11):3167–3174

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Stajković O, De Meyer S, Miličić B, Willems A, Delić D (2009) Isolation and characterization of endophytic non-rhizobial bacteria from root nodules of alfalfa (*Medicago sativa* L.). Botanica Serbica 33(1):107–114

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl Soil Ecol 15(2):183–190

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soil 25(1):13–19

Sturz AV, Christie BR, Matheson BG (1998) Association of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. Can J Microbiol 44:162–167

Sun G, Fang Y, Han DF, Lv M (2008) The Bioturbation effects of *Limnodrilus hoffmeisteri* on the vertical distribution of sediment particles in paddy field. J Changchun Normal University 8:017

Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C, Dreyfus B (2001) *Methylotrophic Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J Bacteriol 183:214e220

Sziderics AH, Rasche F, Trognitz F, Sessitsch A, Wilhelm E (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). Can J Microbiol 53(11):1195–1202

Tani A, Muthuri CW, Ngamau CN, Matiru VN (2015) Potential use of endophytic bacteria as biofertilizer for sustainable banana (*Musa* Spp.) Prod Afr J Hort Sci 8(1):1–11

Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Ludueña L, Pena D, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329(1–2):421–431

Tivendale ND, Ross JJ, Cohen JD (2014) The shifting paradigms of auxin biosynthesis. Trend Plant Sci 19(1):44–51

Tiwari R, Kalra A, Darokar MP, Chandra M, Aggarwal N, Singh AK, Khanuja SPS (2010) Endophytic bacteria from *Ocimum sanctum* and their yield enhancing capabilities. Curr Microbiol 60(3):167–171

Tiwari R, Awasthi A, Mall M, Shukla AK, Srinivas KS, Syamasundar KV, Kalra A (2013) Bacterial endophyte-mediated enhancement of in planta content of key terpenoid indole alkaloids and growth parameters of Catharanthus roseus. Ind Crop Product 43:306–310

Toharisman A, Suhartono MT, Spindler-Barth M, Hwang JK, Pyun YR (2005) Purification and characterization of a thermostable chitinase from *Bacillus licheniformis* Mb-2. World J Microbiol Biotechnol 21(5):733–738

Trujillo ME, Willems A, Abril A, Planchuelo AM, Rivas R, Ludena D, Mateos PF, Martinez-Molina E, Velazquez E (2005) Nodulation of Lupinus albus by strains of *Ochrobactrum lupini* sp. nov. Appl Environ Microbiol 71:1318e1327

Trujillo ME, Alonso-Vega P, Rodríguez R, Carro L, Cerda E, Alonso P, Martínez-Molina E (2010) The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. The ISME J 4(10):1265–1281

Tsavkelova EA, Cherdyntseva TA, Botina SG, Netrusov AI (2007) Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res 162(1):69–76

Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I, Thies C (2005) Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. Ecol Lett 8 (8):857–874

Valverde A, Velazquez E, Gutierrez C, Cervantes E, Ventosa A, Igual JM (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of Phaseolus vulgaris. Int J Syst Evol Microbiol 53:1979e1983

Van Elsas JD, Heijnen CE (1990) Methods for the introduction of bacteria into soil: a review. Biol Fert Soil 10(2):127–133

van Loosdrecht MC, Lyklema J, Norde W, Zehnder AJ (1989) Bacterial adhesion: a physicochemical approach. Microb Ecol 17(1):1–15

Van Wees SC, Luijendijk M, Smoorenburg I, Van Loon LC, Pieterse CM (1999) *Rhizobacteria* mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene Atvsp upon challenge. Plant Mol Biol 41(4):537–549

Verma SC, Singh A, Chowdhury SP, Tripathi AK (2004) Endophytic colonization ability of two deep-water rice endophytes, Pantoea sp. and Ochrobactrum sp. using green fluorescent protein reporter. Biotechnol lett 26(5):425–429

Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. Biol Fertil Soil 40(1):36–43

Wakelin SA, Gupta VVSR, Forrester ST (2010) Regional and local factors affecting diversity, abundance and activity of free-living, N_2-fixing bacteria in Australian agricultural soils. Pedobiologia 53(6):391–399

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root exudation and rhizosphere biology. Plant Physiol 132(1):44–51

Wang S, Wu H, Qiao J, Ma L, Liu J, Xia Y, Gao X (2009) Molecular mechanism of plant growth promotion and induced systemic resistance to tobacco mosaic virus by *Bacillus* spp. J Microbiol Biotechnol 19(10):1250–1258

Wang ET, Chen WF, Sui XH, Zhang XX, Liu HC, Chen WX (2011a) *Rhizobium herbae* sp. nov. and *Rhizobium giardinii*-related bacteria, minor microsymbionts of various wild legumes in China. Int J Syst Evol Microbiol 61(8):1912–1920

Wang F, Wang ET, Wu LJ, Sui XH, Li JrY, Chen WX (2011b) *Rhizobium vallis* sp. nov., isolated from nodules of three leguminous species. Int J Syst Evol Microbiol 61(11):2582–2588

Wang JY, Wang R, Zhang YM, Liu HC, Chen WF, Wang ET, Chen WX (2013a) *Bradyrhizobium daqingense* sp. nov., isolated from soybean nodules. Int J Syst Evol Microbiol 63(2):616–624

Wang R, Chang YL, Zheng WT, Zhang D, Zhang XX, Sui XH, Chen WX (2013b) *Bradyrhizobium arachidis* sp. nov., isolated from effective nodules of *Arachis hypogaea* grown in China. Syst Appl Microbiol 36(2):101–105

Wang YC, Wang F, Hou BC, Wang ET, Chen WF, Sui XH, Zhang YB (2013c) Proposal of *Ensifer psoraleae* sp. nov., *Ensifer sesbaniae* sp. nov., *Ensifer morelense* comb. nov. and *Ensifer americanum* comb. nov. Syst Appl Microbiol 36(7):467–473

Wang W, Zhai Y, Cao L, Tan H, Zhang R (2016) Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). Microbiol Res 188:1–8

Wani ZA, Ashraf N, Mohiuddin T, Riyaz-Ul-Hassan S (2015) Plant-endophyte symbiosis, an ecological perspective. Appl Microbiol Biotechnol 99(7):2955–2965

Watson R, Albon S, Aspinall R, Austen M, Bardgett B, Bateman I, Bullock J (2011) UK National ecosystem assessment: understanding nature's value to society. Synthesis of key findings. http://eprints.lancs.ac.uk/id/eprint/49673

Wei XL, Han MS, Xia CC, Ding SL, Xu L, Lin YB, Wei GH (2015) *Diaphorobacter ruginosibacter* sp. nov., isolated from soybean root nodule, and emended description of the genus Diaphorobacter. Arch Microbiol 197(5):683–692

Welbaum GE, Meinzer FC (1990) Compartmentation of solutes and water in developing sugarcane stalk tissue. Plant Physiol 93(3):1147–1153

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant–endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52(suppl 1):487–511

Will ME, Sylvia DM (1990) Interaction of rhizosphere bacteria, fertilizer, and vesicular-arbuscular mycorrhizal fungi with sea oats. Appl Environ Microbiol 56(7):2073–2079

Wilson M, Lindow SE (1993) Interactions between the biological control agent *Pseudomonas fluorescens* A506 and *Erwinia amylovora* in pear blossoms. Phytopathol 83(1):117–123

Wilson M, Hirano SS, Lindow SE (1999) Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. Appl Environ Microbiol 65:1435–1443

Woodward AW, Bartel B (2005) A receptor for auxin. Plant Cell 17(9):2425–2429

Xie GH, Cai MY, Tao GC, Steinberger Y (2003) Cultivable heterotrophic N2-fixing bacterial diversity in rice fields in the Yangtze River Plain. Biol Fert Soil 37(1):29–38

Xu L, Shi JF, Zhao P, Chen WM, Qin W, Tang M, Wei GH (2011) *Rhizobium sphaerophysae* sp. nov., a novel species isolated from root nodules of Sphaerophysa salsula in China. Antonie Van Leeuwenhoek 99(4):845–854

Xu L, Zhang Y, Deng ZS, Zhao L, Wei X L, Wei GH (2013) *Rhizobium qilianshanense* sp. nov., a novel species isolated from root nodule of Oxytropis ochrocephala Bunge in China. Antonie Van Leeuwenhoek 103(3):559–565

Yang B, Ma HY, Wang XM, Jia Y, Hu J, Li X, Dai CC (2014) Improvement of nitrogen accumulation and metabolism in rice (*Oryza sativa* L.):by the endophyte Phomopsis liquidambari. Plant Physiol Biochem 82:172–182

Yanni YG, Rizk RY, El-Fattah FKA, Squartini A, Corich V, Giacomini A, Vega-Hernandez M (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. trifolii with rice roots. Functional. Plant Biol 28(9):845–870

Zakhia F, Jeder H, Willems A, Gillis M, Dreyfus B, de Lajudie P (2006) Diverse bacteria associated with root nodules of spontaneous legumes in Tunisia and first report for nifH-like gene within the genera *Microbacterium* and *Starkeya*. Microb Ecol 51:375e393

Zhang RJ, Hou BC, Wang ET, Li JrY, Zhang XX, Chen WX (2011) *Rhizobium tubonense* sp. nov., isolated from root nodules of *Oxytropis glabra*. Int J Syst Evol Microbiol 61(3):512–517

Zhang YM, Li JrY, Chen WF, Wang ET, Sui XH, Li QQ, Chen WX (2012) *Bradyrhizobium huanghuaihaiense* sp. nov., an effective symbiotic bacterium isolated from soybean (*Glycine max* L.):nodules. Int J Syst Evol Microbiol 62(8):1951–1957

Zhang YJ, Zheng WT, Everall I, Young JPW, Zhang XX, Tian CF, Chen WX (2015) *Rhizobium anhuiense* sp. nov., isolated from effective nodules of *Vicia faba* and *Pisum sativum*. Int J Syst Evol Microbiol 65(9):2960–2967

Zheng WT, Li JrY, Wang R, Sui XH, Zhang XX, Zhang JJ, Chen WX (2013) *Mesorhizobium qingshengii* sp. nov., isolated from effective nodules of Astragalus sinicus. Int J Syst Evol Microbiol 63(6):2002–2007

Zhou PF, Chen WM, Wei GH (2010) Mesorhizobium robiniae sp. nov., isolated from root nodules of Robinia pseudoacacia. Int J Syst Evol Microbiol 60(11):2552–255

Zhou S, Li Q, Jiang H, Lindström K, Zhang X (2013) *Mesorhizobium sangaii* sp. nov., isolated from the root nodules of Astragalus luteolus and Astragalus ernestii. Int J Syst Evol Microbiol 63(8):2794–2799

Zilli JE, Baraúna AC, da Silva K, De Meyer SE, Farias EN, Kaminski PE, Dourado FDS (2014) *Bradyrhizobium neotropicale* sp. nov., isolated from effective nodules of Centrolobium paraense. Int J Syst Evol Microbiol 64(12):3950–3957

Zimmermann MH (1983) Pathology of the xylem. In: Xylem structure and the ascent of sap. Springer, Berlin, Heidelberg, pp 107–125

Chapter 10
Diversity, Distribution and Functional Role of Bacterial Endophytes in *Vitis vinifera*

Marco Andreolli, Silvia Lampis and Giovanni Vallini

Abstract Associations between microorganisms and botanical species play an important role in the ability of plants to survive and thrive in diverse environments, by better facing unfavorable climatic and edaphic conditions or by determining either a greater vegetative development or possibly the resistance to diseases and pests. In this article, we focus on the relationship between grapevine (*Vitis vinifera*) and its endophytic plant growth-promoting bacteria (PGPB), i.e., the endophytes that stimulate and facilitate grapevine growth. Most previous studies have considered the ability of such microbes to help plants draw nutrients from the soil or to counter the effect of phytopathogens. Here, we discuss recent studies concerning the infection process, the spatiotemporal localization of endophytic PGPB in grapevine, and particularly their contribution to plant growth and defense against pathogens in this important fruit crop.

Keywords Endophytic bacteria · Grapevine · Internal plant tissue colonization modes · Phytopathogen control capacity · Plant growth-promoting activity *Vitis vinifera*

10.1 Introduction

Cultivated vines are predominantly cultivars of the species *Vitis vinifera* L. (the Eurasian grapevine) due to the high quality of its berries. All vines belong to the family *Vitaceae* and together represent the most widely grown and economically important woody fruit crop in the world (Vivier and Pretorius 2002; Mattia et al.

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_10

Table 10.1 Vineyard cultivation areas in hectares between 2011 and 2015 (ranked by country and region based on 2015 data)

Country	2011	2012	2013	2014	2015
Spain	1,031,544	1,016,570	1,020,617	1,021,427	1,020,617
China*	632,928	706,581	756,762	795,612	829,605
France	795,612	791,565	792,779	790,756	785,495
Italy	719,531	712,651	704,558	689,584	681,895
Turkey*	507,880	496,954	503,834	501,810	496,954
USA	412,779	411,970	421,682	418,850	418,850
Argentina	218,935	221,768	223,791	225,815	225,005
Portugal	235,932	233,099	229,052	223,791	216,912
Chile	205,985	205,985	208,008	210,841	210,841
Romania	191,012	191,821	191,821	191,821	191,821
Australia	169,968	161,874	157,018	153,781	148,924
South Africa	133,142	134,760	133,142	131,928	129,904
Greece	110,075	110,075	110,075	110,075	106,837
Germany	101,981	101,981	101,981	101,981	101,981
Brazil	89,840	91,054	89,840	89,031	84,984
Other Europe	976,506	933,610	933,610	918,636	916,613
Other Asia* and Oceania	620,788	634,547	629,691	631,714	632,928
Other Africa*	242,002	236,741	233,908	233,908	233,908
Other North and South America	87,007	89,031	93,078	95,910	97,125
World total	7,483,447	7,482,637	7,535,247	7,537,271	7,531,199

*Primarily consumed as fresh fruit or raisins, although China's wine grape production areas are increasing rapidly [*Source* Modified from International Organization of Vine and Wine (OIV), April 2016, available at http://italianwinecentral.com/top-fifteen-grape-producing-countries/]

2008; Torregrosa et al. 2015). In the year 2015, vineyards covered a total area of ~7.5 million hectares (~18.5 million acres). The area of cultivated vines between 2011 and 2015 is ranked by country/region in Table 10.1, and the proportion of global production by country/region in 2015 is summarized in Fig. 10.1.

By far, the greatest proportion of harvested grapes is used for wine making, and this is probably the most important cultural use of grapes. Nevertheless, table grapes are also cultivated mainly in Italy, Spain and Greece, the USA, Chile and South Africa, with the latter two countries primarily producing for export. Additional uses for grape berries include the production of raisins, juice, vinegar, and distilled spirits (http://faostat.fao.org/, data 2015).

Disease control is an essential part of good quality for grape production. Pesticides and fungicides are applied from early spring until harvest in order to protect vines against a variety of phytopathogens. In the last few decades, the use of synthetic fungicides to control plant diseases in agriculture has increased, although this has made the public more aware of the environmental harm caused by such

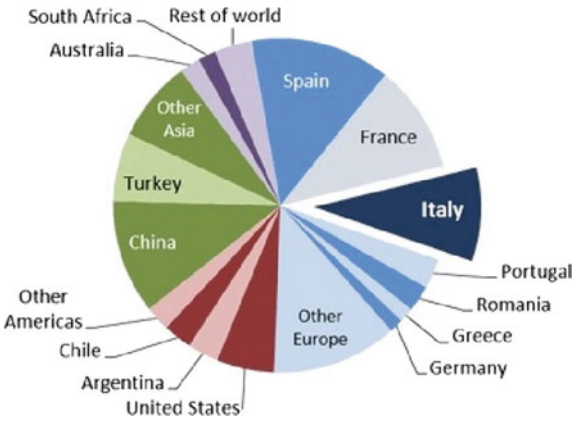

Fig. 10.1 Distribution of global vineyard cultivation area by nation/region in 2015 [*Source* Italian Wine Central™ April 2016, available at http://italianwinecentral.com/top-fifteen-grape-producing-countries/]

chemicals (Goldammer 2015). Indeed, the repeated use of fungicides has resulted in environmental pollution and emergence of resistant microorganisms (Brent and Hollomon 2007; EFSA 2013). Fungicides also have undesirable effects on non-target organisms, including humans (Nicolopoulou-Stamati et al. 2016). Some fungicides even have phytotoxic effects, although little is known about the practical impact of this phenomenon (Dias 2012). These concerns have increased the demand for alternative crop protection products, including biopesticides with active principles of natural origin that are safe for humans and the environment (Yoon et al. 2013). Other researchers have considered the possibility of managing natural microbial endophytes as biological control agents to confer or induce resistance against phytopathogens in crops such as grapevine (Compant and Mathieu 2016).

A common sense definition of endophytes is the community of bacteria and fungi that can be detected at a given time inside the tissues of different anatomic compartments in apparently healthy plant hosts (Schulz and Boyle 2005). More recently, this definition has been updated to consider "all microorganisms which for all or part of their life time colonize internal plant tissues" (Hardoim et al. 2015). Endophytes colonize the majority of wild plant species and also most species of crops (Hallmann et al. 1997; Hallmann and Berg 2006). Until the turn of the millennium, most studies of endophytic microorganisms depended on in vitro cultivation, which is unsuitable for more than 99% of known microbial species and tends to select for the fastest growing organisms (Magnani et al. 2013). In contrast, culture-independent methods allow the identification of a larger portion of the endophytic microbiome (Tian et al. 2007). However, the ability to produce axenic cultures of endophytic microbes remains necessary to assay microbial isolates for plant growth-promoting traits (Liaqat and Eltem 2016). Endophytes include species with diverse behavioral strategies in terms of plant–microbe interactions, ranging from mutualism to latent pathogenicity through to commensalism and unilateral exploitation (Schulz and Boyle 2006). Nevertheless, endophytes often promote the growth of the plants they colonize in various ways, possibly similar to the strategies

of plant growth-promoting rhizobacteria which can enhance plant growth by phosphate solubilization, the production of siderophores or indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, biological nitrogen fixation, or competition with phytopathogens (Kevin 2003; Bhattacharyya and Jha 2012). The mutualistic interactions between endophytes and plant hosts are similarly diverse: the plants provide a variety of protective niches, and the microbes can release useful metabolites and signaling molecules (Gary 2003; Rosenblueth and Martínez-Romero 2006) that increase nutrient uptake (Ramos et al. 2011), with effects on plant growth, development and biomass production (Compant et al. 2005a; Hardoim et al. 2008). They can also induce resistance to pathogens (Sturz and Matheson 1996; Nagarajkumar et al. 2004; Padgham et al. 2005) and insects (Azevedo et al. 2000) and can increase tolerance to osmotic stress (Sziderics et al. 2007), heavy metals (Rajkumar et al. 2009), xenobiotic contaminants (Siciliano et al. 2001; Andreolli et al. 2013), and other forms of abiotic stresses (Xia et al. 2015).

The elicitation of plant defense responses is a desirable trait during grapevine cultivation, particularly to counteract wood rot and trunk diseases whose etiological agents include the fungi *Eutypa lata* (Eutypa dieback), *Botryosphaeria dothidea* (black dead arm), and the agents responsible for esca or black measles (*Togninia minima*, *Phaeomoniella chlamydospora,* and *Phaeoacremonium angustius*). Moreover, endophytes can also make grapevine plants more resistant toward adverse environmental and edaphic conditions such as dehydration, salinity or limited nutrient availability. Vineyard soils in different geographical areas are often characterized by such conditions, which can cause severe abiotic stress that threatens the quality and yield of grapes. This review article considers what is currently known about the distribution and potential functional roles of bacterial endophytes in grapevine cultivars (*V. vinifera* L.) by integrating data from the literature and our original investigations.

10.2 Methods for the Isolation of Endophytes

A typical procedure for the isolation of endophytes from epiphytic microorganisms includes sterilization of the relevant parts of the plant (e.g., the roots, stems, or leaves) followed by immersing the disaggregated tissues in appropriate media (Reissinger et al. 2001; Hallmann et al. 2006; Gaiero et al. 2013). Culture-dependent methods for the identification of endophytes involve the isolation and growth of the microbes (bacteria or fungi) from surface-sterilized plant sections (Coombs and Franco 2003; Qin et al. 2011). Characterization can then be carried out by a number of techniques, such as fatty acid or lipid assays, morphological analysis or enzymatic tests (Garbeva et al. 2001; Berg et al. 2005; Aravind et al. 2009). Despite many attempts to develop adequate protocols for the cultivation of endophytic microorganisms, it appears that most of these microbes remain uncultivable in laboratory settings (Schloss and Handelsman 2005). In

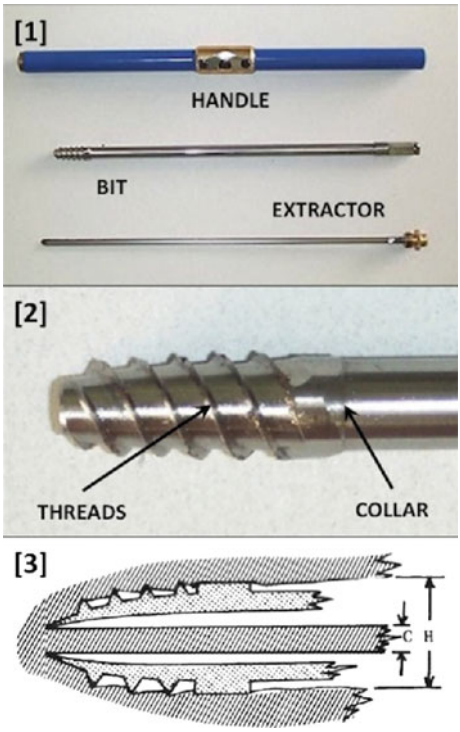

Fig. 10.2 An increment borer, which is used to sample the inner tissues of grapevine stems. [**1**] The tool components. [**2**] Enlargement showing the bit head (threaded auger). [**3**] Sectional schematic showing the cutting and compression of the inner tissues of a grapevine woody stem using the increment borer. The compression of woody tissue by the borer is indicated by the difference in diameter between the ingress hole (H) and the core (C)

particular, obligate endophytes cannot proliferate outside their host and require continuous interaction with the plant for survival, often rendering them viable but uncultivable (Sturz et al. 2000; Hardoim et al. 2008; Croes et al. 2013). This has necessitated the development of metagenomics or culture-independent techniques based on molecular biology.

The isolation of endophytes from grapevine plants using culture-dependent methods has been described by several authors (Bell et al. 1995; Altalhi 2009; Compant et al. 2011). Nevertheless, a standardized procedure has not yet been developed to isolate endophytic bacteria from portions of corky stems measuring a few centimeters in diameter, which are difficult to allow a proper surface sterilization. This issue can be addressed using a sterile increment borer (Fig. 10.2) to sample inner grapevine stem tissues, as recently described by Andreolli et al. (2016).

The same authors also used a heat-sterilized chisel with a shaped cutting edge to generate a longitudinal opening in the surface-sterilized grapevine stems in order to collect samples of core tissues. All the sampling procedures were performed under a laminar flow hood. Sampling using an increment borer equipped with a threaded auger and extractor tray is illustrated in Fig. 10.3.

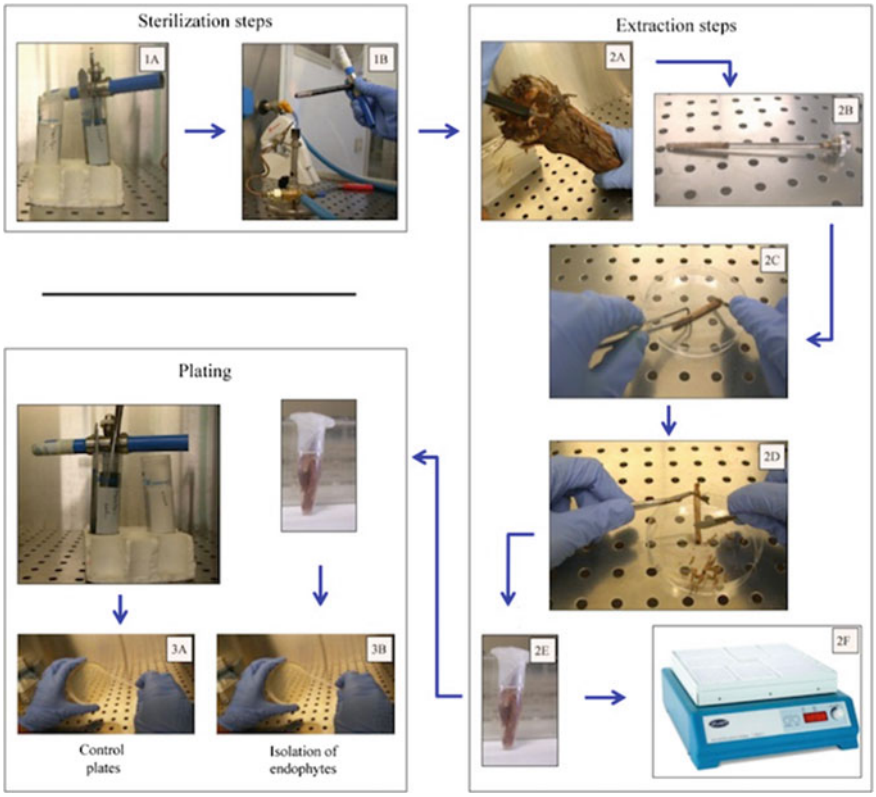

Fig. 10.3 Sampling protocol using an increment borer. (**1A** and **1B**) The sampling device is flame sterilized by dipping in ethanol (95%) followed by ignition in a Bunsen flame. (**2A** and **2B**) Extraction of the core samples from grapevine stem portions is performed using the increment borer. (**2C**) The outer part of the sample (1–2 cm from both ends) is discarded. (**2D**) The inner core samples are scraped to remove 50–100 mg of sawdust which is placed in 2-ml tubes. (**2E**) Physiological solution (0.9% [w/v] NaCl) is added to achieve a dilution of 1:10 [w/v] in each tube. (**2F**) The samples are agitated on an orbital shaker at 250 rpm for 1 h. (**3A**) Serial dilutions are prepared, and 100-µl aliquots are plated onto appropriate culture media. (**3B**) The same media are plated with 250-µl aliquots of physiological solution which was used to rinse the heat-sterilized sampling device to confirm that microbial contamination has been eliminated

10.3 Infection and Colonization

Although microbes can penetrate through wounded zones of the epidermis, the colonization of plants by endophytes is usually achieved through the secondary roots (Hallmann et al. 1997). Infection is associated with the following steps: (i) bacterial attraction by root exudates (Huang et al. 2014), (ii) attachment by adhesion, (iii) penetration with disruption of natural barriers in the host, and (iv) stable establishment in the host (Wilson et al. 2002) (Fig. 10.4).

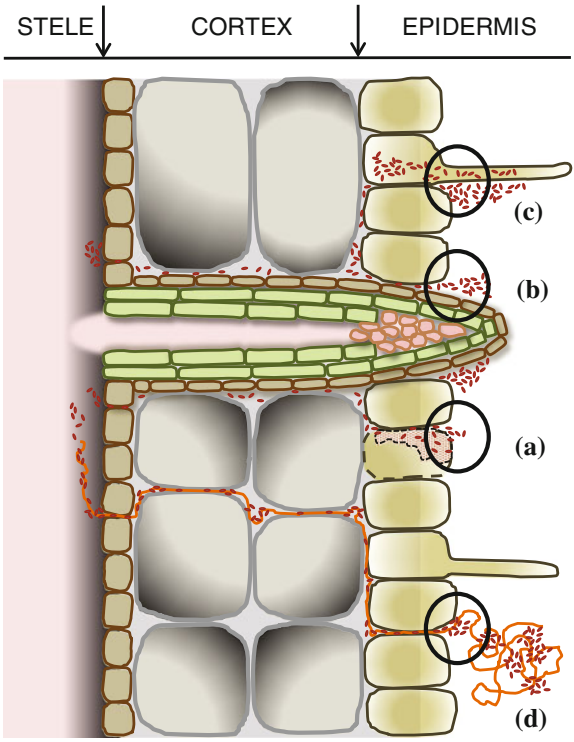

Fig. 10.4 Main root niches for the infection of plants by bacterial endophytes. [**A**] Endophytes generally reach internal plant tissues through damaged areas of root epidermis caused by abiotic stress, such as friction with soil particles at the root tip, or biotic stress, such as wounds inflicted by arthropods and nematodes. Other points of entry include epidermal cracks where the lateral roots emerge [**B**] and either fully elongated or initiating root hairs [**C**] (Mercado-Blanco and Prieto 2012). The colonization of plants by bacteria is also facilitated by fungal penetration of roots [**D**] (van Overbeek and Saikkonen 2016). In this case, bacteria and fungi occupy free spaces in the apoplast, cross the root endodermis, and enter the xylem lumen. Endophytic bacteria can then spread to distant plant organs namely the stem, leaves, seeds, and fruits

The progress of endophytic colonization in grapevine has been monitored using a strain of *Burkholderia phytofirmans* PsJN expressing a green fluorescent protein marker (Compant et al. 2005b, 2008). Infection begins via the non-uniform colonization of the root hair zone surface. Bacteria that survive competition with the natural microbial population can penetrate the roots, often facilitated by the secretion of specific cell wall-degrading enzymes such as endoglucanase, endopolygalacturonase, endo-β-D-cellobiosidase, and/or exo-β-1,4-glucanase (Compant et al. 2005b, 2008). The more limited microbial diversity and smaller population in root tissues compared to the rhizosphere reflect the selection for specific physiological requirements that are necessary to penetrate to the interior of the roots (Hardoim et al. 2008; Marasco et al. 2013).

Endophytic bacteria must avoid the ability of the host plant's innate immune system to recognize them as pathogens (Zeng and He 2010; Zamioudis and Pieterse 2012). Flagellin, the main protein component of the bacterial flagellum, acts as a defense elicitor in many plant species (Boller and Felix 2009). A recent study analyzed the flagellin sensing 2 (FLS2) system in grapevine, its interaction with the active flagellin epitope flg22, and its relationship with *B. phytofirmans* PsJN (Trdá et al. 2014). Unlike flagellin peptides from the pathogenic strains *Pseudomonas aeruginosa* and *Xanthomonas campestris*, the flg22 peptide from *B. phytofirmans* triggered only a weak oxidative burst, causing the transient induction of defense genes. These data suggested that flagellin from the beneficial PsJN strain has evolved to evade the grapevine innate immune system (Trdá et al. 2014).

Once the root system has been penetrated, the PsJN strain migrates from the rhizodermis to the exodermis and to the cortical cell layers through intercellular pathways. The barrier of the endodermis can be broken directly by the PsJN strain, or it can migrate through breaches previously opened by other microorganisms. Once the endodermis has been penetrated, the bacteria are detected mainly within the xylem vessels of the central cylinder, often along with other microorganisms. The PsJN strain was subsequently found in the vascular bundles of grapevine inflorescence stalks, pedicels and young berries, and 5 weeks after soil inoculation, in the inflorescence itself (Compant et al. 2005b, 2008).

A recent study followed the colonization of grapevine by three other endophytes (*Enterobacter ludwigii* EnVs6, *Pantoea vagans* PaVv7, and *Sphingomonas phyllosphaerae* SpVs6). The data indicated that strains EnVs6 and PaVv7 can colonize the root surfaces, the cortex, and the central cylinder up to the xylem vessels, but cannot mount a systemic infection. In contrast, strain SpVs6 efficiently colonized the root surface but not the endorhiza and was, therefore, not detected as an endophyte (Lòpez-Fernàndez et al. 2015a, b). The colonization strategies of endophytes, therefore, appear to differ in a strain-dependent manner. Furthermore, the activation of metabolic pathways in the host plant can also facilitate endophytic colonization. Indeed, the infection of grapevine by the endophyte *Enterobacter ludwigii* EnVs6 triggers the production of vanillic acid and reduces the accumulation of catechin, esculin, arbutin, astringin, pallidol, ampelopsin, D-quadrangularin, and isohopeaphenol (Lòpez-Fernàndez et al. 2015a, b).

10.4 Taxonomy of Bacterial Endophytes in *Vitis vinifera*

A wide diversity of bacterial endophytes in *Vitis vinifera* has been described so far. The major taxonomical information concerning endophyte distribution in grapevine are outlined in the following sections.

10.4.1 Spatial Distribution of Endophytic Bacteria in Grapevine Tissues

Once the plants are infected, the endophytic bacteria can colonize the internal tissues. Endophytes have been isolated from all grapevine tissues, including the reproductive organs. Compant et al. (2011) quantified 1.44 ± 1.44, 2.77 ± 1.08 and 2.87 ± 2.2 $\log_{10}$ colony forming units (CFU) g^{-1} within the seeds, flowers, and harvested berries, respectively, whereas ~3.5 $\log_{10}$ CFU g^{-1} endophytes were found in the grape stalks, 0.5–2 CFU g^{-1} in the shoots, 3.5–7 $\log_{10}$ CFU g^{-1} in the roots, 3–4 CFU g^{-1} in the xylem tissue, and 2–4 $\log_{10}$ CFU g^{-1} in the leaves (Bell et al. 1995; Altalhi 2009; Lo Piccolo et al. 2010; Compant et al. 2011; Marasco et al. 2013; Baldan et al. 2014). Various authors have observed a declining gradient in the number of bacterial cells from the underground to the aerial parts of grapevine plants, as reported in other endophyte-colonized plants (Hallmann and Berg 2006; West et al. 2010).

Compant et al. (2011) showed that several isolates from different plant tissues correspond to identical bacterial groups. Similarly, culture-independent analysis has evidenced that endophytic populations remain homogeneous throughout the woody parts of grapevine plants (West et al. 2010). Fluorescent in situ hybridization (FISH) revealed that *Gammaproteobacteria* and *Firmicutes* were the predominant genera in the epidermis of the flower and inside the xylem of ovaries, whereas large numbers of *Bacillus* spp. has been reported in the flower ovules, in the berry pulp, and inside the seeds. On the other hand, no bacteria were found within the epidermal cell layer of pulp (Compant et al. 2011). Furthermore, endophytic bacteria were detected in the leaves 4–8 μm below the stoma, mainly within the cells, intercellular spaces, veins, hairs, and along the cut edges of leaf fragments (Lo Piccolo et al. 2010).

10.4.2 Distribution of Endophytic Bacteria Among Different Grapevine Cultivars and Geographical Areas

Currently, there are approximately 5000–10,000 different varieties of *V. vinifera*, although only a few are commercially significant for wine and table grape production (*Vitis* International Variety Catalogue 2015). Grapevine is cultivated throughout Asia, North America, and Europe under subtropical, Mediterranean, and continental–temperate conditions (Terral et al. 2010). The analysis of endophytic bacteria among different grapevine cultivars and regions has been carried out using culture-dependent and culture-independent techniques. The bacterial isolates are summarized in Tables 10.2 and 10.3 and Fig. 10.5.

Table 10.2 List of grapevine endophytes isolated by culture-dependent techniques

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
*Achromobacte*r sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Acetobacter sp.	*Vitis vinifera* L.	Stems, leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Acinetobacter sp.	*V. vinifera* subsp. *sylvestris*	Stems	North of Italy	Campisano et al. (2015)
	Vitis vinifera L.	Stems	Taif Governorate, Saudi Arabia	Altalhi (2009)
Acinetobacter baumannii	Different cultivars	Leaves	Sicily Region, Italy	Lo Piccolo et al. (2010)
Acinetobacter/ Prolinoborus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Agrobacterium rhizogenes	Zweigelt	Flowers	Austria	Compant et al. (2011)
Agrobacterium tumefaciens	Zweigelt	Flowers	Austria	Compant et al. (2011)
Agrococcus baldri	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Agrococcus jejuensis	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Bacillus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
	Zweigelt	Berries, flowers	Austria	Compant et al. (2011)
	Vitis vinifera L.	Stems, leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Bacillus amyloliquefaciens	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Bacillus cereus	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Bacillus fastidiosus	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Bacillus herbersteinensis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Bacillus insolitus	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Bacillus megaterium	Zweigelt	Berries	Austria	Compant et al. (2011)
Bacillus pumilus	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Bacillus safensis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Bacillus simplex	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Bacillus siralis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Bacillus subtilis	Wild, Domesticated	Stems	Northern Italy	Campisano et al. (2015)
Bacillus thuringiensis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Biostraticola/ Yersinia sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Brachybacterium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Brevibacillus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Brevundimonas sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Burkholderia phytofirmans	Different cultivars	Leaves	Sicily Region, Italy	Lo Piccolo et al. (2010)
Citricoccus alkalitolerans	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Clavibacter michiganensis	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Comamonas terrigena	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Curtobacterium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris;* Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Curtobacterium flaccumfaciens	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
	V. vinifera subsp. *sylvestris;*	Stems	Northern Italy	Campisano et al. (2015)
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Curtobacterium pusillum	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Enterobacter sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
	Vitis vinifera L.	Stems, leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Enterobacter agglomerans	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Enterobacter cloacae	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Enterobacter ludwigii	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Erwinia sp.	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
	Vitis vinifera L.	Leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Frigoribacterium sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Klebsiella ozaenae	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Klebsiella pneumoniae	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Kocuria sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Leclercia sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Leifsonia sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Leifsonia xyli	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Lysinibacillus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Lysinibacillus fusiformis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Massilia sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Mesorhizobium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Mesorhizobium albiziae	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Methylobacterium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Methylococcus sp.	*Vitis vinifera* L.	Stems	Taif Governorate, Saudi Arabia	Altalhi (2009)
Microbacterium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris;* Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Microbacterium flavum	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Microbacterium laevaniformans	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Microbacterium oxydans	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Microbacterium testaceum	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Micrococcus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Micrococcus luteus	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Moraxella bovis	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Nocardioides sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Nocardioides marinisabuli	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Novosphingobium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Paenibacillus sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Paenibacillus amylolyticus	Zweigelt	Berries, flowers	Austria	Compant et al. (2011)
Paenibacillus lautus	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Paenibacillus massiliensis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Pantoea sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
	Zweigelt	Flowers	Austria	Compant et al. (2011)
Pantoea agglomerans	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
	V. vinifera subsp. *sylvestris;* Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
	Zweigelt	Flowers	Austria	Compant et al. (2011)
Pantoea ananatis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Pantoea eucalypti	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Pantoea stewartii	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Paracoccus sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Planococcus sp.	*Vitis vinifera* L.	Leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Pseudoclavibacter helvolus	*V. vinifera* subsp. *sylvestris*	Stems	North of Italy	Campisano et al. (2015)
Pseudomonas sp.	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
	V. vinifera subsp. *sylvestris;* Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
	Zweigelt	Flowers	Austria	Compant et al. (2011)
Pseudomonas cannabina	Zweigelt	Berries, flowers	Austria	Compant et al. (2011)
Pseudomonas cichorii	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudomonas congelans	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Pseudomonas corrugata	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudomonas fluorescens	Zweigelt	Berries	Austria	Compant et al. (2011)
Pseudomonas marginalis	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudomonas poae	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Pseudomonas psychrotolerans	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Pseudomonas putida	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudomonas reactants	Pinot Noir, Chardonnay and Merlot	Stems	Northern Italy	Campisano et al. (2015)
Pseudomonas syringae	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Pseudomonas syringae pv. *lachrymans*	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudomonas syringae pv. *tabaci*	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Pseudoxanthomonas sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Rahnella aquatilis	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Rhizobium sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
	V. vinifera subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
	Zweigelt	Flowers	Austria	Compant et al. (2011)
Rhodococcus sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Rhodococcus luteus	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Sphingomonas sp.	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Sphingomonas aerolata	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Sphingomonas panni	*V. vinifera* subsp. *sylvestris*	Stems	Northern Italy	Campisano et al. (2015)
Staphylococcus sp.	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
	Vitis vinifera L.	Leaves	Taif Governorate, Saudi Arabia	Altalhi (2009)
Staphylococcus epidermidis	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Stenotrophomonas sp.	Corvina	Stems	Veneto Region, Italy	Andreolli et al. (2016)
Stenotrophomonas maltophilia	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Stenotrophomonas rhizophila	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Streptomyces sp.	*Vitis vinifera* L.	Stems	Taif Governorate, Saudi Arabia	Altalhi (2009)
Variovorax paradoxus	Glera	Different grapevine tissues	Veneto Region, Italy	Baldan et al. (2014)
Vibrio sp.	*Vitis vinifera* L.	Stems	Taif Governorate, Saudi Arabia	Altalhi (2009)

(continued)

Table 10.2 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Xanthomonas campestris pv. *Dieffenbachiae*	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)
Xanthomonas campestris pv. *Celebensis*	Different cultivars	Stems	Nova Scotia, Canada	Bell et al. (1995)

Table 10.3 List of grapevine endophytes isolated by culture-independent techniques

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Acaricomes phytoseiuli	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Agrobacterium sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Bacillus sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2009, 2014)
	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Bacillus gibsonii	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Bacillus megaterium	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Bacillus pumilis	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Bacillus subtilis	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Bradyrhizobiaceae sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Brevibacillus brevis	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Brevundimonas sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Burkholderia sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011, 2014)
	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)

(continued)

Table 10.3 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Burkholderia fungorum	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011, 2014)
Caulobacteraceae sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Chitinophaga ginsengisoli	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Chitinophaga pinensis	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Chitinophaga sancti	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Chryseobacterium wanjuense	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Curtobacterium sp.	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2009)
Dyella sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Enterobacter sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Enterobacter amnigenus	Chardonnay	Roots, stems and leaves	New South Wales, Australia	West et al. (2010)
Enterococcus sp.	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2009)
Erwinia persicina	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2009)
Escherichia coli	Chardonnay	Roots, stems and leaves	New South Wales, Australia	West et al. (2010)
Ewingella americana	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2009)
Flavobacterium subsaxonicum	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Glycomyces scopariae	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Halomonas sp.	Chardonnay	Roots, stems and leaves	New South Wales, Australia	West et al. (2010)
Hydrogenophilus hirschii	Different cultivars	Roots	Northern Tunisia	Marasco et al. (2013)
Limnohabitans sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Mesorhizobium sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Methylobacterium gregans	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011, 2014)

(continued)

Table 10.3 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Micromonospora peucetia	Different cultivars	Roots	Northern Italy	Marasco et al. (2013)
Novosphingobium resinovorum	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Oceanobacillus sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Paenibacillus pasadenensis	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Pantoea agglomerans	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2009, 2014)
Pantoea ananatis	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2009)
Pectobacterium sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011, 2014)
Ralstonia sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Rhizobium radiobacter	Different cultivars	Roots	Northern Tunisia; Northwest of Cairo, Egypt	Marasco et al. (2013)
Rhodospirillaceae sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Roseomonas sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Sphingomonas sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
	Barbera	Leaves	Lombardy Region, Italy	Bulgari et al. (2014)
Sphingomonadaceae bacterium	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Propionibacterium sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Pseudomonas sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Pseudoxanthomonas sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)

(continued)

Table 10.3 (continued)

Closest relative organisms	*V. vinifera* host cultivars	Colonized plant compartments	Geographical location	References
Staphylococcus sp.	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Staphylococcus epidermidis	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Staphylococcus pasteuri	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Stenotrophomonas sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Streptomyces sp.	Chardonnay, Merlot	Lateral stems	Trentino Region, Italy	Campisano et al. (2014)
Streptomyces sodiiphilus	Different cultivars	Roots	Northern Tunisia; Northwest of Cairo, Egypt	Marasco et al. (2013)
Streptomyces violascens	Barbera	Leaves	Northwestern Italy	Bulgari et al. (2011)
Variovorax paradoxus	Different cultivars	Roots	Northwest of Cairo, Egypt	Marasco et al. (2013)
Vibrio salmonicida	Chardonnay	Roots, stems and leaves	New South Wales, Australia	West et al. (2010)

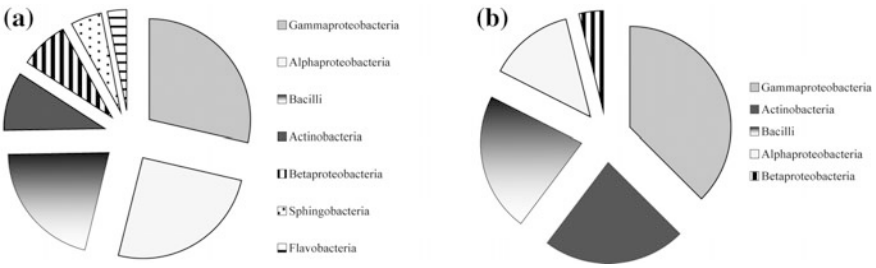

Fig. 10.5 Distribution of endophytic bacterial isolates from grapevine plants through (**A**) culture-independent and (**B**) culture-dependent techniques

V. vinifera cv. Glera is the most widely cultivated grapevine in the "Conegliano-Valdobbiadene DOCG" area (northeastern Italy). A recent manuscript showed that ~30% of the endophytic population was represented by the genus *Bacillus*. Other genera were also isolated, including *Staphylococcus*, *Microbacterium*, *Paenibacillus*, *Curtobacterium*, *Stenotrophomonas*, *Variovorax*,

Micrococcus, and *Agrococcus* (Baldan et al. 2014). The presence of *Bacillus* spp. had already been reported in Australian Chardonnay and the Italian Corvina and Barbera cultivars (Bulgari et al. 2009; West et al. 2010; Andreolli et al. 2016). A metagenomic approach revealed Streptococcaceae, Enterobacteriaceae, Pseudomonadaceae, and Moraxellaceae as dominant families in the Portuguese Tempranillo cultivar (also known as Aragonez and Tinta Roriz) (Pinto et al. 2014). Earlier, Bell et al. (1995) found that Gram-negative bacteria representing the genera *Pseudomonas* and *Enterobacter* were predominant in the Michurinetz and Marechal Foch varieties. The genera *Ralstonia*, *Burkholderia,* and *Pseudomonas* were detected in the Italian Merlot and Chardonnay cultivars.

It is important to highlight the fact that these diverse bacterial communities may reflect differences in environmental and other factors, such as fertilization strategy and/or use of different kinds of pesticides, soil composition, aridity, rhizosphere composition and biotic/abiotic stresses, rather than genotype (Marasco et al. 2013; Zarraonaindia et al. 2015). For example, Campisano et al. (2014) observed differences in the composition of endophytic communities between grapevines cultivated using organic products and integrated pest management strategies. Minor differences in bacterial endophytic communities were found between two cultivars treated with the same pest management strategy (Campisano et al. 2014). A latitudinal gradient effect has also been reported in the distribution of the endophytic community: the bacterial community associated with grapevines in Egypt was found to resemble the community found in Tunisian vines more closely than vines cultivated in Italy (Marasco et al. 2013).

A comparison of the endophytic populations in domesticated (*V. vinifera* ssp. *vinifera*) and wild (*V. vinifera* ssp. *sylvestris*) grapevine plants suggested that there is greater bacterial variability in wild plants: specifically, 118 unique strains representing 25 genera were isolated from wild plants, whereas 37 strains representing six genera were isolated from domesticated plants (Campisano et al. 2015). As stated above, this may in part reflect differences in the environmental context of cultivation, i.e., the greater variability observed in wild grapevines may be a consequence corresponding to greater biodiversity in the wild environment compared to vineyards.

10.4.3 Dynamics of Endophytic Bacterial Communities During the Grapevine Life Cycle

The analysis of endophytic communities during the vine growing season has revealed that the populations are remarkably dynamic. Baldan et al. (2014) found that the predominance of different genera in the bacterial community shifted from *Bacillus* to *Curtobacterium* between the first sample (taken immediately after the emergence of the second leaf) and the second sample (taken after berry harvesting).

A study of grapevine cultivation in the north of Italy revealed that the populations of *Alphaproteobacteria* and *Gammaproteobacteria* showed significant changes between June and August, and the structure of the *Firmicutes* community varied according to the sampling date (Bulgari et al. 2014). In Portugal, the metagenomic analysis of *V. vinifera* cv. Tempranillo revealed that Streptococcaceae, Enterobacteriaceae, Moraxellaceae, and Pseudomonadaceae were more abundant in the month of May, whereas Streptococcaceae and Enterobacteriaceae were more abundant in July (Pinto et al. 2014). Andreolli et al. (2016) investigated the endophytic bacteria isolated from 3-year-old and 15-year-old stems of *V. vinifera* cv. Corvina using culture-dependent techniques. These authors observed a higher microbial biodiversity in young grapevine plants but an increase in the number of bacterial strains within specific genera (e.g., *Pantoea* and *Rhizobium*) in stem parts from the older vines. Genera *Bacillus* and *Actinobacteria* were isolated more frequently from 3-year-old plants, whereas *Alphaproteobacteria* and *Gammaproteobacteria* were more prevalent in the 15-year-old plants (Andreolli et al. 2016).

10.5 Plant Growth-Promoting Endophytic Bacteria in Grapevine

Plant growth-promoting bacteria (PGPB) can improve the growth of grapevine plants by (i) increasing nutrient availability and assimilation, (ii) synthesizing specific compounds that grapevine plants require, and/or (iii) protecting the plants from disease by competing with phytopathogens.

10.5.1 PGPB as Fertilizers and Producers of Beneficial Molecules

PGPB can directly improve the health and support growth of grapevine plants by producing phytohormones or by promoting nutrient assimilation and thereby acting as biological fertilizers. In this manner, the bacteria improve soil fertility and crop yields while reducing the negative impact of chemical fertilizers on the environment (Babalola 2010). Ethylene is a stress hormone in plants that mediate the response to both abiotic and biotic conditions. Bacteria that synthesize the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase can degrade the ethylene precursor ACC for use as a carbon and nitrogen source, and thus present a promising opportunity to increase crop yields (Grichko et al. 2000). Phosphorus is an essential nutrient for plants. Bacteria that can solubilize mineral phosphates enhance the availability and assimilation of this element by plants (Quecine et al. 2012). The auxin indole-3-acetic acid (IAA) is one of the most important

phytohormones because it influences root growth, cell elongation and responses to light and gravity. Beneficial bacteria that produce IAA can stimulate plant growth directly (Nabti et al. 2014). Another important bacterial trait involved in plant growth promotion relies on the production of siderophores. These are small organic molecules that bind ferric iron, making it unavailable to phytopathogens but available to plants, thereby providing plants with nutrients while protecting them from pathogens (Nagarajkumar et al. 2004). The capacity of some bacterial species to produce ammonia can also enhance plant growth (Marques et al. 2010).

Several PGPB have been isolated from grapevine tissues. As stated earlier, Andreolli et al. (2016) investigated both the ecology and the growth-promoting traits of endophytic bacteria isolated from 3-year-old and 15-year-old *V. vinifera* cv. Corvina stems. Approximately 19% of the bacterial strains secreted ammonia, 21% synthesized IAA, 36% produced siderophores but only 1% displayed ACC deaminase activity. There were no differences between the young and old stems in the relative occurrence of these traits. In contrast, 25% of all the bacteria isolated by Andreolli et al. (2016) were able to solubilize phosphate, but there was a significant difference between the old and young stems: only 8.7% of the bacteria in the young stems displayed this trait, but this rose to 41% in the older stems. Furthermore, about half of all the bacterial species that were able to solubilize phosphate represented the genus *Pantoea*, the production of siderophores was attributed mainly by genus *Rhizobium*, and ACC deaminase activity was found only in *Methylobacterium* spp. (Andreolli et al. 2016). A high frequency of growth-promoting traits in endophytic strains was also observed by Campisano et al. (2015) in both domesticated (*V. vinifera* ssp. *vinifera*) and wild (*V. vinifera* ssp. *sylvestris*) plants. Interestingly, endophytes from the domesticated plants displayed more growth-promoting traits than those in wild plants, suggesting that grapevine domestication did not involve a loss of agriculturally relevant traits (Campisano et al. 2015). The distribution of growth-promoting features seems not to differ significantly among vines grown in Italy, Tunisia, and Egypt (96% in Italy, 97% in Tunisia, and 94% in Egypt), indicating that functional growth-promoting potential is maintained in grapevine root systems in different areas (Marasco et al. 2013).

Several studies have demonstrated the beneficial effects of inoculating grapevine plants with PGPB (Subramanian et al. 2015; Abbamondi et al. 2016). The effect of *Burkholderia* sp. IF25 on adventitious root emission was evaluated in micropropagated grapevine explants. This bacterial strain is characterized by multiple growth-promoting traits including phosphate solubilization, IAA synthesis, and siderophore synthesis. After 8 days, no root emergence was observed in the untreated plants, but 30% of the infected grapevine plants showed evidence of root development. After 30 days, 40% of the untreated plants evidenced root neogenesis, but in the inoculated plants incidence of new root formation rose to 80% (Muganu et al. 2015).

It is worth noting that Baldan et al. (2015) found 12 promising PGPB mainly represented by the genera *Bacillus*, *Micrococcus,* and *Pantoea* able to exert beneficial effects on *Arabidopsis thaliana* in terms of structural root development. The

effects of PGPB on grapevine plants were observed in detail using the endophytic strain *Burkholderia phytofirmans* PsJN, which was originally isolated from onion roots infected with *Glomus vesiculiferum* (Nowak et al. 1995). Infection with this strain was able to reduce chilling-induced damage consisting in inhibition of both root growth and plant biomass accumulation. The infection induced starch synthesis in host plants and increased their photosynthetic capacity, as well as acquisition of several traits associated with low-temperature tolerance such as accumulation of proline and phenolic compounds, and modification of carbohydrate metabolism (Ait Barka et al. 2006; Fernandez et al. 2012; Theocharis et al. 2012). Recently, Rolli et al. (2016) have evidenced that PGP bacteria isolated in the laboratory from different geographical origins and derived from different crop plants can be successfully exploited to promote growth of grapevines both in vitro and in the field.

10.5.2 PGPB as Biocontrol Agents in Grapevine

Grapevine plants can be infected and colonized by several pathogens that cause significant losses in the wine industry (Gubler et al. 2005; Ricketts et al. 2015). Pesticides are currently applied in vineyards (Chen et al. 2016), but the continuous use of chemical products over the last few decades has resulted in the accumulation of their residues and the contamination of the environment, ultimately affecting human health (Pérez-Ortega et al. 2012; Lavezzi et al. 2015). Both integrated pest management (IPM) and organic production methods can reduce the use of synthetic pesticides in agriculture (Council Regulation (EC) No 834/2007 (2007); Council Directive 2009/128/EC 2009). Campisano et al. (2014) investigated the impact of these two pest management approaches on the composition of endophytic communities, revealing significant differences in the structure of such bacterial populations. Actually, the genera *Mesorhizobium* and *Staphylococcus* were more abundant in plants from vineyards managed by organic farming, whereas the genus *Ralstonia* was more abundant when IPM was the procedure used (Campisano et al. 2014). Therefore, bacterial endophyte populations are clearly affected by anthropogenic factors such as pest management strategies. An alternative approach to reduce the use of pesticides in vineyards involves the application of beneficial bacteria as biocontrol agents (Compant et al. 2013). Certain bacteria can improve plant growth by reducing the effects of phytopathogens through direct or indirect mechanisms (Compant et al. 2005a). For example, bacteria can compete with pathogens for root niches and nutrients, synthesize allelochemicals such as biocides, antibiotics, or lytic enzymes, or interfere with the quorum sensing ability of pathogens. Furthermore, the interaction between some beneficial bacteria and their host plant can increase host resistance to certain pathogenic bacteria, fungi, and viruses through a mechanism known as induced systemic resistance (ISR) (Lugtenberg and Kamilova 2009).

10.5.2.1 Grapevine Fungal Diseases

A number of fungal taxa caused trunk diseases but the diatrypaceous fungus *Eutypa lata* is known to cause one of the major syndromes, namely the Eutypa dieback, also known as dead arm and grape canker (Trouillas et al. 2010). Ferreira et al. (1991) found that spraying plants with a suspension of an endophytic strain of *B. subtilis* (previously isolated from the Chenin Blanc cultivar) reduced the likelihood of *E. lata* infection. In vitro tests showed that this bacterium induced malformation of fungal hyphae and inhibition of ascospore germination (Ferreira et al. 1991).

Botrytis cinerea is the agent responsible for gray mold, which affects young fruit during the ripening process (Williamson et al. 2007). Andreolli et al. (2016) isolated 11 strains among 196 stem-derived endophytes capable of inhibiting the growth of *B. cinerea*. One strain representing the genus *Lysinibacillus* induced a significantly wider zone of growth inhibition on agar plates than the other strains (Andreolli et al. 2016). In another study, 26 isolates were able to control *B. cinerea* on grapevine leaves, and nine strains showed an antifungal effect in vitro. Among them, the two strains *Pantoea* sp. PTA-AF1 and *Pseudomonas fluorescens* PTA-CT2 were isolated from disinfected leaves and stems, respectively (Trotel-Aziz et al. 2008). Furthermore, 25 endophytic strains isolated from domesticated and wild grapevine plants were highly active against *B. cinerea* in vitro, particularly those strains belonging to the genera *Bacillus* and *Pantoea* (Campisano et al. 2015). Ait Barka et al. (2000, 2002) found that the ability of *B. phytofirmans* PsJN to inhibit the infection of grapevine plants by *B. cinerea* was related to the induction of transient extracellular alkalization, the production of salicylic acid and the expression of defense-related transcripts (Bordiec et al. 2011).

On the other hand, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* are associated with tracheomycosis and the so-called esca disease, grapevine trunk diseases that severely affect vine yield and longevity (Larignon and Dubos 1997). In vitro, tests showed that two endophytic *Bacillus* strains isolated from grapevine stems were effective against *P. chlamydospora* and *P. aleophilum* (Andreolli et al. 2016). Furthermore, Campisano et al. (2015) isolated six strains with potent activity and seven strains with moderate activity against *P. aleophilum*. A recent screen has also shown that a considerable number of endophytic bacterial isolates from grapevine can depress the growth of *Neofusicoccum parvum*, *Botryosphaeria dothidea, Botryosphaeria obtuse, Pochonia chlamydospora,* and *Plasmopara viticola* in vitro (Campisano et al. 2015; Andreolli et al. 2016).

10.5.2.2 Grapevine Bacterial Diseases

Bacteria belonging to different genera are cause of diseases in grapevine during its life cycle. *Rhizobium vitis* (formerly *Agrobacterium vitis*) is the etiological agent of crown gall disease in grapevine nurseries, usually inhibiting growth but even up to killing plants in the most severe cases (Young et al. 2001; Creasap et al. 2005). Bell et al. (1995) identified 24 endophytic strains of *Enterobacter agglomerans*,

Rahnella aquatilis, and *Pseudomonas* sp. with a strong inhibitory effect on *R. vitis*. Moreover, three endophytic bacteria isolated from *Malus domestica* (namely *Pseudomonas fluorescens* 1100-6, *B. subtilis* EN63-1, and *Bacillus* sp. EN71-1) demonstrated to significantly reduce both *R. vitis* population and gall size. Growth chamber studies revealed that *P. fluorescens* 1100-6 persisted in the xylem and pith tissues of grapevine plants for 6 months, evidencing the participation of this beneficial strain in the endophytic community (Eastwell et al. 2006).

The agent responsible for Pierce's disease in grapevines is *Xylella fastidiosa*, which aggressively colonizes xylem vessels following transmission by sharpshooter leafhoppers (Cicadellidae) (Chatterjee et al. 2008). Nevertheless, several avirulent endophytic strains of *X. fastidiosa* can attenuate the severity of Pierce's disease symptoms in the grapevine cultivar Carignan; but only strain EB92-1 (isolated from elderberry) was found capable of an effective disease control in both Flame Seedless and Cabernet Sauvignon vines (Hopkins 2005). Genome sequencing of the avirulent strain EB92-1 evidenced high similarity to pathogenic *X. fastidiosa* strains, but 10 genes associated with virulence factors were missing (Zhang et al. 2011). Therefore, although *X. fastidiosa* EB92-1 appears to be an effective biocontrol agent against Pierce's disease, there is some concern that this strain could revert to virulence via mutation or the acquisition of virulence genes from pathogenic *X. fastidiosa.*

10.5.2.3 Grapevine Phytoplasma Diseases

Grapevine yellow complex is a severe disease caused by obligate bacterial parasites (phytoplasma) that invade plant phloem tissue (Belli et al. 2010), against which no effective control measures or naturally occurring resistance traits exist (Laimer et al. 2009). Recently, ACC deaminase activity of the bacterial endophyte *Pseudomonas migulae* 8R6 was shown to help the plant regulate the level of ethylene, improving resistance to phytoplasma infection (Gamalero et al. 2016).

Phytoplasma-infected plants may spontaneously recover, although the underlying mechanisms and biological factors of such a resilience are unknown (Musetti et al. 2004). Therefore, the effect of endophytic bacteria on the recovery of vines from phytoplasma infection has been investigated. Bulgari et al. (2011, 2014) found significant differences among the endophytic bacterial communities of recovered, infected and healthy (control) grapevine plants, with less bacterial diversity between infected and recovered plants compared to the control. The loss of bacterial richness may reflect the direct interaction between phytoplasma and endophytic bacteria or competition between these species for carbon sources or favorable niches (Bulgari et al. 2011). Recently Bulgari et al. (2014) reported similarity between bacterial communities of control and infected plants only when phytoplasma titers were below the level of detection. This confirms that the proliferation of phytopathogens can affect the structure of plant-associated bacterial communities (Trivedi et al. 2010; Bulgari et al. 2012). Strains of the genus *Burkholderia*, *Bacillus pumilis, Paenibacillus pasadenensis,* and uncultured *Bacillus* sp. could only be isolated

from the recovered grapevine plants (Bulgari et al. 2011). These species are well-known activators of ISR and also produce allelochemicals (Choudhary and Johri 2009; Depoorter et al. 2016). Furthermore, genus-specific PCR analyses revealed that *Burkholderia*, *Methylobacterium,* and *Pantoea* communities were markedly influenced by the phytoplasma infection (Bulgari et al. 2014) indicating how the presence of the phytopathogen affects endophytic communities more than the environmental factors. Furthermore, the presence of ISR-eliciting bacteria specifically in recovered plants may be an indice of the involvement of these endophytes in the resilience of grapevine from the yellow syndrome. Accordingly, these strains may provide an effective strategy for the biocontrol.

10.6 Conclusions and Future Perspectives

The colonization of plant tissues by microbial endophytes confers benefits to the host plant such as enhanced growth and protection against abiotic and biotic stress. Endophytes could, therefore, be exploited to increase the yield of grapevine plants or even to modify the organoleptic properties of harvested fruits. In the last few years, the role of endophytes in vineyards has attracted attention because plants harbor an interesting internal microbiome that could enhance productivity and provide a natural disease control capacity, thus avoiding the widespread use of chemical pesticides. These bacteria could even help to mitigate the impact of climate change, particularly in vineyards affected by encroaching desertification and soil salinization. Efforts to identify endophytic bacteria and the underlying mechanisms of plant–endophyte interactions could, therefore, evolve into strategies for plant protection, ecologically beneficial vineyard management and sustainable viticulture. Analysis of grapevine endophytic microbiome would not only increase our understanding of the equilibrium among the microbial inhabitants of internal plant tissues but would also help to identify strains with potential beneficial traits that could be applied as growth promoters or biological fertilizers.

The modern agricultural economy is based on the extended use of agrochemicals and intensive production practices which have a negative impact on biodiversity, including natural microbial communities. These microbial consortia must urgently be preserved, particularly because some are beneficial to plants by mediating essential processes such as nitrogen fixation, phosphate solubilization, production of growth-promoting phytohormones and siderophores, and protection against plant pathogens. Future investigations should focus on a detailed analysis of the endophytic microbiome of grapevine plants and the interactions between this important fruit crop and its internal microbial inhabitants. This could lead to the development of new biotechnological approaches for an ecologically sound improved productivity, quality, and sustainability of the viticulture industry.

References

Abbamondi GR, Tommonaro G, Weyens N, Thijs S, Sillen W, Gkorezis P, Iodice C, Rangel WdM, Nicolaus B, Vangronsveld J (2016) Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. Chem Biol Technol Agric 3:1

Ait Barka E, Belarbi A, Hachet C, Nowak J, Audran JC (2000) Enhancement of in vitro growth and resistance to gray mould of *Vitis vinifera* co-cultured with plant growth promoting rhizobacteria. FEMS Microbiol Lett 186:91–95

Ait Barka E, Gognies S, Nowak J, Audran JC, Belarbi A (2002) Inhibitory effect of bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biol Control 24:135–142

Ait Barka E, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252

Altalhi AD (2009) Plasmids profiles, antibiotic and heavy metal resistance incidence of endophytic bacteria isolated from grapevine (*Vitis vinifera* L.). Afr J Biotechnol 8:5873–5882

Andreolli M, Lampis S, Poli M, Gullner G, Biró B, Vallini G (2013) Endophytic *Burkholderia fungorum* DBT1 can improve phytoremediation efficiency of polycyclic aromatic hydrocarbons. Chemosphere 92:688–694

Andreolli M, Lampis S, Zapparoli G, Angelini E, Vallini G (2016) Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. Corvina and their potential for plant growth promotion and phytopathogen control. Microbiol Res 183:42–52

Aravind R, Kumar A, Eapen SJ, Ramana K (2009) Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici*. Lett Appl Microbiol 48:58–64

Azevedo JL, Walter M, Pereira JO, Araújo WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J Biotechnol 3:40–65

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Baldan E, Nigris S, Populin F, Zottini M, Squartini A, Baldan B (2014) Identification of culturable bacterial endophyte community isolated from tissues of *Vitis vinifera* "Glera". Plant Biosyst 148:508–516

Baldan E, Nigris S, Romualdi C, D'Alessandro S, Clocchiatti A, Zottini M, Stevanato P, Squartini A, Baldan B (2015) Beneficial bacteria isolated from grapevine inner tissues shape *Arabidopsis thaliana* roots. PLoS One 10:e0140252

Bell CR, Dickie GA, Harvey WLG, Chan JWYF (1995) Endophytic bacteria in grapevine. Can J Microbial 41:46–53

Belli G, Bianco PA, Conti M (2010) Grapevine Yellows in Italy: past, present and future. J Plant Pathol 92:303–326

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol 51:215–229

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Ann Rev Plant Biol 60:379–406

Bordiec S, Paquis S, Lacroix H, Dhondt S, Ait Barka E, Kauffmann S, Jeandet P, Mazeyrat-Gourbeyre F, Clément C, Baillieul F, Dorey S (2011) Comparative analysis of defence responses induced by the endophytic plant growth-promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN and the non-host bacterium *Pseudomonas syringae* pv. pisi in grapevine cell suspensions. J Exp Bot 62:595–603

Brent KJ, Hollomon DW (2007) Fungicide resistance: the assessment of risk. 2nd revised edition, FRAC (Fungicide Resistance Action Committee), Croplife International, Brussels

Bulgari D, Casati P, Brusetti L, Quaglino F, Brasca M, Daffonchio D, Bianco PA (2009) Endophytic bacterial diversity in grapevine (*Vitis vinifera* L.) leaves described by 16S rRNA gene sequence analysis and length heterogeneity-PCR. J Microbiol 47:393–401

Bulgari D, Casati P, Crepaldi P, Daffonchio D, Quaglino F, Brusetti L, Bianco PA (2011) Restructuring of endophytic bacterial communities in grapevine yellows-diseased and recovered *Vitis vinifera* L. plants. Appl Environ Microbiol 77:5018–5022

Bulgari D, Bozkurt AI, Casati P, Caglayan K, Quaglino F, Bianco PA (2012) Endophytic bacterial community living in roots of healthy and '*Candidatus* Phytoplasma mali'-infected apple (*Malus domestica*, Borkh.) trees. Antony von Leeuwenhoek 102:677–687

Bulgari D, Casati P, Quaglino F, Bianco PA (2014) Endophytic bacterial community of grapevine leaves influenced by sampling date and phytoplasma infection process. BMC Microbiol 14:198

Campisano A, Antonielli L, Pancher M, Yousaf S, Pindo M, Pertot I (2014) Bacterial endophytic communities in the grapevine depend on pest management. PLoS One 9:e112763

Campisano A, Pancher M, Puopolo G, Puddu A, Lòpez-Fernàndez S, Biagini B, Yousaf S, Pertot I (2015) Diversity in endophyte populations reveals functional and taxonomic diversity between wild and domesticated grapevines. Am J Enol Vitic 66:1

Chatterjee S, Almeida RPP, Lindow SE (2008) Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. Annu Rev Phytopathol 46:243–271

Chen B, Wu F-q Wu, W-d Jin B-h, L-q Xie, Feng W, Ouyang G (2016) Determination of 27 pesticides in wine by dispersive liquid–liquid microextraction and gas chromatography–mass spectrometry. Microchem J 126:415–422

Choudhary DK, Johri BN (2009) Interactions of *Bacillus* spp. and plants—with special reference to induced systemic resistance (ISR). Microbiol Res 164:493–513

Compant S, Mathieu F (2016) Biocontrol of major grapevine diseases: leading research. CABI (Centre for Agriculture and Biosciences International), Wallingford, UK

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005a) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Ait Barka E (2005b) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71:1685–1693

Compant S, Kaplan H, Sessitsch A, Nowak J, Ait Barka E, Clément C (2008) Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. FEMS Microbiol Ecol 63:84–93

Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A (2011) Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb Ecol 62:188–197

Compant S, Brader G, Muzammil S, Sessitsch A, Lebrihi A, Mathieu F (2013) Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. Biocontrol 58:435–455

Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608

Council Directive 2009/128/EC (2009) Council Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. OJ: 16. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:en:PDF. Accessed from 24.11.2009

Council Regulation (EC) No 834/2007 (2007) Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ: 23. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:189:0001:0023:EN:PDF. Accessed from 20.7.2007

Creasap J, Reid C, Goffinet M, Aloni R, Ullrich C, Burr T (2005) Effect of wound position, auxin, and *Agrobacterium vitis* strain F2/5 on wound healing and crown gall in grapevine. Phytopathology 95:362–367

Croes S, Weyens N, Janssen J, Vercampt H, Colpaert JV, Carleer R, Vangronsveld J (2013) Bacterial communities associated with *Brassica napus* L. grown on trace element-contaminated and non-contaminated fields: a genotypic and phenotypic comparison. Microb Biotechnol 6:371–384

Depoorter E, Bull MJ, Peeters C, Coenye T, Vandamme P, Mahenthiralingam E (2016) *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. Appl Microbiol Biotechnol 100:5215–5229

Dias MC (2012) Phytotoxicity: an overview of the physiological responses of plants exposed to fungicides. J Bot. doi:10.1155/2012/135479

Eastwell KC, Sholberg PL, Sayler RJ (2006) Characterizing potential bacterial biocontrol agents for suppression of *Rhizobium vitis*, causal agent of crown gall disease in grapevines. Crop Protec 25:1191–1200

EFSA (European Food Safety Authority) (2013) The 2013 European Union report on pesticide residues in food. EFSA J 13:4038–4207

FAOSTAT (2015) available to http://faostat.fao.org

Fernandez O, Theocharis A, Bordiec S, Feil R, Jacquens L, Clément C, Fontaine F, Ait Barka E (2012) *Burkholderia phytofirmans* PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. Mol Plant Microbe Interact 25:496–504

Ferreira JHS, Matthee FN, Thomas AC (1991) Biological control of *Eutypa lata* on grapevine by an antagonistic strain of *Bacillus subtilis*. Phytopathology 81:283–287

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750

Gamalero E, Marzachì C, Galetto L, Veratti F, Massa N, Bona E, Novello G, Glick BR, Ali S, Cantamessa S, D'Agostino G, Berta G (2016) An 1-Aminocyclopropane-1-carboxylate (ACC) deaminase-expressing endophyte increases plant resistance to flavescence dorée phytoplasma infection. Plant Biosyst (in press). doi:10.1080/11263504.2016.1174172

Garbeva P, Overbeek LS, Vuurde JWL, Elsas JD (2001) Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microbial Ecol 41:369–383

Gary AS (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Goldammer JG (2015) Vegetation fires and global change-challenges for concerted international action: a white paper directed to the United Nations and International Organizations. Planet Risk 3:45–57

Grichko VP, Filby B, Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd Co, Cu, Ni, Pb, and Zn. J Biotechnol 81:45–53

Gubler WD, Rolshausen PE, Trouillas FP, Urbez JR, Voegel T, Leavitt GM, Weber EA (2005) Grapevine trunk diseases in California. Pract Winery Vineyard Mag 27:6–25

Hallmann J, Berg B (2006) Spectrum and population dynamics of bacterial root endophytes. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, Berlin, Germany, pp 15–31

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Hallmann J, Berg G, Schulz B (2006) Isolation procedures for endophytic microorganisms. In: Schulz B, Boyle C, Sieber N (eds) Soil biology, vol 9. Springer. Berlin, Germany, pp 299–319

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Hardoim PR, van Overbeek LS, 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

Hopkins DL (2005) Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plan Dis 89:1348–1352

Huang X-F, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM (2014) Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany 92:267–275

Kevin VJ (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Laimer M, Lemaire O, Herrbach E, Goldschmidt V, Minafra A, Bianco P, Wetzel T (2009) Resistance to viruses, phytoplasmas and their vectors in the grapevine in Europe: a review. J Plant Pathol 91:7–23
Larignon P, Dubos B (1997) Fungi associated with esca disease in grapevine. Eur J Plant Pathol 103:147–157
Lavezzi AM, Cappiello A, Pusiol T, Corna MF, Termopoli V, Matturri L (2015) Pesticide exposure during pregnancy, like nicotine, affects the brainstem α7 nicotinic acetylcholine receptor expression, increasing the risk of sudden unexplained perinatal death. J Neurol Sci 348:94–100
Liaqat F, Eltem R (2016) Identification and characterization of endophytic bacteria isolated from in vitro cultures of peach and pear rootstocks. 3 Biotech. doi:10.1007/s13205-016-0442-6
Lo Piccolo S, Ferraro V, Alfonzo A, Settanni L, Ercolini D, Burruano S, Moschetti G (2010) Presence of endophytic bacteria in *Vitis vinifera* leaves as detected by fluorescence in situ hybridization. Ann Microbiol 60:161–167
Lòpez-Fernàndez S, Compant S, Vrhovsek U, Bianchedi PL, Sessitsch A, Pertot I, Campisano A (2015a) Grapevine colonization by endophytic bacteria shifts secondary metabolism and suggests activation of defense pathways. Plant Soil. doi:10.1007/s11104-015-2631-1
Lòpez-Fernàndez S, Sonego P, Moretto M, Pancher M, Engelen K, Pertot I, Campisano A (2015b) Whole-genome comparative analysis of virulence genes unveils similarities and differences between endophytes and other symbiotic bacteria. Front Microbiol 6:419
Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556
Magnani GS, Cruz LM, Weber H, Bespalhok JC, Daros E, Baura V, Yates MG, Monteiro RA, Faoro H, Pedrosa FO, Souza EM (2013) Culture independent analysis of endophytic bacterial communities associated to Brazilian sugarcane. Genet Mol Res 12:4549–4558
Marasco R, Rolli E, Fusi M, Cherif A, Abou-Hadid A, El-Bahairy U, Borin S, Sorlini C, Daffonchio D (2013) Plant growth promotion potential is equally represented in diverse grapevine root-associated bacterial communities from different biopedoclimatic environments. Biomed Res Int. doi:10.1155/2013/491091
Marques APGC, Pires C, Moreira H, Rangel AOSS, Castro PML (2010) Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. Soil Biol Biochem 42:1229–1235
Mattia F, Imazio S, Grassi F, Doulati H, Scienza A, Labra M (2008) Study of genetic relationships between wild and domesticated grapevine distributed from middle east regions to European countries. Rendiconti Lincei 19:223–240
Mercado-Blanco J, Prieto P (2012) Bacterial endophytes and root hairs. Plant Soil 361:301–306
Muganu M, Paolocci M, Bignami C, Di Mattia E (2015) Enhancement of adventitious root differentiation and growth of in vitro grapevine shoots inoculated with plant growth promoting rhizobacteria. Vitis 54:73–77
Musetti R, di Toppi LS, Ermacora P, Favali MA (2004) Recovery in apple trees infected with apple proliferation phytoplasma: an ultrastructure and biochemical study. Phytopathology 94:203–208
Nabti E, Bensidhoum L, Tabli N, Dahel D, Weiss A, Rothballer M, Schmid M, Hartmann A (2014) Growth stimulation of barley and biocontrol effect on plant pathogenic fungi by a *Cellulosimicrobium* sp. strain isolated from salt-affected rhizosphere soil in northwestern Algeria. Eur J Soil Biol 61:20–26
Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159:73–81
Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L (2016) Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front Public Health. doi:10.3389/fpubh.2016.00148

Nowak J, Asiedu SK, Lazarovits G, Pillay V, Stewart A, Smith C, Liu Z (1995) Enhancement of in vitro growth and transplant stress tolerance of potato and vegetable plantlets co-cultured with a plant growth-promoting rhizobacterium. In: Carré F, Chagvardieff P (eds) Proceedings of the international symposium on ecophysiology and photosynthetic in vitro cultures, CEA, Aix-en-Provence, France, pp 173–180

Padgham J, Le H, Richard AS (2005) Opportunities for nematode biocontrol in lowland rainfed rice using bacterial endophytes. In: Tielkes E, Hülsebusch C, Häuser I, Deininger A, Becker K (eds) The global food and product chain-dynamics, innovations, conflicts, strategies: international research on food security, natural resource management and rural development. Book of abstract, Tropentag 2005, Stuttgart, Hohenheim, October 11–13, 2005, University of Hohenheim, Stuttgart, Germany, p 293

Pérez-Ortega P, Gilbert-López B, García-Reyes JF, Ramos-Martos N, Molina-Díaz A (2012) Generic sample treatment method for simultaneous determination of multiclass pesticides and mycotoxins in wines by liquid chromatography–mass spectrometry. J Chromatog A 1249:32–40

Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC (2014) Unravelling the diversity of grapevine microbiome. PLoS One 9:e85622

Qin S, Xing K, Jiang J-H, Xu L-H, Li W-J (2011) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89:457–473

Quecine MC, Araújo WL, Rossetto PB, Ferreira A, Tsui S, Lacava PT, Mondin M, Azevedo JL, Pizzirani-Kleinera AA (2012) Sugarcane growth promotion by the endophytic bacterium *Pantoea agglomerans* 33.1. Appl Environ Microbiol 78:7511–7518

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160

Ramos PL, Van Trappen S, Thompson FL, Rocha RCS, Barbosa HR, De Vos P, Moreira-Filho CA (2011) Screening for endophytic nitrogen-fixing bacteria in *Brazilian sugar* cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov. Int J Syst Evol Microbiol 61:926–931

Reissinger A, Vilich V, Sikora RA (2001) Detection of fungi in planta: effectiveness of surface sterilization methods. Mycol Res 105:563–566

Ricketts KD, Gomez MI, Atallah SS, Fuchs MF, Martinson TE, Battany MC, Bettiga LJ, Cooper ML, Verdegaal PS, Smith RJ (2015) Reducing the economic impact of grapevine leafroll disease in California: identifying optimal disease management strategies. Am J Enol Viticolt. doi:10.5344/ajev.2014.14106

Rolli E, Marasco R, Saderi S, Corretto E, Mapelli F, Cherif A, Borin S, Valenti L, Sorlini C, Daffonchio D (2016) Root-associated bacteria promote grapevine growth: from the laboratory to the field. Plant Soil. doi:10.1007/s11104-016-3019-6

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837

Schloss PD, Handelsman J (2005) Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome Biol 6:229

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–687

Schulz B, Boyle C (2006) What are Endophytes? In: Schulz B, Boyle C, Sieber TN (eds) Microbial root endophytes, Springer, Berlin, Heidelberg, pp 1–13

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CW (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. J Appl Environ Microbiol 67:2469–2475

Sturz AV, Matheson BG (1996) Populations of endophytic bacteria which influence host-resistance to *Erwinia*-induced bacterial soft rot in potato tubers. Plant Soil 184:265–271

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Subramanian P, Kim K, Krishnamoorthy R, Sundaram S, Sa T (2015) Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regul 76:327–332

Sziderics AH, Rasche F, Trognitz F, Sessitsch A, Wilhelm E (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). Can J Microbiol 53:1195–1202

Terral J-F, Tabard E, Bouby L, Ivorra S, Pastor T, Figueiral I, Picq S, Chevance J-B, Jung C, Fabre L, Tardy C, Compan M, Bacilieri R, Lacombe T (2010) Evolution and history of grapevine (*Vitis vinifera*) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. Ann Bot 105:443–455

Theocharis A, Bordiec S, Fernandez O, Paquis S, Dhondt-Cordelier S, Baillieul F, Clément C, Ait Barka E (2012) *Burkholderia phytofirmans* PsJN primes *Vitis vinifera* L. and confers a better tolerance to low non-freezing temperatures. Mol Plant Microbe Interact 25:241–249

Tian X, Cao L, Tan H, Han W, Chen M, Liu Y, Zhou S (2007) Diversity of cultivated and uncultivated actinobacterial endophytes in stems and roots of rice. Microb Ecol 53:700–707

Torregrosa L, Vialet S, Adivèze A, Iocco-Corena P, Thomas MR (2015) Grapevine (*Vitis vinifera* L.). In: Wang K (ed) *Agrobacterium* protocols, vol 1224, Methods in molecular biology. Springer, Berlin, New York pp 177–194

Trdá L, Fernandez O, Boutrot F, Héloir MC, Kelloniemi J, Daire X, Adrian M, Clément C, Zipfel C, Dorey S, Poinssot B (2014) The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. New Phytol 201:1371–1384

Trivedi P, Duan Y, Wang N (2010) Huanglongbing, a systemic disease, restructures the bacterial community associated with citrus roots. Appl Environ Microbiol 76:3427–3436

Trotel-Aziz P, Couderchet M, Biagianti S, Aziz A (2008) Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. mediating grapevine resistance against *Botrytis cinerea*. Environ Exp Bot 64:21–32

Trouillas FP, Urbez-Torres JR, Gubler WD (2010) Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102:319–336

van Overbeek LS, Saikkonen K (2016) Impact of bacterial–fungal interactions on the colonization of the endosphere. Trends Plant Sci 21:230–242

Vitis International Variety Catalogue (2015) Available to www.vivc.de

Vivier MA, Pretorius IS (2002) Genetically tailored grapevines for the wine industry. Trends Biotechnol 20:472–478

West ER, Cother EJ, Steel CC, Ash GJ (2010) The characterization and diversity of bacterial endophytes of grapevine. Can J Microbiol 56:209–216

Williamson B, Tudzynski B, Tudzynski P, Van Kan JAL (2007) *Botrytis cinerea*: the cause of grey mould disease. Mol Plant Pathol 8:561–580

Wilson M, Rod McNab, Henderson B (2002) Bacterial invasion as a virulence mechanism. In: Bacterial disease mechanisms. Cambridge University Press, Cambridge, pp 405–465

Xia Y, DeBolt S, Dreyer J, Scott D, Williams MA (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front Plant Sci. doi:10.3389/fpls.2015.00490

Yoon M-Y, Cha B, Kim J-C (2013) Recent trends in studies on botanical fungicides in agriculture. Plant Pathol J 29:1–9

Young JM, Kuykendall LD, Martinez-Romero E, Kerr A, Sawada H (2001) A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter, R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*. Int J Syst Evol Microbiol 51:89–103

Zamioudis C, Pieterse C (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microb Interac 25:139–150

Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-Marcell J, Lax S, Bokulich NA, Mills DA, Martin G, Taghavi S, van der Lelie D, Gilbert JA (2015) The soil microbiome influences grapevine-associated microbiota. MBio 6:e02527–e14

Zeng W, He S (2010) A prominent role of the flagellin receptor flagellin-sensing2 in mediating stomatal response to *Pseudomonas syringae* pv tomato DC3000 in *Arabidopsis*. Plant Physiol 153:1188–1198

Zhang S, Flores CZ, Kumar D, Chakrabarty P, Hopkins DL, Gabriel DW (2011) The *Xylella fastidiosa* biocontrol strain EB92-1 genome is very similar and syntenic to Pierce's disease strains. J Bacteriol 193:5576–5577

Chapter 11
Biology, Diversity and Promising Role of Mycorrhizal Endophytes for Green Technology

Kamal Prasad

Abstract Arbuscular mycorrhizal fungal symbiosis formed by majority of vascular plants has played a key role in evolution of land plants. An understanding of the manifold advantages of mycorrhizal symbiosis can be helpful in utilizing them as a significant microbe in green technology for sustainable agriculture development which has become an absolute requirement in current environmental scenario. The manuscript discusses the implication of recent results and ideas on symbiosis that are relevant for plant community establishment under natural environmental condition and way the process are interlinked. Mycorrhizal symbiosis also opens a way to a pollution-free environment by playing a magnificent role in nutrient uptake, interacts to affect plant community composition by changing relative species abundance and consequently above-ground productivity, thereby replacing the chemical input and saving the fertilizers subsidiary of government and save the environment.

Keywords Biology · Diversity · Mycorrhizae · Green technology Environment

Abbreviation

AL	*Acaulospora laecunosa*
At	*Acaulospora tuberculata*
Ga	*Glomus aggregatum*
Gc	*Glomus constrictum*
Gca	*Glomus caledonium*
Gf	*Glomus fasciculatum*
Gi	*Glomus intraradices*
Gia	Gigaspora albida
Gge	*Glomus gerdemanil*

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_11

Gic *Gigaspora candida*
Gm *Glomus mosseae*
Gma *Glomus macrocarpum*
Gmi *Glomus microcarpum*
Gco *Gmomus coronatum*
Get *Glomus etunicatum*
Gle *Glomus lept*oticum
Gs *Glomus species*
Gci *Gigaspora calaspora*
Gg *Gigaspora gigantea*
Gsp *Gigaspora spp.*
Sn *Sclerocystis nigra*
Sc *Sclerocystis spp.*

11.1 Introduction

Mycorrhizae are highly evolved soil fungi involved in tripartite interaction mutualistic associations amid soil and plant. The associations formed by Glomeromycota fungi in plants usually colonize in arbuscules and often vesicles thus, known as vesicular mycorrhiza (AM) and vesicular-arbuscular mycorrhizas (VAM). These are members of Zygomycetes, Ascomycetes and Basidiomycetes classes of fungi kingdom (Kendrick 1985; Brundrett 2006). The knowledge updated so far in the context mycorrhizal literature, the term symbiosis in mycorrhiza association is used to describe their highly interdependent or obligatory mutualistic relationships with the plants where the host plant receives mineral nutrients and in turn fungus harness photosynthetically derived carbon compounds (Harley and Smith 1983; Prasad 1993; Gautam and Prasad 2001; Prasad 2015). The most common associations are (i) vesicular-arbuscular mycorrhizas (VAM) in which zygomycetous fungi produce arbuscules, hyphae and vesicles within root cortex cells, (ii) ectomycorrhizal (ECM) where Basidiomycetes and other fungi form a mantle around roots and a Hartig net between root cells, (iii) orchid mycorrhizas where fungi produce coils of hyphae within roots (or stems) of orchidaceous plants and (iv) ericoid mycorrhizas involving hyphal coils in outer cells of the narrow "hair roots" of plants in the Ericales. Hyphae of a mycorrhizal fungus originating from one entry point in roots or one propagule in soil are referred to as colonies, and colonization refers to the ability of root occupation by mycorrhizal fungi.

Arbuscular mycorrhizal (AM) fungi found in rhizosphere and associated with several vascular plants have tremendous contribution in sustainable agriculture as well as agricultural ecosystems management. The beneficial effects of indigenous AM fungi on the nutrition replenishment for plants depend on both the abundance and type of fungi present in the soil (Abbott and Robson 1982; Prasad and Gautam 2000; Prasad 2000c, 2005). However, the potential of AM fungi to be employed on

a wide scale in agriculture solely depends on the development corroborating crop growth promotion. (Menge 1983; Prasad 1993; Prasad 2000c, 2005; Prasad and Kaushik 2004). Therefore, field study becomes necessary to understand the abundance and type of indigenous AM fungi present in the plant rhizosphere. AM fungi also benefit plants by increasing nutrient water uptake, resistance against phytopathogens, adaptation to a variety of environmental stresses such as drought, heat, salinity and heavy metal contamination, production of growth hormones and certain enzymes and even in the uptake of radioactive elements. Thus, incorporation of the natural roles of beneficial microorganisms in maintaining soil fertility and plant productivity is gaining importance and can be an important approach towards a clean and green environment. In addition, we have identified efforts to determine key areas where sincere research efforts are still needed to develop strategies for manipulating mycorrhizae application in such a way that it could be more efficiently utilized in managing soil and sustainable development for green technology.

11.2 The Biology of Mycorrhizal Fungi

Mycorrhizal symbioses are ubiquitous system of green technology. In these symbioses, the fungal mycelia scavenge through soil for resources (often phosphorus or nitrogen) and provide these resources to plants in exchange of organic carbon. The associations are mutualistic most often but sometimes exist as parasitism depending upon fungal nature. Mycorrhizal associations may involve any of four different fungal phyla as mentioned earlier and a broad range of plants including mosses and liverworts, ferns, seed plants, etc. The mycorrhizal status of many plants is yet to be explored; about 90% plant species have mycorrhizal association and rest only 10% do not form mycorrhizal symbiosis. The symbioses among plant and fungi are often classed by either arbuscular mycorrhizal fungi (AMF) or ectomycorrhizal fungi (EMF), defined by both the taxonomy of the fungi and the structures formed in or around plant roots. In addition to AMF and EMF symbioses, mycorrhizal associations include arbutoid, monotropoid, ericoid and orchid forms. This mode of entry has more focuses on AMF and EMF symbioses; because much information has been explored about these mycorrhizal types, it is increasingly clear that other forms also involve the same fungal species as an associate of AMF and EMF symbioses. Studies on AMF have been revealed fascinating insights into the plasticity of plant cell development and of inter-organism communication, driven by the prospect for increased exploitation of AMF and further benefits for sustainable agriculture. In the matter of fact, the plant provides intracellular accommodation to the AMF via genetically defined signalling pathways which involve calcium spiking in the nucleus as second messenger. The calcium spiking is another molecular dialogue that directly initiates by chit oligosaccharides release by AMF which is supposed to be produced via receptor *LysM* domain receptor kinases. The fungal infection and calcium spiking are spatiotemporally coordinated, and only cells committed to accommodation undergo high-frequency spiking. Further, the

delivery of mineral nutrients by AMF occurs at arbuscules in the plant cortical cells. First, nutrients are consumed up by tree-shaped hyphal structures, the arbuscules, in plant cortical cells. Later by, nutrients are taken up at a plant-derived peri-arbuscular membrane which surrounds fungal hyphae and carries a specific transporter composition that is of direct importance for symbiotic efficiency.

11.3 Reproductive Structure of Mycorrhizal Fungi

Fungi reveal several different types of life cycles (asexual and sexual) but mycorrhizal fungi reproduce by asexual spores and its called as asexual life cycle (Fig. 11.1). The chlamydospores present in the rhizosphere region, influenced by the root exudates and germinate on the root surface. Flavonoids compounds exuded by the roots may strongly stimulate AM fungi (Gianinazzi-Pearson et al. 1989). They produce appressoria from which penetration pegs are produced. The hyphae establish longitudinally within the cortical region of root tissues and are mostly intercellular or rarely intracellular (Fig. 11.2). Mycorrhizal fungal species can produce asexual spores by mitosis in specialized spore-producing structures. This structure allows the organism to clone itself producing very large numbers of asexual spores. The hyphae of many species are haploid during the majority of their life cycles. Many fungi spend a good portion of their life in the asexual mode. The

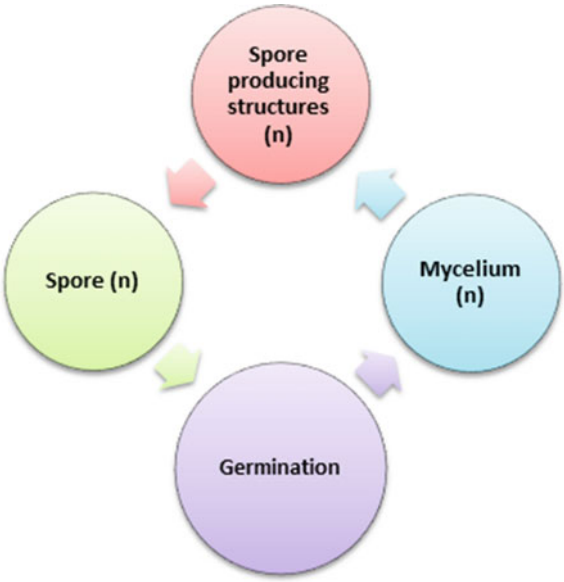

Fig. 11.1 Schematic diagram of a sexual life cycle of mycorrhizal fungi

transition to the sexual mode can be triggered by certain conditions (e.g. light, temperature, moisture, availability of a sexually compatible partner and limited nutrient availability).

The intracellular hyphae produce short branches that penetrate the cell wall (Fig. 11.3). When the short hyphae penetrate the cell, the host cell plasmalemma invaginates and extends in all endomycorrhizal associations; an interface is formed between the fungal wall and the newly formed plasmalemma. With the invaginated plasmalemma, the short hyphae branch dichotomously several times, leading to bunch of branches having the size of host cell mitochondria. This increases the exterior for incorporation of carbohydrate beginning with supply of water and minerals into the plant root. The physical change that takes place due to the entry of the endophytes into the host cell is the investigation of plasmalemma around it (compact mutualisation) and the deposition of an osmophilic fungal infection. This osmosphili fibrillar material deposition is continuous with the host primary wall having similar composition. The cell cytoplasm increases (in volume) with increase

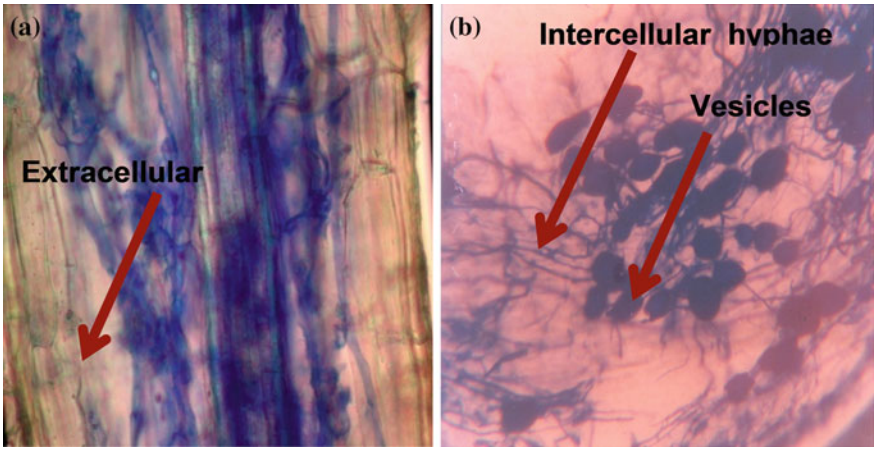

Fig. 11.2 (**a**) extracelluar hyphae (**b**) intercellular hyphae and vesicles

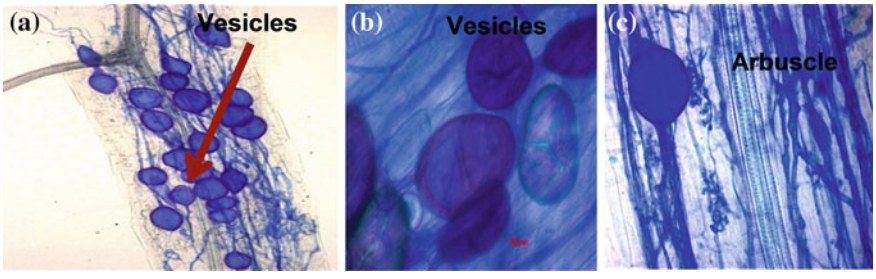

Fig. 11.3 (**c**) vesicle with intercellular hyphae; (**d**) mature bladder-like structurevesicles; (**e**) branched finger-like hyphae called arbuscules

in the size of nucleus. After active transportation, a reaction septum is formed. The cytoplasm of the branches of arbuscules is withdrawn. The AM fungi are formed by the symbiotic association between certain phycomycetous fungi and angiosperm roots. They are globular to elongate, swellings produced on the hyphae, mostly intracellular and are the storage organs of the fungus. When primary cortex sloughs off some of the soil, develops thick walls and functions as infective propagules aschlamydospores. The fungus colonizes the root cortex forming a mycelial network and characteristic bladder-like structure called vesicles, and branched finger-like hyphae called arbuscules. It starts from the fine branches off towards the trunk; the arbuscules collapse and the host cell returns to normal. The fungi colonize the root system of a host plant, providing increased water and nutrient absorption capabilities while the plant provides the fungus with carbohydrates synthesized during photosynthesis. One of the most important parts of AM fungus is the extrametrical mycelium. This extends beyond the zone of root and serves as absorbing structures of the fungus which conveys irrigate and raw materials from the dirt to the fix which is otherwise inaccessible to plant roots (Mosse 1978). Effectiveness of the fungus can be correlated with its ability to produce more extrametrical hyphae (Schellema et al.1985).

11.4 Taxonomy of AM Fungi

Hayman identified different genera based on colour and the size of the spore, wall layers and their structure, cytoplasmic inclusion, subtending hyphae, sporocarps and subsidiary spores and the method of germination. The presence or absence of subtending hyphae and their morphology are important features in distinguishing different genera. The genera *Glomus* and *Sclerocysti*s have simple hyphae, whereas the genera *Gigaspora* and *Scutellospor*a have hyphae with bulbous base. On the other hand, *Acaulospora* and *Entrophospora* do not have hyphal attachment. *Glomus* produces chlamydospores singly and wherever sporocarpus are produced the spores occur in loose clusters. The genus *Sclerocystis* produces porocarps having district peridium, and spores are wall-oriented around central plexus hyphae. The spores with hyphae having bulbous base and laterally placed hyphae are placed under the genus *Scutellospora* (Prasad and Rajak 1999). An investigation was carried out for twenty-two plant species, cultivated widely as vegetable crops in arid region of Rajasthan in India, belonged to eight different families to measure their affinity in harbouring symbiotic association with AMF and nutrient status in rhizospheric soil (Tables 11.1 and 11.2). Twenty out of twenty-two species were developed AMF colonization in their root tissues with a range of 16.33–91.33%. The mycorrhizal spore density in the soil was not found to have any effect on symbiotic colonization in root tissues of studied vegetable crop plants by mycorrhizae. The soil chemical analysis was also found to have no correlation with both infections of root tissues by AMF and spore densities in the soil. Plant species had a significant role in root tissue colonization by mycorrhizal fungi.

Table 11.1 Chemical analysis of rhizospheric soils (pH. EC and OC) of different species of vegetable species growing in the arid region of Rajasthan, India

Plant species/Family	**Soil pH**	**EC (dSm-1)**	**OC (%)**
Daucuscarota ssp. Sativa (Apiaceae)	6.50a ± 0.15	0.123a ± 0.001	1.62bcd ± 0.09
Allium cepa L. (Amaryllidaceae)	6.97ab ± 0.22	0.123a ± 0.001	1.41abc ± 0.10
Allium sativum L. (Amaryllidaceae)	6.97ab ± 0.22	0.150abcd ± 0.021	1.49abcd ± 0.04
Lablab purpureus (L.) Sweet (Fabaceae)	6.90ab ± 0.61	0.130ab ± 0.006	1.62bcd ± 0.09
Pisumsativum L. (Fabaceae)	6.77ab ± 0.38	0.131abc ± 0.003	1.47abcd ± 0.20
VignasinensisPrain (Fabaceae)	7.30ab ± 0.26	0.164ef ± 0.035	1.55abcd ± 0.16
Lycopersicumesculentum L. (Solanaceae)	7.37ab ± 0.30	0.185abcd ± 0.30	1.23a ± 0.12
Solanummelongena Linn. (Solanaceae)	7.6b ± 0.38	0.136f ± 0.38	1.33ab ± 0.06
Solanumtuberosum Linn. (Solanaceae)	7.23ab ± 0.22	0.198abcd ± 0.22	1.40abc ± 0.04
Pomoeabatatas (Solanaceae)	7.23ab ± 0.18	0.138def ± 0.18	1.41abc ± 0.03
Capsicum spp. (annuum) (Solanaceae)	7.23ab ± 0.18	0.180abcd ± 0.18	1.44abcd ± 0.06
Solanummelongina (Solanaceae)	7.07ab ± 0.17	0.138abcd ± 0.004	1.45abcd ± 0.01
Brassica oleracea L. var. botrytis (Brassicaceae)	7.00ab ± 0.21	0.134ab ± 0.006	1.77d ± 0.06
Brassica oleracea L. var. capitata (Brassicaceae)	7.00ab ± 0.15	0.130ab ± 0.004	1.66bcd ± 0.06
Raphanussativus L. (Brassicaceae)	7.17cde ± 0.28	0.129cdef ± 0.003	1.70 cd ± 0.14
Abelmoschusesculentus (Linn) Moench (Malvaceae)	6.83abcd ± 0.38	0.176abcd ± 0.020	1.33ab ± 0.07
Basella alba (Basellaceae)	7.43abcd ± 0.33	0.156abcd ± 0.021	1.54abcd ± 0.17
Cucurbita maxima (Cucurbitaceae)	7.33abcd ± 0.29	0.134abcd ± 0.001	1.73 cd ± 0.13
Cucumissativus (Cucurbitaceae)	7.40abcd ± 0.25	0.153bcde ± 0.021	1.63bcd ± 0.07
Momordicacochinchinensis (Cucurbitaceae)	7.40bcde ± 0.25	0.171bcde ± 0.024	1.66bcd ± 0.06
Momordicacharantia L. (Cucurbitaceae)	7.53abcd ± 0.09	0.138abcd ± 0.003	1.73 cd ± 0.13
Luffaacutangula L. (Cucurbitaceae)	7.00ab ± 0.30	0.131abc ± 0.004	1.73 cd ± 0.13

Values are mean of four replicates. ± *SE* Std error; Values in a column followed by the same letter are not significantly different at $P < 0.05$ according to DMRT

Table 11.2 Chemical analysis of rhizospheric soils (available NPK) of different species of vegetables species growing in the arid region of Rajasthan, India

Plant species/Family	Available N (%)	Available P (ppm)	Available K (ppm)
Daucuscarota ssp. Sativa (Apiaceae)	0.0072a ± 0.00034	8.95a ± 1.32	212.33a ± 21.67
*Allium cepa L. (*Amaryllidaceae)	0.0067a ± 0.0001	8.18a ± 2.17	163.33a ± 9.39
Allium sativum L. (Amaryllidaceae)	0.0074a ± 0.00037	11.07a ± 0.91	185.00a ± 26.08
Lablab purpureus (L.) Sweet (Fabaceae)	0.0071a ± 0.00037	11.07a ± 0.91	188.00a ± 23.86
Pisumsativum L. (Fabaceae)	0.0071a ± 0.00037	9.76a ± 1.70	186.33a ± 24.13
VignasinensisPrain (Fabaceae)	0.0071a ± 0.00037	10.02a ± 0.16	185.00a ± 22.81
Lycopersicumesculentum L. (Solanaceae)	0.0071a ± 0.00037	10.62a ± 1.62	206.00a ± 26.03
Solanummelongena Linn. (Solanaceae)	0.0067a ± 0.00031	9.85a ± 0.29	180.00a ± 25.01
Solanumtuberosum Linn. (Solanaceae)	0.0074a ± 0.00037	10.95a ± 0.86	186.33a ± 24.13
Pomoeabatatas (Solanaceae)	0.0076a ± 0.00039	10.62a ± 1.92	185.67a ± 24.55
Capsicum spp. (annuum) (Solanaceae)	0.0075a ± 0.00036	11.88a ± 0.77	212.33a ± 21.67
Solanummelongina (Solanaceae)	0.0071a ± 0.00037	10.62a ± 1.92	185.66a ± 24.55
Brassica oleracea L. var. botrytis (Brassicaceae)	0.0073a ± 0.00036	10.79a ± 0.93	186.33a ± 24.13
Brassica oleracea L. var. capitat a (Brassicaceae)	0.0067a ± 0.00037	9.51a ± 1.63	182.00a ± 25.01
Raphanussativus L. (Brassicaceae)	0.0078a ± 0.00039	9.85a ± 1.65	179.34a ± 25.33
Abelmoschusesculentus (Linn) Moench (Malvaceae)	0.0071a ± 0.00037	9.85a ± 0.29	186.33a ± 24.13
Basella alba (Basellaceae)	0.0074a ± 0.00033	10.95a ± 0.86	186.33a ± 24.13
Cucurbita maxim a (Cucurbitaceae)	0.0072a ± 0.00035	10.70a ± 0.91	180.00a ± 25.01
Cucumissativus (Cucurbitaceae)	0.0067a ± 0.00037	9.76a ± 1.70	180.00a ± 25.01
Momordicacochinchinensis (Cucurbitaceae)	0.0074a ± 0.00036	10.95a ± 0.86	186.33a ± 24.13
Momordicacharantia L. (Cucurbitaceae)	0.0071a ± 0.00037	8.82a ± 1.86	164.67a ± 4.33
Luffaacutangula L (Cucurbitaceae)	0.0072a ± 0.00039	9.76a ± 1.70	186.33a ± 24.13

Values are mean of four replicates. ± *SE* Std error; Values in a column followed by the same letter are not significantly different at $P < 0.05$ according to DMRT

11.5 Physiology of Mycorrhizal Fungi

The development of AMF prior to root colonization, known as presymbiosis, consists of three stages: spore germination, hyphal growth, host recognition and appressorium formation (Prasad 1993; Prasad 1995; Douds and Nagahashi 2000; Prasad and Kaushik 2004; Zubek et al. 2016). Spores of the AM fungi are thick-walled multi-nucleate resting structures (Wright 2005). The germination of the spores does not depend on the plant as spores have been germinated under experimental conditions in the absence of plants both in vitro and in situ. However, the rate of germination can be increased by host root exudates (Douds and Nagahashi 2000; Prasad and Rajak 2000; Prasad et al. 2005a; Prasad and Pandey 2012; Prasad 2015; Rafiq et al. 2016). AM fungal spores germinate at given suitable conditions of the soil matrix, temperature, carbon dioxide concentration, pH and phosphorus concentration (Prasad 1993; Prasad and Rajak 2001; Wright 2005; Prasad et al. 2005b). Low phosphorus concentrations in the soil increase hyphal growth and branching as well as induce plant exudation of compounds which control hyphal branching intensity (Prasad 1993; Nagahashi et al. 1996; Douds and Nagahashi 2000; Prasad 2006a, b). Excess phosphorus concentration occurs in natural soil conditions and could thus contribute to reduced mycorrhiza colonization (Nagahashi et al. 1996).

Root exudates from AM fungal host plants grown in a liquid medium with and without phosphorus have been shown to influence hyphal growth (Diop et al. 1990; Nagahashi et al. 1996). Pre-germinated surface-sterilized spores of *Gigaspora magarita* which were grown in host plant exudates from roots starved of phosphorus had increased hyphal growth and produced tertiary branches compared to those grown in exudates from plants given adequate phosphorus (Nagahashiet al. 1996). When the growth-promoting root exudates were added in low concentration, the AMF produced scattered long branches. As the concentration of exudates was increased, the fungi produced more tightly clustered branches. At the highest concentration, the AMF structures of phosphorus exchange were formed arbuscules. This chemotaxic fungal response to the host plants exudates is thought to increase the efficacy of host root colonization in low phosphorus soils (Douds and Nagahashi 2000). It is an adaptation for fungi to efficiently explore the soil in search of a suitable plant host (Nagahashiet al. 1996). More evidence that AM fungi exhibit host-specific chemotaxis spores of *Glomus mosseae* where separated from the roots of a host plant, non-host plants and dead host plant by a membrane only permeable to hyphae. Douds et al. (2002) observed spore population of *Glomus intraradices* in split-plate monoxenic culture system by repeated harvest, gel replacement and resupply of glucose to the mycorrhiza. In the treatment with the host plant, the fungi crossed the membrane and always emerged within 800 µm of the root, whereas in the treatments with non-host plants and dead plants, the hyphae did not cross the membrane to reach the roots (Prasad 1993; Sbrana and Giovannetti 2005). This demonstrates that AMF have chemotaxis abilities which enable hyphal growth towards the roots of a potential host plant.

11.6 Mycorrhizal Fungi in Ecosystems

The ecology of mycorrhizal fungi is yet to be well-documented (Abbott and Gazey 1994; Francis and Read 1995, Prasad 2000c; Prasad et al. 2006a, b; Prasad et al. 2011). In nature, the situation is far more complex as a single tree may have fungal partners which can vary in time and space. The fungal/plant interface provides a conduit for the movement of carbon from the plant to the fungus and for movement between plants linked by mycelia (Simard et al. 1997; Van der Heijder 1998a; Wu et al. 2001; Prasad et al. 2005b). The nature of the interface and its mode of regulation are still being elucidated (Hall and Williams 2000). It is generally believed that mycorrhizal plants direct more of their photosynthates into the soil than non-mycorrhizal plants. This extra carbon accumulates in patches and at the edge of hyphal mats (Finlay and Read 1984), and boosts the energy supply to the detrital food web, benefiting saprophytic microbes and other soil organisms (Barea 2000). Because the chemical (Dieffenbach and Matzner 2000) and physical environment around mycorrhizas differ from non-mycorrhizas, presumably it provides microhabitats for soil biota that are not present in the rhizosphere of non-mycorrhizal roots. Mycorrhizal fungi are estimated to consume from 15 to 50% of net primary production (Vogt et al. 1982; Baltruschat and Dehne 1988). Mycorrhizal fungi act as decomposer by producing several classes of enzymes and converted polymeric constituents into soluble forms suitable for absorptions and utilization as sources of carbon and energy in plants. These fungi exist in organic contents with the roots provide a direct pathway of channelling nutrients into plants for benefitting their growth. The concept that mycorrhizal fungi are involved in nutrient cycling came following the finding that the fungi forming Ectomycorrhiza with the plant family Ericaceae can be cultured. These meant that mycorrhizal mycelium could be obtained for protein extraction and assay of enzyme activities. Finley and David (1986) inferred that mycorrhizas have scavenging nutrients from litter and translocation of N and P from the plants via the roots. Role of mycorrhiza in determination of species composition and plant communities was studied via determining associations among plants in the field experiments. Mycorrhizal hyphae connect plants roots of the same or different species and serve as conduits for distribution of photosynthetically made carbon compound between plants. The modifying influence indicates that the fungus is able to down-regulate (silence) the gene encoding P transporter in the plant. In the other word, new capabilities are achieved through a molecular cross-talk between the mycorrhizal partners.

11.7 Plant Host Physiology

The physiology of mycorrhizal associations has been well discussed by Hayman (1983), Harley and Smith (1983), Smith and Gianinazzi-Pearson (1988). Mycorrhizal associations are generally considered to benefit host plants by

enhancing mineral nutrient acquisition, especially with regards to phosphorus. Nitrogen supply by EMF and ericoid associations is also considered to improve nitrogen uptake (Barea et al. 2002a, b; Prasad 2015; Arul and Nelson 2016). Increase in the absorption of minor nutrients such as Mg, Cu and Zn has also been observed, effect (Killham 1985; Pacovsky 1986). Other less specific change to host physiology includes alterations in nutrient requirements. Membrane composition and metabolite levels occur even when nutrient input is negligible (Dehne 1986; Pacovsky 1986). Mycorrhizal fungi (ECM and ericoid) apparently influence host morphology and physiology by producing plant hormones such as ethylene and auxins, which are responsible for the reduced apical growth of mycorrhizal short roots (Gay and Debaud 1987; Berta et al. 1988; Rupp et al. 1989). Root growth is usually only slightly affected by AMF but in some detrimental reduction root elongation occurs (Jones and Hendrix 1987). Mycorrhizal associations have been implicated in increased host resistance to disease and other stresses condition (Prasad 1993)

11.8 Building Bridges Due to Mycorrhiza for Green Technology

A plant feeds through the outer surface of its roots. The effect of the mycorrhizae around the root's surface serves to expand this surface area in many directions while permitting more nutrients to be absorbed and contained within the rhizosphere. In the case of phosphorus, which is difficult for a plant to absorb due to its immobility, it forms a bridge that directly seeks out phosphoric sources, sometimes at great distances. In turn, the fungi are able to transform it in a way that is mobile and in an accessible and digestible form for the plant. Alongside, these mycorrhizae enable the mineralization of nitrogen and carbon by naturally composting decaying plant matter in the soil and re-delivering it to the plant and surrounding soil as available and useable food sources. Mycorrhiza is a remarkable, natural phenomenon that connects all growth and life, providing for itself and its environment and sustaining and regenerating itself through its myriad connections. Serious research on the workings of mycorrhizae only commenced since 40 years. Its many benefits are now known; particular strains are grown and colonies applied in situations of low microbial activity such as barren landscapes with no nutritional content or overworked farmland. This has far-reaching possibilities in the area of agriculture and particularly food production where the introduction of beneficial mycorrhizae could assist in sustaining third world countries and feeding their people. For the hobby grower, strains of mycorrhizae can be purchased and added to garden beds and potting mixes to colonize and assist in plant development.

11.9 Mycorrhiza Compatibility and Specificity for Green Technology

Initially, mycorrhiza infection process, root and mycorrhizal activity are independently initiated and regulated (both partners may be responding to the same soil or environmental conditions), but there is strong evidence of genetic interactions between the mutualistic partners in the later stages of this process. Evidence of genome expression changes in the fungal partners is provided by hyphal structure and behaviour at the root surface, but the response by roots apparently is largely restricted to individual cells forming exchange sites (Gianinazzi-Pearson 1984). The widespread susceptibility of plant roots to colonization by mycorrhizal fungi explained by specific comparability systems or because of mycorrhizal fungi somehow avoids or fails to elicit host defence mechanisms (Gianinazzi-Pearson and Gianinazzi 1986). There is little evidence of host–fungus specificity in most type's mycorrhizal associations (Harley and Smith 1983; Gianinazzi-Pearson 1984; Duddridge 1987). Ineffective AMF associations have been discovered in only a few of the many host plant and mycorrhizal fungus combinations tried in synthesis experiments (Johnson 1977; Giovannetti and Hepper 1985). Thus, relatively few endophytes (± 150 members of the Glomales) can form associations with majority of members of the plant kingdom (Morton 1990). Genotypic variations within a host species can influence the degree of AMF formation (Azcon and campo 1981; Krishna et al. 1985; Thomas and Ghai 1987; Sieverding and Galvez 1988). Some hosts provide more benefit to AMF than others, as is suggested by differences in the magnitude of spore production. But in most cases, spore formation is loosely related to the length of mycorrhizal roots produced by a given host (Pellet and Sieverding 1986; Howeler et al. 1987; Giovannetti et al. 1988; Struble and Skipper 1988; Simpson and Daft 1990a). The adaptation of mycorrhizal fungi to particular soil conditions apparently is more common than specific interactions with host plants. Thus, in experimental systems incompatible host–fungus combinations are rare, but in ecosystems, many of these combinations may be less successful because the fungi are poorly adapted to the normal habitat of plants. However, even if environmental and soil conditions could somehow be excluded from consideration, particular entophytes are also likely to exhibit differences in metabolic competence (Smith and Gianinazzi-Pearson 1988). McGonigle and Fitter (1990) observed the preferential association between a AM fungus and a grass species, but there have been few other attempts to identify the AM fungus associates of plants in natural ecosystems. The assertion that AMF associations lack host–fungus specificity may well be a reflection of how little we known about these fungi. Observations of the occurrence of above-ground fructifications of ECM fungi have provided much information about associated host plants and the geographic ranges (Mason et al. 1987; Prasad 2010a; Prasad 2013). There is usually a high correlation between the occurrence of fruiting structures and mycorrhizal formation by ECM fungi (Trappe 1987; Gardner and Malajczuk 1988), On the other hand, sometimes erroneous reports involved ash trees *(Fraxinus* sp.), known to have AMF associations, and the

fungus *Boletinellus merulioides*, but now known to associate with root-feeding aphids (Brundrett and Kendrick 1987). Most ECM fungi associate with a broad range of host plants, but incompatible host–fungus combinations have been found (Duddridge 1987). Clonal variations within *Sitka spruce* influence populations of ECM fungi associated with their roots (Walker et al. 1986). The compatibility of host plant–ECM fungus combinations has been tested using artificial conditions (host seedlings grown in aseptic media), and fungi that colonize roots best under these conditions are often those that form sporocarps in close association with the same host in the field (Molina and Trappe 1982b). There is strong evidence of cellular and genetic interactions between host plants and mycorrhizal fungi (Gianinazzi-Pearson and Gianinazzi 1989), but these relatively delicate interactions may be different to different from environmental/edaphic on the occurrence of mycorrhizal fungi in natural ecosystems.

11.10 The Activity of Mycorrhizal Fungus Hyphae in Soils

Mycorrhizal fungi form a hyphal network in soil which can obtain and transport nutrients, propagate the association and interconnect plants (Newman 1988; Read et al. 1989; Prasad and Meghavansi 2005; Prasad and Bilgrami 2006; Prasad 2007; Prasad 2015). Production of external hyphae varies between species, and isolates of AM fungi are influenced by soil properties is important determinant of mutualistic effectiveness (Graham et al. 1982b; Abbot and Robson 1985; Gueye et al. 1987). Mycorrhizal fungus hyphae are normally thought to obtain poorly uptake of nutrients from beyond the zone of nutrient depletion surrounding roots in soils but may also respond to soil heterogeneity. Harvey et al. (1976) observed that most of the ECM roots in a forest soil occurred within organic soil fractions, due litter, woody debris and charcoal decomposition. Hyphae of these fungi may exploit soil heterogeneity by occupying substrates with lower carbon/nutrient ratios (Coleman et al. 1983) and also preferentially occupy soil organic material (Mosse 1959; StJohnet al.1983; Warner 1984), where they produce fine, highly branched, septate hyphae that may have an absorptive function (Mosse 1959; Nicolson 1959). Roots also respond to spatial and temporal variations in soil nutrient supply, but less efficient than mycorrhizal hyphae. Mycorrhizal associations provide the greatest benefit when plants are supplied with forms of phosphorus that dissolve very slowly (Bolan et al. 1987; Harwani et al. 2009; Prasad and Pandey 2012). Some mycorrhizal fungi apparently utilize organic or insoluble nutrient sources that are normally thought to be unavailable to plants. Absorption of inorganic nutrients by mycorrhizal hyphae and their transport through soil to roots over distances measured in centimetres' have been demonstrated by tracers such as ^{32}P (Harley and Smith 1983; Hayman 1983). The quick transport of carbon, nitrogen, phosphorus and water by hyphal networks of AM and ECM fungi has also been (Finlay and Read 1986; Francis et al. 1986; Ritz and Newman 1986; Finlay 1988; Haystead et al. 1988; Newman, 1988). Francis et al. (1986) reported that mycorrhizal-mediated

inter-plant nutrient transfer significantly enhanced the growth of recipient plants. Ritz and Newman (1986) considered the P-transfer rates measured to be substantially less than uptake rates in the field. The hyphal networks may facilitate the absorption and transport of nutrients in soil, since their disruption can reduce the efficacy of mycorrhizal associations in a way that is independent of colonization levels (Evans and Miller 1990). Mycorrhizal fungus hyphae can influence soil structure by helping to produce humic acids, weathering soil minerals and stabilizing large soil aggregates (Oades 1984; Perry et al. 1987), but organic acids and polysaccharides of the soil animal activity are also important components of soil structural stability (Lynch and Bragg 1985a, b; Perry et al. 1987). Major structural contributions to soils by hyphae of AM or ECM fungi have been observed in arctic communities (Miller 1982b), sand dunes (Rose 1988), deserts (Went and Stark 1968), revegetating mine sites (Rothwell 1984) and agricultural fields (Tisdall and Oades 1979). The abundance of mycorrhizal fungus hyphae in many soils suggests that they may be important as source of humic acids as well as influencing soil structural properties.

11.11 Mycorrhizal Fungi in Soil Improvement

Restoration of native AM fungi increases the success of ecological restoration project and the rapidity of soil recovery (Jeffries et al. 2003). There is evidence about enhancement of soil aggregate stability due to the production of a soil protein known as glomalin. Glomalin-related soil proteins (GRSP) have been identified using a monoclonal antibody (Mab32B11) raised against crushed AMF spores. There is other circumstantial evidence that glomalin is of AM fungal origin. When AM fungi are eliminated from soil through incubation of soil without host plants the concentration of GRSP declines. Similar declines in GRSP have been observed in incubated soils from forested, afforested and agricultural land (Rilliget al. 2003) and grassland streaked with fungicide, etc. (Rillig 2004). Glomalin is hypothesized to improve soil, aggregate water stability and decrease soil erosion. A strong correlation has been found between GRSP and soil aggregate water stability in a wide variety of soils where organic material is the main binding agent, although the mechanism is yet to come out. The protein Glomalin has not yet been isolated and described and, the link between Glomalin, GRSP and arbuscular mycorrhizal fungi is not yet clear.

11.12 Nutrient Transfer Through AM Fungi

AM fungi are well known as mediators of nutrient transfer from soil to plant. The majority of vascular plants (80–90%) are able to form associations with AM fungi, for nutrient exchange. Initial studies on nutrient transfer focused on their ability to

provide roots with phosphate, and it was found that the arbuscules of these fungi, specifically the peri-arbuscular membrane, contained phosphate transporters (Gaudeet al. 2012; Prasad 2015). Providing the fungus with increased levels of plant-derived carbon resulted in increased transference of phosphate (Bucking and Shachar-Hill 2005). *Glomus* species have the ability to transfer nutrients, including nitrogen to the roots of leguminous plants (Gaude et al. 2012). Some fungal endophytes affect plant growth and responses to pathogens, herbivores and environmental change; others produce useful or novel secondary metabolites. Root endophyte colonizes healthy plant roots. An increase in nutrient content and growth was observed for *Carex* sp., *Pinuscontorta* and *Vulpiaciliata* when inoculated with dark septate endophytic fungi (Haselwandter and Read 1982). The dark septate endophytic fungus *Heteroconium chaetospira* forms a functional symbiosis with *Brassica campestris* where the fungus transfers nitrogen to, and receives carbon from, the plant (Usuki and Narisawa 2007). The Brassicaceae do not usually form mycorrhizal associations so this association with *H. chaetospira,* as well as others (Behie et al. 2012), suggests that endophyte can also transfer nutrients to plants. Endophytic associations can also result in more efficient nutrient acquisition since root associated fungal hyphae are able to obtain soil nutrients from areas too small for plant roots to penetrate (Majdiet al. 2001).

Recently, some insect pathogenic fungi have shown to form endophytic associations with plant roots (Akello et al. 2007; Sasan and Bidochka 2012). In particular, one EIPF, *Metarhizium robertsii*, can transfer insect-derived nitrogen to plants (Behie et al. 2012). *M. Robertsii* infected a soil insect after which the fungal mycelia colonized the host (switch grass and haricot bean), where nitrogen transfers were detected (Behie et al. 2012). Three ectomycorrhizal fungi were including was also able to transfer insect-derived nitrogen from springtails to the roots of white pine trees (Klironomosand Hart 2001). The ability of EIPF to transfer insect-derived nitrogen to plant roots indicates a fundamental shift in the way these fungi are viewed within the ecosystem as they represent the ability of the plant to regain nitrogen previously lost by insect herbivory.

11.13 Mycorrhizal Function for Green Technology

AM fungi absorb N, P, K, Ca, S, Cu and Zn from the soil and translocate them to the plant (Tinker and Gildon 1983; Prasad and Deploey 1999; Gautam and Prasad 2001; Prasad 2002; Prasad and Kaushik 2004; Prasad and Gautam 2005; Prasad and Pandey 2012; Prasad 2015). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of insoluble soil immobile nutrients, particularly P, Cu and Zn (Pacovsky 1986; Manjunath and Habte 1988a, b; Prasad 2013; Prasad 2015). The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root. The supply of immobile nutrients to roots is largely determined by the rate of diffusion. In soils not adequately supplied with nutrients, uptake of nutrients by plants far exceeds the rate at which the nutrients diffuse into

the root zone, resulting in a zone around the roots depleted of the nutrients. Mycorrhizal fungi help overcome this problem by extending their external hyphae to areas of soil beyond the depletion zone, thereby exploring a greater volume of the soil than is accessible to the unaided root. Enhanced nutrient uptake is often associated with dramatic increase in dry matter yield, typically amounting to several-fold increases for plant species having high dependency on mycorrhizae. AM fungi capabilities may involve increases in root phosphatase activity, excretion of chelating agents and rhizosphere acidification. However, these mechanisms do not appear to explain the very pronounced effect the fungi have on plant growth (Perez-Moreno and Read 2000). They have been associated with enhanced chlorophyll levels in leaves and improved plant tolerance to diseases, parasites, water stress, salinity and heavy metal toxicity (Bethlenfalvay 1992, Prasad 2013, Prasad 2015). Moreover, there is increasing evidence that hyphal networks of AM fungi contribute significantly to the development of soil aggregates, and hence to soil conservation (Miller and Jastrow 1992; Bethlenfalvay et al. 1999).

11.14 Ecological and Physiological Aspects of Mycorrhizal Fungal Symbiosis

In the ecosystem concept, agro ecosystems are characterized by major dependence on and influenced by factor external to the system such as energy and agricultural chemicals and their residues (Odum 1984). The challenge confronting the agricultural community is to reduce the input and output costs to the agricultural system so that costs are integrated compatibility at the farming scale (Wright and Millner 1994). Mutualistic AM fungi have been studied extensively at a global scale not only on account of their ability to help plant withstand various kinds of abiotic and biotic stresses but also with their new found role in evolution, ecosystem dynamics and plant community establishment (Prasad and Deploey 1999; Manoharachary et al. 2005; Prasad 2005, 2015). Van der Heijden (1998b) proved the singular relationship among mycorrhizal fungal diversity, ecosystem variability and productivity studies of the central European agro ecosystem that has led to descriptions of not only new species of *Glomus* (Oehl et al. 2002, 2003) but also establishment of a new genus *Pacispora*. In view of established significance in plant productivity and stress management, AM fungal diversity has been studied extensively in various natural and man-made ecosystems and some new forms discovered. Within the agro ecosystem, in general terms, productivity can be assessed as primary plant productivity with inputs delivered in such a manner so as to uncouple dependence of productivity which encompasses the concept of maintaining soil quality in microbial interactions to sustain production. AM fungi and AM fungal biomass are integral parts of soil-plant productivity because of their roles in (i) amelioration of environmentally induced plant stress, (ii) soil structure development and

(iii) carbon, nitrogen and phosphorus cycling. At the present stage, investigators must assess the impact of particular disturbances, for example, tillage, on specific plant fungus soil combination. Effort is needed to acquire information on system-level impacts of disturbances, with consideration given to variably in AM fungal isolate effectiveness. Also, future research needs to address the overall contribution of AM fungi to the rates, amount and forms of carbon, nitrogen and phosphorus cycling through the rhizosphere with particular attention on soil productivity along with plant productivity in the long-term perspectives. In this context, greater interactions with molecular biologists and their powerful techniques are critical for further our understanding and application of mycorrhizal technology. It has been established that AM fungi help plants in nutrient uptake resulting in improved growth and yield. Recent researches, however, focus seed attention on observations pertaining to absorption efficiency, partitioning and the biochemical fate of these nutrients in the AM fungal systems. There are reports which suggest an efficient role of AM fungi in improving rates of photosynthesis under both, natural- and water-stressed conditions (Sharma et al. 1990; Potty and Indira 1990). Similarly, movement of water from soil to roots through the AM hyphal pathway has also been the subject of considerable experimentation. Some other aspects such as the quantitative and qualitative influence of root exudates as measured by total sugar or total sugar and amino acids, are also very quite significant contributing to rhizosphere dynamics. An interaction between root exudates and elevated CO_2 level for growth of hyphae has been demonstrated. Similarly, flavonoid involved in the chemical dialogue stimulate AM fungi hyphal growth and root colonization in vitro studies has also been shown by Nair et al. (1991). Compounds may be, however, significant to have better understanding of molecular signalling between the host and fungus, employing advanced molecular biological techniques.

11.15 Mycorrhizal Fungi for Plant's Benefits

AM fungi colonizing internal tissues of root and develop thread-like structures called "hyphae" that extend into the soil. These filamentous structures explore a far greater volume of soil than root hairs can, coming into contact with nutrients such as phosphorus, copper, and zinc that do not move easily through the soil solution. AM fungal hyphae transport the nutrients back to the released into the root cells. This increase in nutrition contributes to the plant's ability to resist disease and avoid water stress. As AM fungi grow through the soil, they also modify the balance of micro biome in the soil. AM fungi appear to selectively enhance populations of soil bacteria that inhibit the growth of plant pathogens and reduce disease pressure (Duchesne et al. 1989; Farguhar and Peterson 1991; Prasad 1993; Prasad 2000a, b, 2006b). AM fungal hyphae also stabilize soil particles by physically "wrapping" the particles into small clusters or clumps (aggregates) and release a glue-like substance called glomalin that binds the soil particles together. Soil aggregates increase the number of empty spaces (pores) in

the soil's structure. These pores, in turn, allow the soil to hold more air (needed for root and microbial activity), and improve the soil's ability to absorb and retain water during periods of heavy rain or snow melt. In these ways, soil aggregates promote better plant growth and reduce soil erosion. As part of their symbiosis with plants, AM fungi depend on plant roots to supply the sugars the fungi need to grow and reproduce. Clearly, plants and fungi benefit from their symbiotic relationship, by supporting AM fungus populations in soil with fungus-friendly farm management practices. Ectomycorrhizal fungi exhibit adaptive tolerance to toxic metal (Hartley et al. 1997; Prasad et al. 2005c).

11.16 Mycorrhizal Fungi and Plant Diversity

Van der Heijden et al. (1998b) have provided evidence that the community of AM fungi determines plant community structure by the response of individual plant species to colonization by single or multiple species of AM fungi. This is certainly a point which also needs attention in agrosystems via better screening of plants and AM fungi for functional compatibility. In further work, Van der Heijden et al. (1998b) showed that belowground diversity of AM fungi is a major factor in the maintenance of plant biodiversity and to ecosystem stability and function. AM fungi enter the roots of many plant species in the same community resulting in simultaneous colonization by several species of AM fungi. This results in interconnections of plants via the ERM of each. The conclusion being that increasing the species richness of AM fungi in grasslands leads to the increased spread of highly responsive herb species at the expense of relatively unresponsive grasses. Surveys of the architectures of the ERM produced by species of AM fungi from different genera provide indications that each can exploit soil resources in different ways.

11.17 Mycorrhizal Fungi and Soil Aggregation

Miller and Jastrow (1992) investigated a prairie ecosystem and showed that root and extra radical mycelium lengths were correlated with increased water stable macro aggregates and their geometric diameters. The major component causing this was, in fact, the ERM of AM fungi. A switch in dominance from *Glomus* spp. to *Gigaspora* spp. was also positively correlated with increased length of the ERM and macro aggregation. Mycorrhiza formation in soils results in an increased movement of C into roots and rhizosphere via better root growth and respiration provides a physical structure which can entangle soil particles and lead to micro then macro aggregate production. The recent finding that a glycoprotein called "Glomalin" is produced by AM fungi soil-based mycelium. It is a major binding agent in soils and adds further weight to the importance of AM fungi in stabilizing soil ecosystems. Soil tillage in agricultural production may reduce the subsequent

rate of colonization of plants by AM fungi by breaking up the living ERM in the soil. The result of this disturbance will be a reduction in propagule of "susceptible" AM fungi (Acaulosporaceae and Gigasporaceae) but may increase those of more resistant species of *Glomus*. This reduction in diversity is supported PCR-based techniques to detect rDNA sequences in roots (McGonigle 1998). The over winter survival of the extra radical mycelium and its non-disturbance seem to be a vital agronomic practice for the subsequent colonization of spring crops and optimum functioning of the mycorrhizas (Leyvalet al. 1997). It appears that the survival of the extra radical mycelium, intact, allows plants to be incorporated into functional mycorrhizal associations early in spring.

11.18 Green Technology for Sustainable Production Using AM Fungi

In natural ecosystems or low-tillage agriculture, young seedlings can germinate and effectively "plug" into an already established "motorway" of hyphae of AM fungi which permeate the soil and link different plant species. The lack of host specificity is the secret to the success of AM fungi in mixed plant communities (Prasad et al. 2011). The benefit to plants in natural plant communities is because less carbon from the plant photosynthates is required by AM fungi colonization, since it is plugged into a pre-established mycelium. In contrast, agricultural crops are frequently sown into tilled soil where the mycelium got completely disrupted. Agriculture would, therefore, allow AM fungi having aggressive colonization strategies to produce a new ERM (Gray and Read 1995). There is conflict of interest in the idea of maximizing plant production against an aim of maintaining a high biodiversity of AM fungi in soils. The latter maybe a necessity in natural ecosystems or restoration of degraded natural habitats but selection for efficient populations of AM fungi compatible with the aim of maximizing yields of certain crops may require a different management approach in agrosystems. Modern agricultural practices, such as high levels of fertilizer and pesticide inputs and long-term monocultures, have proven adverse effects on the diversity of soil micro biota. It is becoming clear that sustainable production practices, e.g. crop rotations with legumes, would benefit the survival of inoculum of AM fungi from season to season and hence import their effect on subsequently. One potential weakness is that both systems are using varieties (genotypes) of crops bred for high inputs. This is a selection process driven by conventional plant production, and the varieties may not be suitable for optimal production under organic or other sustainable systems. There are commercial productions of plants which appear to be less susceptible to colonization by AM fungi as a result of breeding programmes. The inbred lines of *Zea mays* L. with resistance to fungal pathogens were less able to form mycorrhizas compared with disease susceptible lines (Toth et al. 1990; Prasad 1998; Prasad and Bilgrami 2005; Prasad 2011b). The relationship, however, between reduced colonization and nutrient uptake ability of AM fungi is uncertain

and maybe uncoupled genetically. However, there is evidence for increased root fibrosity to compensate for the reduced role of AM fungi. These traits will operate fine under high-input agricultural production but same varieties produce high yields under reduced input systems.

11.19 Mycorrhizal Fungi and Soil Fertility

A key factor which affects the potential for mycorrhizas to benefit plants in particular sites is the supply of phosphate and nitrogen in soil (George et al. 1994; George et al. 1996; Prasad 2002; Meghavansi et al. 2010; Prasad 2010b; 2011a). Phosphorus is generally considered to be the most important plant growth limiting factor which can be supplied by mycorrhizal associations, because of the many a biotic and biotic factors which can restrict its mobility in soils (Harley and Smith 1983; Bolan 1991; Marschner and Dell 1994; Marschner 1995). Reductions in the benefit provided by mycorrhizal associations to plants are caused by increasing soil phosphorus levels (Birch 1988; Jones et al. 1990; Prasad and Bilgrami 2004; 2007). High rates of P and N fertilizers suppress endo/ectomycorrhizal development in the field (Menge et al. 1977; Newton and Pigott 1991) as well as influence the relative abundance of different ECM types (Bowen 1973; Bougher 1995; Brazantiet al. 1999).

11.19.1 Mycorrhizal Fungi and Adverse Soil Conditions

Land degradation due to soil salinity, water logging, erosion, etc. is serious and growing problems in all over the world. Excessive NaCl levels in soil inhibit mycorrhizal formation and restrict the activity of most mycorrhizal fungi (Read and Boyd 1986; Malajczuk et al. 1987; Juniper and Abbott 1993). Observations in natural ecosystems have shown that plants with mycorrhizal associations are often less common than non-mycorrhizal species in soils which are waterlogged or saline (Brundrett 1991). ECM fungi can be highly sensitive to water logging of soils, while AM fungi comparatively less sensitive to abiotic stresses (Bowen 1973; Prasad et al. 2005a; Prasad 2010a).

11.20 Phosphorous on Phosphatase Production by AM Fungi

Rubio et al. (1990) described induced and constitutive effect of phosphorus in seedlings of wheat cultivars viz. Dalcahue, Malihue, Carahue and Naofen underlow phosphorus volcanic soil in greenhouse condition. AM fungi root colonization was

increased up to 63 days later steadily decrease up to 84 days. Root surface acid phosphatase activity of root surface did not exhibit variable with the variations and not influenced by supplemented phosphorus. When plants were grown without phosphorus fertilizer, maximum enzyme activity was reached at 63 days for Carahue and Dakahue and 43 days for Naofen and Malihue. Phosphatase activity (micro P-nitro phenol released per gram of dry root) was highest at 21 days but quickly declined in the later samplings. When phosphorus was applied, total infected root length increased up to 63 days. Ezawa and Yoshida (1994) noticed that the phosphatase specific to infection of marigold (*Tagetespatula* cv. Bonanza) roots by the AM fungus, *Glomus etunicatum*, the infection-specific phosphatase (ISPase) was detected in the mycorrhizal root extract of 2–10 week old plants from the beginning of the infection by an electrophoretical technique. Studies on the effect of phosphorus fertilization on ISPase activity and mycorrhizal growth promotion in 4–8 week old plants showed that mycorrhizal plants without phosphorus fertilization showed a greater increase in shoot fresh weight and higher ISPase activity than the plants with phosphorus fertilizer. The optimum pH was 7.5, 1 M phosphate ion inhibited half of the activity. Phosphatase activity was studied in three acid steam-sterilized soils, in which mycorrhizal and non-mycorrhizal red clover plants had been grown for 5.5 months at different Ca $(H_2PO_4)2$ H_2O (calcium dihydrogen phosphate) doses (0, 25, 50, 100, and 200 ppm). At low phosphorus doses (0–50 ppm) in the sterilized soil, AM fungal symbiosis effective to enhance plant growth determined less acid phosphatase activity in soil (Sainet al. 1987).

Fries et al. (1998) described that plants were grown under five different levels of soil phosphorus, either in the presence or absence of formononetin or the AM fungus, *Glomus intraradices*. Formononetin influence physiological consequences in mycorrhizae and their intimate symbiosis with plants, before the onset, of nutrient-dependent responses. Under low phosphorus levels, Formononetin treatment enhanced colonization of the root by *G. intraradices* and partially overcame inhibition of AM fungal colonization by high soil phosphorus concentrations. ACP (acid phosphatase) and ALP (alkaline phosphatase) activities were closely related to the level of fungal colonization in maize roots. ACP activity in maize roots responded more to soil phosphorus availability than did ALP activity (38% more). These results suggest that ACP was involved in the increased uptake of phosphorus from the soil, while ALP may be linked to active phosphate assimilation or transport in mycorrhizal roots. Thus, soil phosphorus directly affected a number of enzymes essential in host–endophyte interplay, while formononetin enhanced fungal colonization. Bhadraiah et al. (1999) explained that the seedlings of *Terminalia arjuna*, grown in polythene bags in sterilized soil treated with *Glomus mosseae*, *G. fasciculatum* and rock phosphate separately, or in various combinations showed that acid phosphatase activity increased to a maximum in *G. fasciculatum* roots followed by *G. mosseae* + phosphorus and *G. mosseae* + *G. fasciculatum* treated roots. The acid phosphatase activity in shoots was maximum in *G. mosseae* + phosphorus treated plants. All other combinations had reduced

acid phosphatase activity. Alkaline phosphatase activity was considerably lower than the acid phosphatase activity in the roots and shoots of all the AM fungi treated *Terminalia* plants. Alkaline phosphatase activity was maximum in roots of *G. fasciculatum* + phosphorus and in shoots of *G. mosseae* + phosphorus, followed by phosphorus treated plants. Positive correlation was noted between acid phosphatase activity and phosphorus concentration.

11.21 Nitrogen Fertilization on Phosphatase Production by AM Fungi

Variable calcium (Ca) and magnesium (Mg) ration in urea and ammonium tarterate as nitrogen source supplemented to evaluate the ectomycorrhizal cell associated phosphatase activity by insulating in one-year-old seedlings of pine up to 10 months revealed that the Ca: Mg ratio influence acid phosphatase activity in the pine roots (Kieliszewska-Rokicka 1990).

11.22 Mycorrhizal Fungi and Land Management for Green Technology

All types of land management that involve tillage, timber harvesting, vegetation clearing or other forms of disturbance can affect mycorrhizal populations. Severe soil disturbance, such as agricultural soils (Thomson 1987), crop rotation with non-host species (Gavito and Miller 1998) or topsoil stripping and storage during mining (Bowen 1973; Jasper et al. 1987; Gardner and Malajczuk 1988), markedly reduces populations of mycorrhizal fungi. Unlike AM fungi, ECM fungi may be able to quickly invade disturbed soils (Jasper 1994). This is often the case have been termed “early colonizing” genera such as *Laccaria*, *Pisolithus, Rhizopogon, Scleroderma* and *Thelephora*. Recolonisation mostly results from spore dispersal by wind and animal vectors from sporocarps in adjacent vegetation.

Studies in a number of ecosystems (Reeveset al. 1979; Allen et al. 1987) show that in climax communities, most often dominated by heavily colonizing mycorrhizal fungi, lead to a successional sequence in which re-colonization is initiated by plant (Read and Birch 1988). The abandonment of agricultural land resulted in succession from non-mycorrhizal ruderal annuals to AMF colonized perennials and an increase in floristic richness (Barbi and Siniscalo 2000). Increasing soil fertility, especially P and N, can suppress mycorrhiza formation and/or mycorrhizal diversity but the effects are often host and fungal dependent (Prasad 1993).

11.23 Improvement of Soil Quality, Increase Yields and Reduce Expenses with AM Fungi

High-quality crop yields depend, in part, on good soil nutrient management. Most farmers rely on nutrient inputs to manage soil nutrients. However, many farmers adopt organic and sustainable farming also contributes to enhance natural biological processes in the soil in order to provide natural nutrients to the crops. Many of these biological processes are powered by mutually beneficial relationships (symbioses) that develop between plants and bacteria (such as nitrogen fixing bacteria) or beneficial soil fungi. One of the most important of these symbioses is that these are developing between plant roots and fungi, producing structures called mycorrhizas.

11.24 Mycorrhizal Fungi and Food for Animals

Long-distance dispersal of spores from ECM fungi with hypogeal (truffle-like) sporocarps depends largely on mammal mycophagy (Kotter and Farentinos 1984; Claridge and May 1994; Claridge et al. 1999). Mycophagy is widespread and has been demonstrated in Europe, Asia, Australasia and North America. Mycophagy serves to maintain populations of ECM fungi and provides nourishment to small mammals (Malajczuk et al. 1987). Sporocarps are good sources of water, protein, carbohydrates and minerals (Johnson 1994; Claridge et al. 1999). The tripartite relationship between truffles/truffle-like fungi, vertebrates such as squirrels and many ground-dwelling marsupials, and the host trees, are well known (Harwani et al. 2009, Prasad 2015).

11.25 Mycorrhizal Fungi and Value for Human

The highest diversity of edible fungi is collected from mixed forests in India, China, and the lowest diversity from areas of tropical pine and dipterocarps. In general, traded fresh sporocarps are 2–20 times more valuable, by weight, than local seasonal fruits and vegetables. International trade in a small number of species is having a major impact on the quality and sustainability of the mushroom harvest from some collecting sites. Forest fungi are also valued for medicine, for their aesthetics, as bio-indicators of environmental quality and for bio-remediation.

11.26 Past and Future of AM Fungi in Plant Production for Green Technology

Research on AM fungi in the 1970s and 2000s was dominated by the search for "super strains" capable of increasing plant biomass under any environmental and soil conditions. The desire to exploit AM fungi as a natural biofertilizer for the agricultural biotechnology industry was understandable, but it became clear that more knowledge was needed of the fungi themselves to allow commercial exploitation. Many inoculant companies have tried to commercialize the use of AM fungi with limited success. This has masked the importance of the symbiosis for normal plant growth and development in natural ecosystems where mycorrhizal plants dominate climax vegetation. Many mycorrhiza inoculants use the same fungal consortia for all environments. The benefits of the symbiosis for nutrient uptake by plants in agrosystems are important but a more complete understanding of how to manage arbuscular mycorrhizas for optimum plant growth and development and general health is needed urgently, as high-input plant production practices are challenged by more sustainable approaches.

11.27 Conclusion

Much attention has to be paid on mycorrhizae to exploit as a tool for improving the growth and health of plants. The use of mycorrhizae in agriculture, horticulture and forestry has been described. The significance of AM fungi in sustainable agriculture combined with technology was a subject of growing interest from several decades. In sustainable agriculture, it is imperative to maximize benefits with low input costs. The fact remains that stable and lower human population is an integral component of sustainable agriculture and more so in an Indian context. Therefore, it is imperative to collect further information on the different aspects of AM fungal symbiosis so as to utilize this plant microbe symbiotic system for the increased production and productivity in a sustainable manner. This may become possible when the integrated approach is made to study of AM fungi right from the isolation of AM fungal spores to the high-quality inoculum's production and its applications in the field. It is also imperative to stimulate new mutualism between mycorrhizal scientists and ecologists. In this regard, recent advancement can be made in the molecular techniques Adequate field testing of mycorrhizae inoculation and commercial exploitation of the potential benefits of mycorrhizae still rest on the development of suitable technology for mycorrhizae inoculum preparation as green technology (Table 11.3).

Table 11.3 AM fungi association in roots and spore propagules and species in soil for different vegetable species growing in the arid region of Rajasthan, India

Plant species/ Family	Family	% Infection level	Spores/100 g soil	AMF species
Daucus carota ssp. sativa	Apiaceae	[a]41.33 cd ± 3.67	145.00 cd ± 6.35	Gc, Gi, Gs. Gca
Allium cepa L.	Amaryllidaceae	85.00j ± 9.45	168.00e ± 11.85	Gmi,Gf,Gi, Gs
Allium sativum L.	Amaryllidaceae	91.33a ± 2.85	190.67f ± 3.67	Gf,Gma,Gm, Gs
Lablab purpureus (L.) Sweet	Fabaceae	62.33 fg ± 3.71	166.33e ± 5.78	Gf,Gm,Gs, Gg
Pisum sativum L.	Fabaceae	72.66jhi ± 2.85	160.67de ± 3.28	Gf,Gc,Gm, Gs,Gic
Vigna sinensis Prain	Fabaceae	58.67fgh ± 3.71	199.00f ± 0.58	Ga, Gm, Gs, At
Lycopersicum esculentum L.	Solanaceae	55.33de ± 5.78	130.67bc ± 2.40	Gi, Gf, Gs, Sc
Solanum melongena Linn.	Solanaceae	34.00bcd ± 5.77	120.33b ± 1.45	Ga, Gm, Gi, At
Solanum tuberosum Linn.	Solanaceae	44.33cde ± 6.06	138.00bc ± 7.51	Gi,Gmi,Get, Gi,Gs
Pomoea batatas	Solanaceae	56.66def ± 6.67	130.00bc ± 4.00	Gi, Gs, Gci, Gco.
Capsicum spp. (annuum)	Solanaceae	51.66de ± 6.67	137.00bc ± 4.04	Ga, Gge, Sn, Sc
Solanum melongina	Solanaceae	52.00de ± 3.51	148.00 cd ± 9.07	Ga, Gi, Sn, Gci
Brassica oleracea L. var. botrytis	Brassicaceae	0.00	80.33a ± 9.60	Gc, Gf, Gi Gs, Sc
Brassica oleracea L. var. capitata	Brassicaceae	19.66ab ± 3.84	145.67 cd ± 15.30	Gia,Gc,Gsp, Gma, Gic, Gma
Raphanus sativus L.	Brassicaceae	19.67ab ± 2.03	133.67bc ± 6.06	Ga, Gle, Gi, Gco
Abelmoschus esculentus (Linn) Moench (Malvaceae)	Malvaceae	16.33a ± 0.88	163.00de ± 3.51	Gge, Gma, Gs,Get,
Basella alba	Basellaceae	0.00	130.33bc ± 2.73	Ga, Gc, Gs,, Sc
Cucurbita maxima	Cucurbitaceae	74.33ij ± 6.36	134.33bc ± 6.06	Gi, Gf, Gs, Gg
Cucumis sativus	Cucurbitaceae	19.67ab ± 2.03	133.67bc ± 6.06	Ga, Gc, Gs, Gle
Momordica cochinchinensis	Cucurbitaceae	77.33ij ± .78	91.33a ± 2.85	Gia, Gs, Gci, Al

(continued)

Table 11.3 (continued)

Plant species/ Family	Family	% Infection level	Spores/100 g soil	AMF species
Momordica charantia L.	Cucurbitaceae	70.33ghi ± 3.84	126.67bc ± 3.67	Ga, Gf, Gi, Gca,
Luffa acutangula L.	Cucurbitaceae	65.67gh ± 0.67	126.67bc ± 3.67	Gc, Gf, Gs, At

Values are mean of four replicates. ± *SE* Std error; Values in a column followed by the same letter are not significantly different at $P < 0.05$ according to DMRT

References

Abbott LK, Gazey C (1994) An ecological view of the formation of VA mycorrhizas. Plant Soil 159:69–78

Abbott LK, Robson AD (1982) Infectivity of vesicular-arbuscular mycorrhizal fungi in agricultural soils. Aust J Agric Res 33:1049–1959

Abbott LK, RobsonAD (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytol 99:245–255

Akello J, Dubois T, Gold CS, Coyne D, Nakavuma J, Paparu P (2007) *Beauveriabassiana* (Balsamo)Vuillemin as an endophyte in tissue culture banana (Musa spp.). J Invertebr Pathol 96:34–42

Allen EB Chambers JC, Connor KE, Allen MF, Brown RW (1987) Natural reestablishment of mycorrhizae in disturbed alpine ecosystems. Arctic Alpine Res 19:11–22

Arul A, Nelson R (2016) Diversity of arbuscular mycorrhizal fungi in the cement dust polluted sites of Ariyalur District, Tamil Nadu. Int J Adv Res Biol Sci 3(1):215–219

Azcon R Ocampo JA (1981) Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol 87:677–685

Baltruschat H, Dehne HW (1988) The occurrence of vesicular-arbuscular mycorrhiza in agro-ecosystem, manure in continuous monoculture and crop rotation on the inoculum potential of winter wheat. Plant Soil 107:279–284

Barbi E, Siniscalco C (2000) Vegetation dynamics and arbuscular mycorrhiza in oldfield successions of the western Italian Alps. Mycorrhiza 10:63–72

Barea JM (2000) Rhizosphere and mycorrhiza of field crops. Biologic Res Manag pp. 81–92

Barea JM, Azcon R, Azcon-Aquilar C (2002a) Mycorrhizosphere interaction to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81:343–351

Barea M, Toro MO, Orozco E, Campos R, Azcón A (2002b) The application of isotopic ^{32}P and ^{15}N dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops. Nutr Cycl Agroecosyst 63:35–42

Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577. doi:10.1126/science.1222289

Berta G, Gianinazzi-Pearson V, Gay G, Torri G (1988) Morphogenetic effects of endomycorrhizal formation on the root system of *Calluna vulgaris* (L.) Hull. Symbiosis 5:33–44

Bethlenfalvay Gabor J (1992) Mycorrhiza and crop productivity. In: Bethlenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. ASA/CSSA/SSSA, Madison, WI, pp 1–27

Bethlenfalvay GJ, Cantrell IC, Mihara KL, Schreiner RP (1999) Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. Biol Fertil Soil 28:356–363

Bhadraiah B, Kankadurga VV, Ramarao P, Manoharachary C (1999) Effect of VAM fungi and rock phosphate on phosphatase activities in *Terminalia arjuna*. Natl Conf Mycorr Section 3 (Poster): Physiol Biochem 5–7 March 1999

Birch CPD (1988) The effects and implications of disturbance of mycorrhizal mycelial systems. Proc Royal Soc Edinburgh 94B:13–24

Bolan NS (1991) A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207

Bolan NS, Robson AD, Barrow NJ (1987) Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant Soil 99:401–410

Bougher NL (1995) Diversity of ectomycorrhizal fungi associated with eucalyptusinAustralia. In: Brundrett M, Dell B, Malajczuk N, Gong M (eds) Mycorr Res Forest Asia. ACIAR Proc No. 62, Canberra, pp 8–15

Bowen GD (1973) Mycorrhizae Symbiosis. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae – their ecology and physiology. Academic Press, London, pp 151–205

Brazanti MB, Rocca E, Pisi E (1999) Effect of ectomycorrhizal fungi on chestnut ink disease. Mycorrhiza 9:103–109

Brundrett MC (1991) Mycorrhizas in natural ecosystems. In: MacFayden AM, Begon Fitter AH (eds) Advances in ecological research. Academic Press, London, pp 171–133

Brundrett MC (2006) Understanding the roles of multifunctional mycorrhizal andendophytic fungi. In: Schulz B, Boyle C, Seiber TN (eds) Microbial root endophyts, vol 9. Springer, Soil Biol, Berlin, pp 281–298

Brundrett MC, Kendrick B (1987) The relationship between the ash bolete (*Boletinellusmerulioides*) and an aphid parasitic on ash tree roots. Symbiosis 3:315–319

Bucking H, Shachar-Hill Y (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. New Phyto1 65:899–911

Chandreshekara CP, Patil VC, Sreenivasa MN (1995) VA-mycorrhiza mediated P effect on growth and yield of sunflower (*Helianthus annus* L.) at different P levels. Plant Soil 176:325–328

Claridge AW, May TW (1994) Mycophagy among Australian mammals. Aust J Ecol 19:251–275

Claridge AW, TrappeJM Cork SJ, Claridge DL (1999) Mycophagy by small mammals in the coniferous forests of North America: nutritional value of sporocarps of Rhizopogonvinicolor, a common hypogenous fungus. J Compl Physiol Biol 169:172–178

Coleman DC, Reid CPP, Cole CV (1983) Biological strategies of nutrient cycling in soil systems. Adv Ecol Res 13:1–55

Dehne WH (1986) Influence of VA mycorrhizae on host plant physiology. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiology and genetical aspects of Mycorrhizae. INRA, Paris, pp 431–435

Dieffenbach A, Matzner E (2000) In situ soil solution chemistry in the rhizosphere of mature Norway spruce (*Piceaabies* [L.]Kars trees. Plant Soil 222:149–161

Diop TA, Becard G, Piche Y (1990) Long-term in vitro culture of an endomycorrhizal fungus, *Gigaspora margarita*, on Ri-T-DNA transformed roots of carrot. Symbiosis 12:249–259

Dodd JC, Dougall TA, Clapp JP, Jeffriues P (2002) The role a species richness of AMF in plant community establishment the Eurotunnel site of special scientific interest, Samphire Hoe Kent, UK. Biodivers Conserv 11:39–58

Douds DD, Nagahashi G (2000) Signalling and recognition events prior to colonization of roots by arbuscular mycorrhizal fungi. In: Podila GK, Douds DD (eds) Current advances in mycorrhizae research. APS Press, Minnesota, pp 11–18

Duchesne LC, Peterson RL, Ellis BE (1989) The time course of disease suppression and antibiosis by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol 111:693–698

Duddridge JA (1987) Specificity and recognition in ectomycorrhizal associations. In: Pegg GF, Abres PG (eds) Fungal infection of plants. Cambridge University Press, Cambridge, pp 25–44

Evans DG, Miller MH (1990) The role of the external mycelial network in the effect of soil disturbance upon vesicular-arbuscular mycorrhizal colonization of maize. New Phytol 114:65–71

Ezawa T, Yoshida T (1994) Acid phosphatase specific to arbuscular mycorrhizal infection in marigold and possible role in symbiosis. Soil Sci Plant Nutr 40(4):655–665

Farquhar ML, Peterson RL (1991) Later events in suppression of *Fusarium* root rot of red pine seedlings by the ectomycorrhizal fungus *Paxillusinvolutus*. Can J Bot 69:1372–1383

Finlay RD, EK H, Odham G, Soderstrom B (1988) Mycelial uptake translocation and assimilation of nitrogen from 15 N-labelled ammonium by *Pinussylvestris* plants infected with four different ectomycorrhizal fungi. New Phytol 110:56–66

Finlay RD, Read DJ (1986) Hyphal mats. New Phyto 40:276–279

Francis R, Read DJ (1984) Direct transfer of carbon between plants connected by vesicular-arbuscular mycorrhizal mycelium. Nature (London) 307:53–56

Francis R, Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Can J Bot-revue Canadienne de batanique 73:1301–1309

Francis R, Finlay RD, Read DJ (1986) Vesicular-arbuscular mycorrhiza in natural vegetation systems IV. Transfer of nutrients in inter- and intra-specific combinations of host plants. New Phytol 102:103–111

Fries LL, Pacovsky M, Safir RS, Kaminski GR (1998) Phosphorus effect on phosphatase activity in endomycorrhizal maize. Physiol Plant 103(2):162–171

Gardner JH, Malajczuk N (1988) Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. Forest Ecol Manag 24:27–42

Gaude N, Bortfeld S, Duensing N, Lohse M, Krajinski F (2012) Arbuscules-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. Plant J 69:510–528

Gautam SP, Prasad K (2001) VA mycorrhiza—importance and biotechnological application. In: Maheshwari DK, Dube RC (eds) Innovative approaches in microbiology. Bishon Singh Mahendra Pal Singh, Dehradun, India, pp 83–114

Gavito ME, Miller MH (1998) Changes in mycorrhiza development in maize induced by crop management practices. Plant Soil 198:185–192

Gay G, Debaud JC (1987) Genetic study on indole-3-acetic acid production by ectmycorrhizal *Hebeloma* species: inter- and interspecific variability in homo- and dikaryotic mycelia. Appl Microbial Biotechnol 26:141–146

George E, Romheld V, Marschner H (1994) Contribution of mycorrhizal fungi to micronutrient uptake by plants. In: Monthey JA, Crowely DE, Luster DG (eds) Biochemistry of metal micronutrient in the rhizosphere. Boca Raton FL, CRC Press, pp 93–109

George E, Gorgus E, Schmeisser A, Marschner H (1996) A method to measure nutrient uptake from soil by mycorrhizal hyphae. In: Azcon A, Beare JM (eds) Mycorrhizas in Integrated System from Genes to plant Development. Luxembourg, European Community

Gianinazzi S, Gianinazzi- Pearson V (1986) Symbiosis 2:139–149

Gianinazzi-Pearson V (1984) Host-fungus specificity, recognition and compatibility in mycorrhizae. In: Verma DPS, Hohn T (eds) Genes involved microbe plant interaction. Springer, New York, pp 225–249

Gianinazzi-Pearson V, Gianinazzi S (1989) Cellular and genetic aspects of interactions between hosts and fungal symbionts in mycorrhizae. Genoine 31:336–341

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S (1989) In-vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. Symbiosis 7:243–255

Giovannetti M, Hepper CM (1985) Vesicular-arbuscular mycorrhizal infection in *Hedysarum coronarium* and *Onobrychisviciae folia*: host-endophyte specificity. Soil Biol Biochem 17:899–900

Giovannetti M, Schubert A, Cravero MC, Salutini L (1988) Spore production by the vesicular-arbuscular mycorrhizal fungus *Glomus monosporum* as related to host species, root colonization and plant growth enhancement. Biol Fertil Soils 6:120–124

Graham JH, Linderman RG, Menge JA (1982) Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of troyercitrange. New Phytol 91:183–189

Gray BD, Read DJ (1995) The structure and function of the vegetative mycelium of ectomycorrhizal plants. New Phytol 130(3):411–417

Gueye M, Diem HG, Dommergues YR (1987) Variation in N_2 fixation, N and P contents of mycorrhizal *Vigna unquiculatain* relation to the progressive development of extra-radical hyphae of *Glomus mosseae*. MIRCEN J 3:75–86

Hall JL, Williams LE (2000) Assimilate transport and partitioning in fungal biotrophic interactions. Aust J Plant Physiol 27:549–560

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Hartley J, Cairney JWG, Meharg AA (1997) Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment. Plant Soil 189:303–319

Harvey AE, Larsen MJ, Jurgensen MF (1976) Distribution of ectomycorrhizae in a mature douglas-fir/larch soil in western Montana. Forest Sci 22:393–398

Harwani, D, Choudhary P, Dhaker S, Prasad K, Mahna SK (2009) Tripartite symbiotic association: legume-rhizobia-mycorrhiza—a review. In: Maheshwari DK, Dube RC (eds) Biotechnology for agricultural microorganisms–An Agro-Industry Approach, I.K. International Pvt. Ltd. New Delhi, India, pp 406–435

Haselwandter K, Read DJ (1982) The significance of a root-fungus association in two *Care* species of high-alpine communities. Oecologia (Berl) 53:352–354

Hayman DS (1983) The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can J Bot 61:944–963

Haystead A, Malajczuk N, Grove TS (1988) Underground transfers of nitrogen between pasture plants infected with vesicular- arbuscular mycorrhizal fungi. New Phytol 108:417–423

Howeler RH, Sieverding E, Saif S (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant Soil 100:249–283

Jasper DA (1994) Bioremediation of agricultural and forestry soils with symbiotic micro-organisms. Aust J Soil Res 32:345–348

Jasper DA, Robson AD, Abbott LK (1987) The effect of surface mining on the infectivity of vesicular arbuscular mycorrhizal fungi. Aust J Bot 35:642–652

Jeffries P, Gianinazzi S, Perotto S, Tumau K, Barea JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soil 37:1–16

Johnson PN (1977) Mycorrhizal Endogonaceae in a New Zealand forest. New Phytol 78:161–170

Johnson CN (1994) Mycophagy and spore dispersal by a rat-kangaroo: consumption of ectomycorrhizal taxa in relation to their abundance. Funct Ecol 8:464–468

Jones K, Hendrix JW (1987) Inhibition of root extension in tobacco by the mycorrhizal fungus *Glomus macrocarpum* and its prevention by benomyl. Soil Biol Biochem 19:297–299

Jones MD, Durall DM, Tinker PB (1990) Phosphorus relationships and production of extrametrical hyphae by two types of willow ectomycorrhizae at different soil phosphorus levels. New Phytol 115:259–267

Juniper S, Abbott L (1993) Vesicular-arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45–57

Kendrick B (1985) The Fifth Kingdom. Mycologue Publications. Waterloo, Ontario

Kieliszewska-Rokicka B (1990) Acid phosphatase activity in roots of Scots pine (*Pinussylvestris* l) seedlings fertilized with different nitrogen sources. Agric Ecosyst Environ 17(1–4):229–234

Killham K (1985) Vesicular-arbuscular mycorrhizal mediation of trace and minor element uptake in perennial grasses: relation to livestock herbage. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soils: plants microbes and animals. Blackwell, Oxford, pp 225–232

Klironomos JN, Hart MM (2001) Food-web dynamics. Animal nitrogen swaps for plant carbon. Nature 410:651–652. doi:10.1038/35070643

Kotter MM, Farentinos RC (1984) Tassel-eared squirrels as spore dispersal agents of hypogeous mycorrhizal fungi. J Mammal 65:684–687

Krishna KR, Shetty KG, Dart PJ, Andrews DJ (1985) Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. Plant Soil 86:113–125
Lackie SM, Bowley SR, Peterson RL (1988) Comparison of colonization among half-sib families of *Medicago sativa* L. by *Glomus versiforme* (Daniels and Trappe) Berch. New Phytol 108:477–482
Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153
Lynch JM, Bragg E (1985a) Microorganisms and soil aggregate stability. Advances in soil science, vol 2. Springer, New York, pp 133–171
Lynch JM, Bragg E (1985) Microorganisms and soil aggregate stability. In: Advances in Soil Science Vol2, Springer-Verlag, New York, pp. 133-171
Majdi H, Damm E, Nylund J (2001) Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytol 150:195–202
Malajczuk N, Trappe JM, Molina R (1987) Interrelationships among some ectomycorrhizal trees, hypogenous fungi and small mammals: Western Australian and north western American parallels. Aust J Ecol 12:53–55
Manjunath A, Habte M (1988) Development of vesicular arbuscular mycorrhizal infection and the uptake of immobile mycorrhizas in forestry and agriculture. ACIAR Monograph 32, Australian Centre for International Agricultural Research, Canberra, pp 374
Manjunath A, Habte M (1988b) The development of vesicular arbuscular mycorrhizal infection and the uptake of immobile nutrients in *Leucaenaleucocephala*. Plant Soil 106:97–103
Manoharachary C, Sridhar K, Singh R, Adholeya A, Suryanarayanan TS, Rawat S, Johri BN (2005) Fungal biodiversity: distribution, conservation and prospecting of fungi from India. Curr Sci 89(1):58–71
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London
Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102
Mason PA, Last FT, Wilson J, Deacon JW, Fleming LV, Fox FM (1987) Fruiting and successions of ectomycorrhizal fungi. In: Pegg GF, Ayes PG (eds) Fungal infection of plants. Cambridge University Press, Cambridge, pp 253–268
McGonigle TP (1998) A numerical analysis of published field trials with vesicular arbuscular mycorrhizal fungi. Functional Ecol 2:473–478
McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol Res 94:120–122
Meghvansi MK, Prasad K, Mahna SK (2010) Symbiotic potential, competitiveness and compatibility of indigenous *Bradyrhizobium japonicum* isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan, India. Saudi J Biologic Sci 17:303–310
Menge JA (1983) Utilization of vesicular arbuscular mycorrhizal fungi in agriculture. Can J Bot 61:1015–1024
Menge JA, Grand LF, Haine LW (1977) The effect of fertilization on growth and mycorrhiza numbers in 11-year-old loblolly pine plantations. Forest Sci 23:37–44
Miller OK Jr (1982) Mycorrhizal fungi and fungal biomass in subalpine tundra at Eagle Summit, Alaska. Holarctic Ecol 5:125–134
Miller RM, Jastrow JD (1992) The role of mycorrhizal fungi in soil conservation. In: Bethlenfalvay GJ, Linderman GR (eds) Mycorrhizae in sustainable agriculture. Agronomy Society of America special Publication No. 54, Madison, WI, pp 29–44
Molina R, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. Forest Sci 28:423–458
Morton JB (1990) Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): Their role in macro and micro evolutionary processes. Mycotaxon 37:493–515
Morton JB, Benny GL (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes); a new order, Glomales, two new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae, with anemendation of Glomacea. Mycotaxonomy 37:471–491

Mosse B (1959) Observations on the extra-matrical mycelium of a vesicular-arbuscular endophyte. Truns Br Mycol Soc 42:439–448

Mosse B (1978) Mycorrhiza and plant growth. In: Freysen AHJ, Woldendorp JW (eds) Structure and functioning of plant populations. North Holland Publishing Co, Amsterdam, pp 269–289

Nagahashi G, Douds DD, Abney GD (1996) Phosphorus amendment inhibits hyphal branching of VAM fungus Gigaspora margarita directly and indirectly through its effect on root exudation. Mycorrhiza 6:403–408

Nair MG, Safir GR, Siqueira JO (1991) Isolation and Identification of vesicular Arbuscular mycorrhizal- stimulatory compounds from clover (*Trifolium repens*) roots. Appl Environ Microbiol 57:434–439

Newman EI (1988) Mycorrhizal links between plants: their functioning and ecological significance. Adv Ecol Res 18:243–270

Newton AC, Pigott CD (1991) Mineral nutrition and mycorrhizal infection of seedling oak and birch. II. Effect of fertilizers on growth, nutrient uptake and ectomycorrhizal infection. New Phytol 117:45–52

Nicolson TH (1959) Mycorrhiza in the Gramineae I. vesicular-arbuscular endophytes, with special reference to the external phase. Trans Br Myco Soc 42:421–438

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76:319–337

Odum EP (1984) Properties of agro ecosystem. In: Lowrance R, Stinnerand BR, House GJ (eds) Agriculteral ecosystems. J Wiley and Sons, New York, pp 5–11

Oehl F, Wiemken A, Sieverding E (2002) *Glomus caesaris*, a new arbuscular mycorrhizal fungus from the Kaiserstuhl in Germany. Mycotaxonomy 84:379–385

Oehl F, Wiemken A, Sieverding E (2003) *Glomus spinutiferum*: A new ornamental species in the Glomales. Mycotaxonomy 86:157–162

Pacovsky RS (1986) Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans. Plant Soil 95:379–388

Pellet D, Sieverding E (1986) Preferential multiplication of fungal species of the Endogonanceae in the field Demonstrated with weeds. In: Gianinazzi S (ed) Gianinazzi-Pearson V. Physiol Genet Aspec Mycorrhizae INRA, Paris, pp 555–557

Perez-Moreno J, Read DJ (2000) Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol 145:301–309

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres and reforestation: current knowledge and research needs. Can J For Res 17:929–940

Potty VP, Indira P (1990) Influence of vesicular arbuscular mycorrlrizae on the photosynthesis and photorespiration of sweet potato (*Ipomea batata*). In: Jalali BL, Chand H (eds) Curr Trend Mycorrhizal Res. Haryana Agricultural University Press, Hisar, India, p 73

Prasad K (1993) Ecological factors affecting vesicular arbuscular mycorrhiza infection in sugarcane. Ph.D Thesis, B.R. Ambedkar Bihar University, Muzaffarpur, India

Prasad K (1995) Physico chemical characteristics of soil in relation to vesicular arbuscular mycorrhizal (*Glomus fasciculatum*) colonization in Saccharum officinarum L. J Phytol Res 8(2):201–205

Prasad K (1998) Effect of *Glomus fasciculatum* VAM form and *Rhizobium* on biomass yield and nutrient uptake of *Dalbergia sissoo* L. J Trop Forestry 14(111):143–148

Prasad K (2000a) Growth responses of *Acacia nilotica* (L.) Del. inoculated with *Rhizobium* and *Glomus fasciculatum* VAM fungi. J Trop Forestry 16(1):22–27

Prasad K (2000b) Impact of arbuscular mycorrhizal (AM) fungi on biomass yield of *Terminalia arjuna* under field conditions. Vislesana, Res J (Sci) 7(2):73–79

Prasad K (2000c) Occurrence of vesicular arbuscular mycorrhizal fungi in some cultivated crop plants. In: Maheshwari DK, Dube RC, Prasad G, Navneet (eds) Microbes: agriculture, industry and environment. Bishen Singh Mahendra Pal Singh Publishers, Dehra Dun, pp 65–69

Prasad K (2002) Effect of arbuscular mycorrhizae on biomass yield, uptake and translocation of nitrogen, phosphorus and potassium in *Azadirachta indica* L. In: Purohit DK, Reedy SR,

Singaracharya MA, Girisham S (eds) Manoharachary C. Front Microbial Biotechnol Plant Pathol, Scientific Publishers (India) Jodhpur, pp 187–191

Prasad K (2005) Arbuscular mycorrhizal fungal occurrence in non-cultivated disturbed and non-fertile land of Bettiahraj, Bettiah, Bihar. Mycorrhiza News 16(4):12–14

Prasad K (2006a) Impact of arbuscular mycorrhizal fungus (*Glomus fasciculatum*) and phosphate solubilizing bacterium (*Pseudomonas striata*) on growth and nutrient status of *Azadirachta indica* L. Mycorrhiza News 18(2):10–12

Prasad K (2006b) Influence of indigenous VAM fungus (*Glomus fasciculatum*) and Rhizobium on growth, nutrient uptake and nodulation in *Acacia nilotica*. In: Prakash A, Mehrotra VS (eds) Mycorrhiza. Scientific Publishers (India), Jodhpur, pp 139–144

Prasad K (2007) Improvement in bioavailability of nutrition and biomass production to *Azadirachta indica* (Neem) by arbuscular mycorrhizal symbiosis. J Basic Applied Mycology 6 (I &II):96–101

Prasad K (2010a) Ectomycorrhiza symbiosis: possibilities and prospects. In: Rao MK, Kovices G (eds) Progress in mycology. Scientific Publisher, Jodhpur, India, pp 290–308

Prasad K (2010b) Responses of dual inoculation of arbuscular mycorrhizal fungi on the biomass production, phosphate, roots and shoots phenol concentrations of *Terminalia arjuna* under field conditions. Mycorrhiza News 22(2):13–17

Prasad K (2011a) Effect of *Glomus intraradices* AM fungi on the shoot dry matter, seed dry yield, and nitrogen and phosphorus uptake of soybean (*Glycine max* (L.) Merrill) inoculated with cultivar-specific *Bradyrhizobium japonicum*. Mycorrhiza News 23(3):13–18

Prasad K (2011b) Interaction between arbuscular mycorrhizal fungus (*Glomus fasciculatum*) and rhizospheric fungi in *Saccharum officinarum* L. Amravati Univ Res J 5:53–65

Prasad K (2013) Arbuscular mycorrhizal fungus plays a major role in agriculture and natural ecosystems to improve production in sustainable manner In: Jamaluddin and Singh AK (eds) Microbes and sustainable plant productivity. Scientific Publication (India) Jodhpur, pp 113–138

Prasad K (2015) Biofertilizers: a new dimension for agriculture and environmental development to improve production in sustainable manner. J Basic Applied Mycol 11(1&II):5–13

Prasad K, Bilgrami RS (2004) Impact of *Glomus fasciculatum* (AMF) and phosphates on biomass yield of *Saccharum officinarum L.* J Basic Applied Mycol 3(I&II):108–110

Prasad K, Bilgrami RS (2005) Interaction between arbuscular Mycorrhizal fungus (Glomus fasciculatum) and rhizospheric fungi in Saccharum officinarum L. Special, vol. of Rhizosphere 2004. J Soil Sci Plant Nutr 56:1234–1239

Prasad K, Bilgrami RS (2006) Impact of *Glomus fasciculatum* and potash on biomass yield and sucrose contents of *Saccharum officinarum* L. In: Prakash A, Mehrotra VS (eds) Mycorrhiza. Scientific Publishers (India), Jodhpur, pp 45–50

Prasad K, Bilgrami RS (2007) Effect of *Glomus fasciculatum* AM fungus and nitrogen's on biomass yield, chlorophyll, juice and sucrose contents of *Saccharun officinarum* L. In: Ram RC, Sinha A (eds) Modern trends in microbial diversity. Daya Publishing House, New Delhi, India, pp 224–233

Prasad K, Deploey JJ (1999) Incidence of arbuscular mycorrhizae and their effect on certain species of trees. JPA Acad Sci 73(3):117–122

Prasad K, Gautam SP (2000) Arbuscular mycorrhizal spore types present in the root zone of *Dalbergia sissoo* L. Vislesana, Res J (Sci) 7(2):013–018

Prasad K, Gautam SP (2005) Effect of inoculation of arbuscular mycorrhizal fungus (*Glomus macrocarpum*) on the growth and nutrients uptake of *Dendrocalamus strictus* (Roxb.) Ness under field conditions. Anusandhan 1:53–62

Prasad K, Kaushik S (2004) Ecology, physiology, biochemistry and taxonomy of arbuscular mycorrhizal fungi. In: Gautam SP, Bansal YK, Pandey AK (eds) Biological diversity: current trends, Prof. R.C. Rajak Festschrift volume. Shree Publication & Distributors, New Delhi, pp 134–141

Prasad K, Meghvansi MK (2005) Interaction between indigenous *Glomus fasciculatum* (AM fungus) and *Rhizobium* and their stimulatory effect on growth, nutrient uptake and nodulation in *Acacia nilotica* (L.) Del. Flora and Fauna 11(1):51–56

Prasad K, Pandey AK (2012) Mycorrhizal symbiosis: A new dimension for agriculture and environmental development to improve production in sustainable manner. In: Bagyaraj DJ, Tilak KVBR, Kehri HK (eds) Microbial diversity and function, New Delhi. New India Publishing Agency, New Delhi, India, pp 389–402

Prasad K, Rajak RC (1999) Recent advances in mycorrhizal taxonomy: morphological and molecular criteria. In: Rajak RC (ed) Microbial biotechnology for sustainable development and productivity. Prof. SK. Hasija Festschrift. Scientific Publishers (India), Jodhpur, pp 62–72

Prasad K, Rajak RC (2000) Biotechnological application of mycorrhizae in reclamation of mined dumps. In: Rai MK, Varma A, Rajak RC (eds) Integrated management of plant resources. Scientific Publishers (India), Jodhpur, pp 283–292

Prasad K, Rajak RC (2001) Microbes and wasteland management: Challenge ahead. In: Roy AK, Varma SK (eds) Wasteland management and environment. Scientific Publishers (India), Jodhpur, pp 27–39

Prasad K, Meghvansi MK, Harwani D, Mahna SK, Werner D (2005a) Synergistic effect of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on growth, yield and nutrient status of soybean [*Glycine max* (L.) *Merrill*] Anusandhan 1(1):13–19

Prasad K, Meghvansi MK, Choudhary KK (2005b) Arbuscular mycorrhizal symbiosis: an overview of research and extension needs. In: Tiwari M, Sati SC (eds) The mycorrhizae: diversity, ecology, and application. Daya Publishing House, New Delhi, pp 87–101

Prasad K, Pandey AK, Rajak RC (2005c) Ectomycorrhizal diversity and its role in the productivity of forest plants. In: Mukherjee KG, Tilak KVBR, Reddy SM, Gangwane LV, Prakash P, Kunwar IK (eds) Frontiers in plant sciences. I. K. International Pvt Ltd, New Delhi, India, pp 617–642

Prasad K, Meghvansi MK, Harwani D, Mahna SK, Werner D (2006a) Distribution of arbuscular mycorrhizal fungi in soybean (*Glycine max* (L.) *Merrill*) rhizosphere. Mycorrhiza News 17 (4):14–17

Prasad K, Kaushik S, Rajak RC (2006b) Performance of arbuscular mycorrhizal biofertilizers and their role in sustainable development and productivity. In: Prakash A, Mehrotra VS (eds) Mycorrhiza. Scientific Publication (India), Jodhpur, pp 281–289

Prasad K, Meghvansi MK, Khan AA (2011) Incidence of arbuscular mycorrhizal fungi (AMF) in tree species in arid zones of Ajmer region of Rajasthan. Mycorrhiza News 22(4):12–15

Rafiq Lone, Shuab R, Malla NA, Gautam AK, Koul KK (2016) Beneficial effects of arbuscular mycorrhizal fungi on underground modified stem propagule plants. J New Biologic Rep 5 (1):41–51

Read DJ, Birch CPD (1988) The effects and implications of disturbance of mycorrhizal mycelial systems. Proc Royal Soc Edinburgh 94B:13–24

Read DJ, Boyd R (1986) Water relations of mycorrhizal fungi and their host plants. In: Ayres P, Boddy L (eds) Water, fungi and plants. Cambridge University Press, Cambridge, pp 287–303

Read DL, Leake JR, Langdale AR (1989) The nitrogen nutrition of mycorrhizal fungi and their host plants. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge University Press, Cambridge, pp 181–204

Reeves BF, Wagner D, Moorman T, Keil J (1979) The role of endo-mycorrhizae in revegetation practices in the semi-arid west. I. A comparison of the incidence of mycorrhizae in severely disturbed vs. natural environments. Am J Bot 66:6–13

Rilling M (2004) Arbuscular mycorrhizae, glomalin and soil aggregation. Can J Soil Sci 84:355–363

Rilling MP, Ramsey S, Morris Paul E (2003) Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. Plant Soil 253:293–299

Ritz K, Newman EI (1986) Nutrient transport between ryegrass plants differing in nutrient status. Oecologia (Berl) 70:128–131

Rose SL (1988) Above and below ground community development in a marine sand dune ecosystem. Plant Soil 109:215–226

Rothwell FM (1984) Aggregation of surface mine soil by interaction between VAM fungi and lignin degradation products of Lespedex. Plant Soil 80:99–104
Rubio R, Moraga E, Borie F (1990) Acid phosphatase activity and vesicular arbuscular mycorrhizal infection associated with roots of 4 wheat cultivars. J Plant Nutr 13(5):585–598
Rupp LA, Mudge KW, Negm FB (1989) Involvement of ethylene in ectomycorrhiza formation and dichotomous branching of roots of mugo pine seedlings. Can J Bot 67:477–482
Sainz MJ, Trasar MC, Arines, Gil-Stores F (1987) Phosphatase activity in three acid soils as affected by VAM symbiosis and soil steam-sterilization. In: Proceedings of Seventh North American Conference on Mycorrhiza 3–8 May 1987, Gainesville, Florida
Sasan RK, Bidochka MJ (2012) The insect- pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. Am J Bot 99:101–107
Sbrana C, Giovannetti M (2005) Chemotropism in the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycorrhizae 15:539–545
Scheltema MA, Abbott LK, Robson AD (1985) Seasonal variation in the infectivity of VA mycorrhizal fungi in annual pastures in a mediterranean environment. Aust J Agric Res 38:70–715
Sharma AK, Srivastava PC, Johri BN (1990) Influence of *Glomus macrocarpum* on growth and physiology of *Sesbania aculenta.* In: Bagyaraj DJ, Manjunath A (eds) Mycorrhiza symbiosis and plant growth. Proc Sec Nat Conf on Mycorrhizae Nov. 21–23. 1990. Reproduction/ Graphis Type Setters, Bangalore, and India, pp 86–87
Sievarding E, Glavez A (1988) Performance of different cassava clones with various VA mycorrhizal Fungi. Angew Botanik 62:273–282
Simard SW, Perry DA, Smith JE, Molina R (1997) Effects of soil trenching on occurrence of ectomycorrhizas on *Pseudotsuga menziesii* seedlings grown in mature forests of *Betulapa pyrifera* and *Pseudotsuga menziesii*. New Phytol 136:327–340
Simpson D, Daft MJ (1990a) Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. Plant Soil 121:171–178
Simpson D, Daft MJ (1990b) Interactions between water-stress and different mycorrhizal inoculum on plant growth and mycorrhizal development in maize and sorghum. Plant Soil 121:179–186
Smith SE, Gianinazzi-Pearson V (1988) Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Ann Rev Plant Physiol Plant Mol Biol 39:221–244
St John TV, Coleman DC, Reid CPP (1983) Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. Plant Soil 71:487–493
Struble JE, Skipper HD (1988) Vesicular-arbuscular mycorrhizal fungal spore production as influenced by plant species. Plant Soil 109:277–280
Thomas GV, Ghai SK (1987) Genotype dependent variation in vesicular arbuscular mycorrhizal colonisation of coconut seedlings. Proc Indian Acad Sci (Plant Sci) 97:289–294
Thompson JP (1987) Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. Aust J Agric Res 38:847–867
Tinker PB, Gilden A (1983) Mycorrhizal fungi and ion uptake. In: Robb DA, Pierpoint WS (eds) Metals and micronutrients, uptake and utilization by plants. Academic Press, NY, pp 21–32
Tisdall JM, Oades JM (1979) Stabilization of soil aggregates by the root systems of ryegrass. Aust J Soil Res 17:429–441
Toth R, Toth D, Stark D, Smith DR (1990) Vesicular-arbuscular mycorrhizal colonisation in *Zea mays* affected by breeding for resistance to fungal pathogens. Can J Bot 68:1039–1044
Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the Angiosperms from an evolutionary standpoint. In: Safir GR (ed) Eco physiology of VA mycorrhizal plants. CRC Press, Boca Raton, Florida, pp 5–25
Usuki F, Narisawa KA (2007) Mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99:175–184
Van der Heijden MG, Klironimos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998a) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Van der Heijden MG, Boller T, Wiemken A, Sanders IR (1998b) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091

Vogt KA, Grier CC, Meir CE, Edmonds RL (1982) Mycorrhizal role in net production and nutrient cycling in Abiesamabilis ecosystems in western Washington. Ecology 63:370–380

Walker C, Biggin P, Jardine DC (1986) Difference in mycorrhizal status among clones of Sitka spruce. Forest Ecol Manag 14:275–283

Warner A (1984) Colonization of organic matter by vesicular-arbuscular mycorrhizal fungi. Trans Br Mycol Soc 82:352–354

Went FW, Stark N (1968) The biological and mechanical role of soil fungi. Proc Natl Acad Sci 60:497–504

Wright SF (2005) Roots and soil management: interactions between roots and the soil. In: Zobel RW, Wright SF (eds) Management of arbuscular mycorrhizal fungi. American Society of Agronomy, USA, pp 183–197

Wright SF, Millner PD (1994) Dynamic processes of vesicular-arbuscular. Mycorrhizae: a mycorrhizasystem within agro ecosystem. In: Hatfield JL, Stevart BA (eds) Soil biology: effects on soil quality. Lewis Publishers, Boca Raton, pp 29–57

Wu B, Nara K, Hogetsu T (2001) Can 14C-labelled photosynthetic products move between *Pinus densiflora* seedlings linked by ectomycorrhizal mycelia? New Phytol 149:137–146

Zubek S, Majewska ML, Błaszkowski J, Stefanowicz AM, Nobis M, Kapusta P (2016) Invasive plants affect arbuscular mycorrhizal fungi abundance and species richness as well as the performance of native plants grown in invaded soils. Biol Fertil Soils 52:879–89893

Chapter 12
Endophytic Fungi: Eco-Friendly Future Resource for Novel Bioactive Compounds

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Abstract The current research focuses on the isolation of bioactive compounds from the natural sources which have immense potential for pharmaceutical value. Pharmaceutical biology perceives plants as a unique resource of potentially precious remedial bioactive metabolites. But due to slow growth and harvest of endangerd plants species pose a threat and inbalance in the biodiversity of plants. However, most of the plant species occur on the earth to be a reservoir of vast numbers of endophytic microorganisms like bacteria, actinomycetes, and fungi that play an imperative role in the production of novel secondary metabolites for the defense of host and can be utilized for treatment of a number of ailments. Search for isolation and characterization of different plant-associated fungal origin novel bioactive metabolites are given an immense attention to global investigators. The endophytic fungi are an enormous manufacturer of bioactive compounds which can be widely used in the medical, agricultural, and industrial application. Therefore, there is a need to isolate, identify, and characterize these bioactive compounds from the endophytic fungi. Further, research on the biology of endophytes is also required to saturate at the molecular level for a better understanding of host–endophyte interactions and biosynthesis of secondary metabolites thereby. Modern technologies have opened new avenue on endophytic research as natural "warehouse" with very little has been able to tap from this source so far and among the reported natural bioactive metabolites. Thus, there is more research and studies on these groups of endophytic microorganisms are required. The collaboration among chemists and mycologists are needed to comprehend the biology of endophytic

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_12

fungi and may help to learn the different pathways involved in synthesis of bioactive compounds, and the ecology of the organisms will help to understand the optimization parameters of the organism for the maximum metabolites production, and mycologist will have the chance to increase further imminence into the multifarious diversity of endophytic fungal species. The present review is made on endophytic fungi, biosynthetic pathways responsible for the production of novel bioactive compounds from these microorganisms and their applications.

Keywords Endophytic fungi · Pharmacophores · Microorganisms
Taxol · Antimicrobial

12.1 Introduction

From thousands of year, mankind is using natural goods, different types of phytochemicals, drugs, food, hallucinogens along with microbial products of fungi, bacteria, algae, and other living organisms in a variety of applications. Natural products generally show a new method of the deed and exhibit novel therapeutic activities with significant probability than that of synthetic compounds. These have been proved to serve as promising sources of chemotherapy especially in the case of cancer due to their novel bioactive compounds with structural intricacy and biological activeness (Verdine 1996). A source of pharmacophores having multiple activities works as powerful biochemical tools and plays the role of "guide" to assist molecular biologists and chemists in their investigation of cellular function (Bram et al. 1993). Because of the various benefits, these novel bioactive compounds dignified over synthetic alternatives, and their isolation from natural resources on large scale is on the swing. In the present scenario, a huge work has been carried out in the field of mycology for extraction of bioactive compounds for commercial purposes. Fungi having rigid cell wall full of chitin, polysaccharide, and cytoplasmic membrane contain steroids (sterols). There is a huge diversity of fungi exists on the earth, and many of them have a contribution in therapeutic use against pathogenic organisms; hence, the endophytic fungi became the center of attraction for researchers (Dias et al. 2012; Golinska et al. 2015).

12.2 What Are Endophytic Fungi

In 1884, the word endophyte was introduced by De Barry (1884), and some early publications on the endophytic fungi were reviewed by Freeman (1904). During 1930–1990, a number of asymptomatic endophytic microorganisms were isolated from a variety of grasses and plants. These studies encouraged the isolation of these unique microorganisms for different purposes such as extraction of novel bioactive compounds too.

The endophytes are a group of microorganisms which exist inside the plants without causing any sign or symptoms. De Barry (1884) defined endophytes as "*any living form found in the cell tissue of the plant or organisms that reside in plant organs at some time in their life and can colonize into the internal plant cell, tissues without causing any harm to the host.*" Another definition of endophytes given by Hirusch et al. (1992) as "*a cluster of organisms that form colonies in the internal living tissue of plants without causing any apparent harm and negative effect.*" In the following year, Cabral et al. (1999) have defined "*endophytes are those microorganisms that isolated from internal tissues of the plant without any symptoms.*" Earlier Wilson (1993) has been described endophytes as "*the microorganisms such as fungi and bacteria which inhabitant in internal tissues of the plant for all or part of their life cycle and cause asymptomatic infections entirely within plant tissues, but remain without symptom/disease.*"

12.3 Types of Endophytic Fungi

The endophytic fungi are categorized into various classes on the sources of their relation to plant organs that belong to distinct classes like fungal endophytes from dicot, bryophytes, ferns, lichens, tree bark, etc. Stone et al. (2000) classified endophytes into distinct classes related to their plant organ as depicted in Fig. 12.1. Endophytic fungi are generally divided into two categories like Balansiaceous (grass endophytes) and non-Balansiaceous (endophytic fungi). The Balansiaceous is a class of endophytes due to their environmental and fiscal effect. These fungi

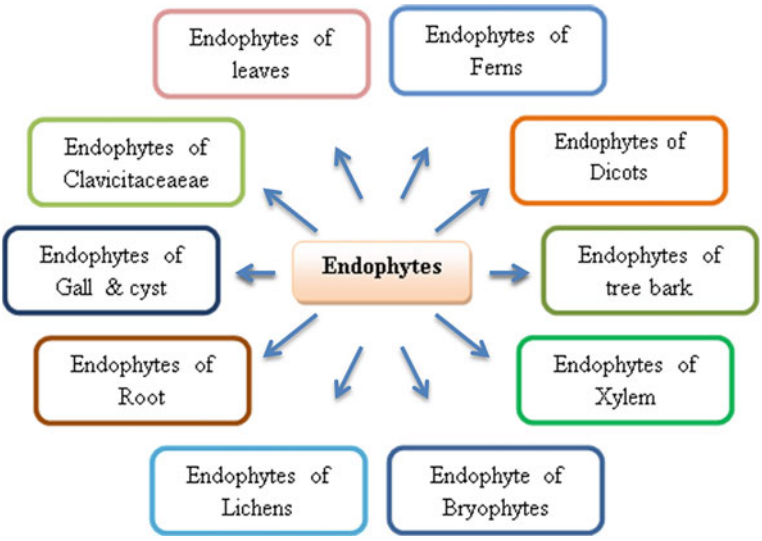


Fig. 12.1 Classification of endophytes according to Stone et al. (2000)

form a distinctive group with environmental need and their adaptation so as to separate them from the other endophytes (Petrini 1986). The classification of fungal endophytes has been elaborated in Fig. 12.1.

Endophytic fungi belong to *Clavicipitaceous* genera growing in Pasteur lands of all part of world (Schardl et al. 2004). These fungi produce a different type of secondary metabolites like poisonous compounds (alkaloids) such as anti-vertebrate alkaloids lolitrem B and ergovaline, anti-insect alkaloids—lolitrem and lolines (Schardl 2001). These provide nutrition to their host as well as provide protection against abiotic stress (Bultman and Murphy 2000). The category of non-Balansiaceous endophytes is the diverse concern to their phylogeny as well as their life cycle. Non-Balansiaceous endophytes are prominently the member of Ascomycota but the preponderance of these also belongs to a ubiquitous group of genera, e.g., *Acremonium* sp., *Alternaria* sp., *Cladosporium* sp. Most of the fungal species are common in hot, humid (tropical), and moderate climate (*Fusarium* sp., *Phomopsis* sp.) whereas *Colletotrichum guignardia* is common in the tropics (Schulz and Boyle 2005).

Rodriguez et al. (2008) has re-classified endophytic fungi on the basis of their role and location at which these have been isolated from plant materials (leaf, root, stem, bark, etc.) and arranged endophytes into four classes. The class I consisted endophytes often enhance biomass of plants, increase specificity to survive in drought tolerance condition, and secret toxic chemicals that are harmful to grazing animals. Therefore, these groups of fungi help their host to defend themselves from grazing animals and other organisms. The class II of endophytic fungi is special type endophytes that grow in both upper and underneath the ground tissues. These types of endophytes also have potential to provide habitat-specific stress tolerance to host against pH, temperature, and salinity. The class III bears endophytic fungi which are characterized on the basis of their presence mainly in mid-air tissues, straight transmission, and the pattern of exceeding localization. This class also includes hyper-diverse endophytic microorganisms associated with leaves of hot and humid trees above ground tissues of non-vascular plants, woody and herbaceous angiosperms, seedless vascular plants, and conifers. The class IV is the most important category of endophytic fungi because these endophytes can mimic as of their host plant and produce almost similar metabolites or constituents. This is demonstrative with the case of Taxol extracted from the yews and also being effective anticancer compounds. Taxol is produced by number of endophytic but maximally by *Taxomyces andreanae* associated with yews as well as other plant sources.

12.4 Endophytes and Plant Relationship

The fossilized tissue of different plants' parts has been provided strong evidence of plants–endophytes relationships (Taylor et al. 1999). Intimate and prolong relationship between plant and microbe observed as a genetic exchange among plant and microbes to transfer information inherent among both organisms. The host–endophytes relationships and their application have been summarized in Fig. 12.2.

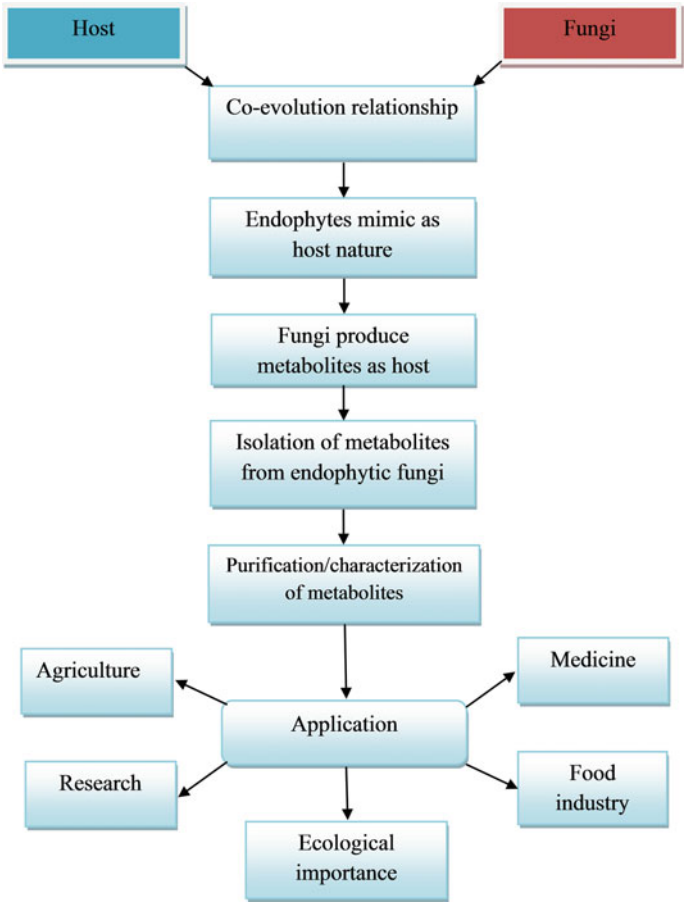


Fig. 12.2 Host–endophytes relation and their application

The exchanges of information are responsible for the adoption of surviving in adverse and favourable ecological conditions more professionally so as to increase intimacy of the association for better adaptation. Moreover, the evolutionary relationship of endophytic fungi with plants may also have allowed improved adaptation, and endophytes could help by secreting chemical substances that protect the host from pathogen and insect (Strobel 2003; Kusari and Spiteller 2012). Therefore, endophytic fungi produce a variety of bioactive compounds to give their contributions to host plant as shown in Fig. 12.2. According to the plant endophyte coevolution, endophytes may able to produce bioactive secondary metabolites which help plant in chemical defense (Carroll 1988; Li et al. 2008). Thus, provide protection, growth, and survival to their host by providing an access of substance that can also be isolated and characterized to harness their immense potential

industrial use including the area of agriculture, and medicine (Strobel 2003; Aharwal et al. 2016).

In early years, endophytic fungi *Piriformospora indica* exploited for the production of pyriform chlamydospores. *P. indica* has significant capacity to colonize in the root of the plant and enhance growth and development of host plant (Verma et al. 1998; Rai et al. 2001). In many respect, *P. indica* is similar to arbuscular mycorrhizal fungi (Rai and Verma 2005; Deshmukh et al. 2006). *P. indica* also acts as a multifunctional fungus because of its role as a biofertilizer, bioprotector, growth regulator or it can increase drought tolerance (Sun et al. 2010). The *P. indica* also plays an important role in the transportation of phosphate from fungus to host plant, through a phosphate transporter gene (PiPT); hence, it also provided a new insight for understanding the mechanism of phosphate transfer in host plants.

12.5 Why Only Endophytes?

Due to excessive deforestation and extinction of few important plant species resulted in the loss of useful preparations of medicine and drugs in pharmaceutical industries. Further, the extraction of novel compounds from plants to be utilized for pharmaceutical industry is time consuming, costly, and laborious process. Therefore, harnessing the endophytic fungi for the production of the similar bioactive compound has emerged as an alternative pathway as few genes involved in the biosynthetic pathways of production of secondary metabolite in plants also appear expressible in endophytic fungi and bacteria (Keller et al. 2005). The genetic screening methods have gained attention due to rapid, economical, and sensitive. The endophytes are biochemical factories inside the plants which secreted natural metabolites and have low toxic effect to higher organisms (Owen and Hundley 2004). These compounds bear diverse chemical structures and have often evolved to possess biological activities with roles as defensive compounds against competitors/parasites/predators, growth and reproduction facilitators or as cell signaling compounds (Vining 1990). The endophytic microorganisms provided a variety of novel bioactive metabolites with inimitable structure, synthesized via various biosynthetic pathways. The bioactive compounds isolated from these endophytic fungi not only have sensory properties, but also contained some attractive properties such as antiviral, antibacterial, antifungal, somatic fat reducing, antioxidant, blood pressure regulating, and anti-inflammatory properties indicating the pharmaceutical significance of compounds extracted from endophytes summarized in Fig. 12.3.

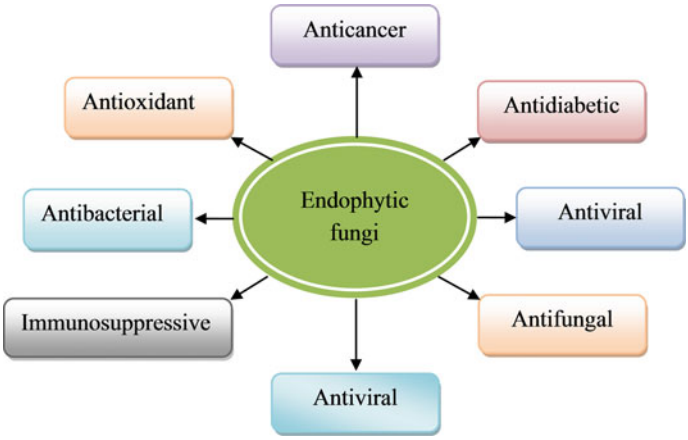


Fig. 12.3 Indicating the pharmaceutical significance of compounds extracted from endophytes

12.6 General Pathways of Synthesis of Secondary Metabolites from Endophytic Fungi

Endophytic fungi provide a variety of bioactive metabolites with unique structure, synthesized via various metabolic pathways, e.g., polyketide, isoprenoid, and amino acid derivatives (Tan and Zou 2001). These fungi have caliber to produce different types of secondary bioactive metabolites, providing opportunity to researchers for dealing with bioactive compounds of pharmaceutical significance and avenue of possible development of novel drugs (Strobel 2003). Natural products segregated into several classes evident with a plethora of microbial secondary metabolites are polyketides and non-ribosomal peptides, which are biosynthesized by polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) systems, respectively (Sauer 2002; Rakshith and Sreedharamurthy 2010). But the most of bioactive metabolites production in plants, fungi, and some bacteria occur by enzymatic pathway as shown in Fig. 12.4.

12.6.1 Non-ribosomal Polyketide Synthesis Mechanism (NRPS)

NRPS/PKS biosynthetic pathways play important roles for the synthesis of bioactive compounds in bacteria and fungi. Non-ribosomal peptide synthetase gene (NRPS gene) exists as multi-gene cluster that encodes NRP-synthetases. The NRP-synthetases with separate domains like adenylation, thiolation,

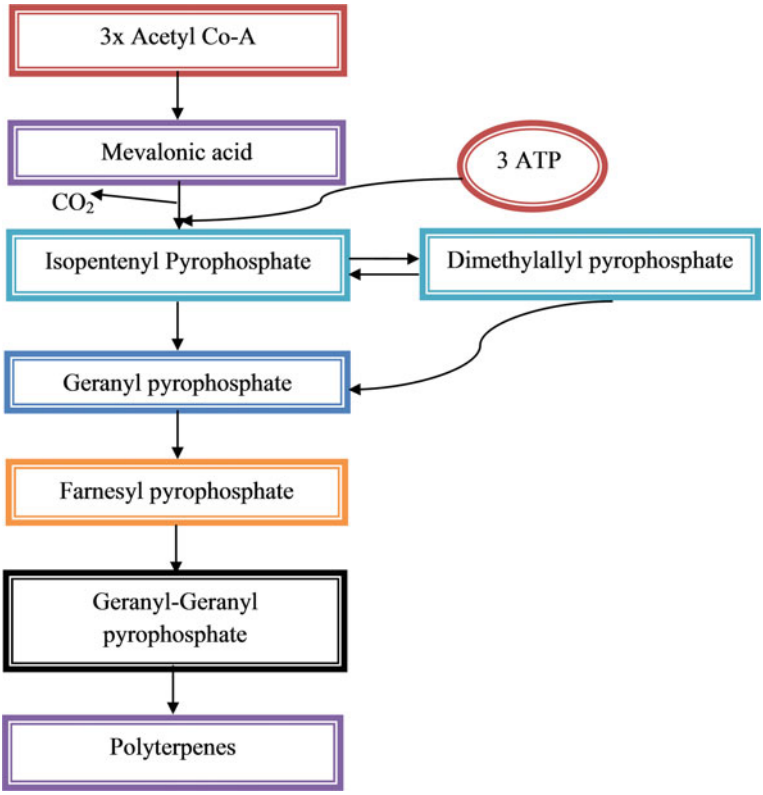


Fig. 12.4 Scheme of enzymatic pathway for secondary metabolites' production in fungi (Manitto and Sammes 1981; Dewick 1997; Hanson 2003)

[PCPPeptide Carrier Protein], and condensation domains together form a single module, e.g., NRP-synthetase encoded by *pesM* in *Aspergillus fumigatus.* These modules help in recognition and integration of an amino acid into the growing peptide product. Therefore, NRP-synthetase is usually made up of one or more module and can finish in a thioesterase—a domain that liberates freshly synthesized peptide chain from an enzyme as shown in Fig. 12.5. In addition, all fungi and bacteria NRP-synthetases involved in post-translational 4, Phospho-pantetheinylation to facilitate metabolic production. The 4, Phospho-pantetheinylation transferases [4pp-tase4,phospho-pantetheinylation transferases] catalyze the transfer of 4-Phosphopantethiene from coenzyme A to conserve serine residue within thiolation domains of NRP-synthetases to yield activated Holo NRP-synthetases. The 4pptase activated during NRPS via thioester formation and assist their movement between active site within NRP-synthetase (Fitriani and Herdiansyab 2016).

12.6.2 Polyketide Synthesis Mechanisms (PKS)

PKSs and non-ribosomal peptide synthetase (NRPSs) are large multi-modular enzymes that participate in the production of secondary metabolites from bacteria and fungi as displayed in Fig. 12.6. The natural products synthesized by bacteria and fungi have extensive functional pharmaceutical properties, viz., antibacterial, anticancer, cholesterol-lowering abilities similar to lovastatin agents. Some of the bioactive compound synthesized by PKs and NRPs mechanism in endophytic

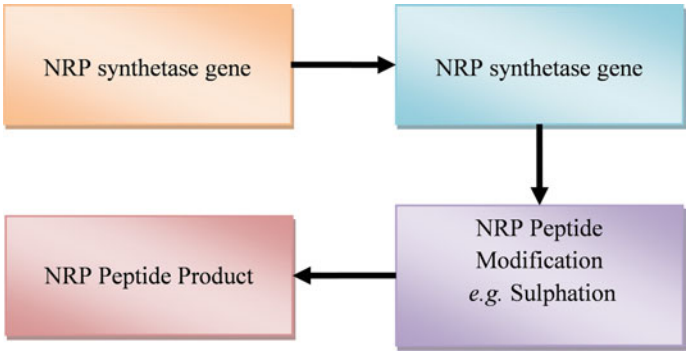


Fig. 12.5 Schematic representation of enzyme involved in NRPs system (Stack et al. 2007)

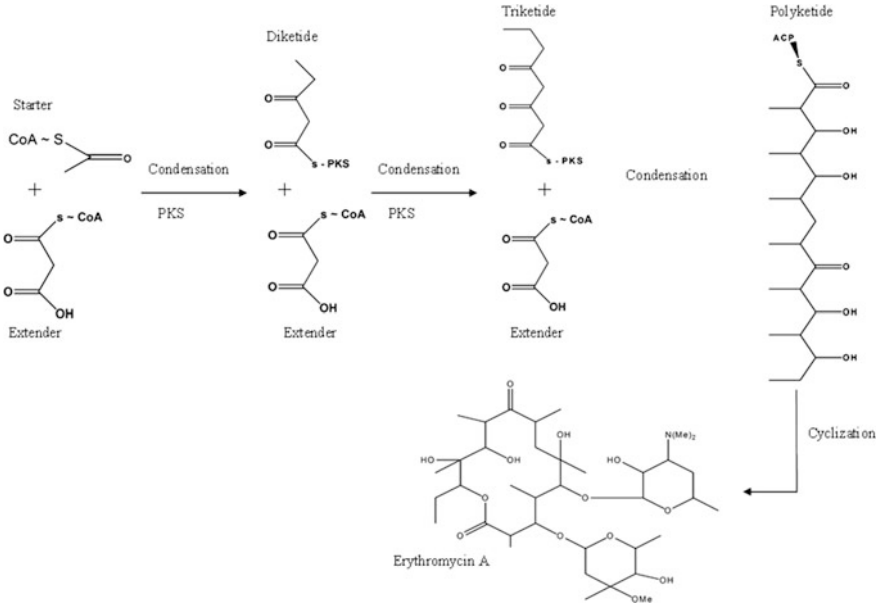


Fig. 12.6 Mechanism of polyketide biosynthesis (Hranueli et al. 2001; Sandhu et al. 2014a)

microorganisms showed negative mycotoxic properties, such as aflatoxins, ochratoxin A (OTA), fumonisin, and patulin. These enzymes have vast strategies to form a diverse array of compounds similar to carboxylic and amino acid building blocks (Finking and Marahiel 2004; Hertweck et al. 2009). The endophytic fungal polyketides comprise a verity of bioactive metabolites that play vital function for drug discovery. A lot of polyketide mycotoxins are produced by fungi using PKS system like aflatoxin (Hertweck et al. 2007), fumonisin (Hoffmeister and Keller 2007), zearalenone (Schumann and Hertweck 2006), and the 6-methylsalicylic acid (Smith 2007) derived patulin (Cronan and Thomas 2009). In PKS, the monomers of acyl-CoA thioesters (acetyl-CoA, malonyl-CoA, methyl malonyl-CoA) are derived from the primary metabolites of microbes. The NRPS monomers consist of proteinogenic and non-proteinogenic amino acids and also have carboxylic acids (Fischbach and Walsh 2006). Polyketide synthases can be divided into three types like PKSI, PKSII, and PKSIII, which have similar enzymatic abilities, but differ in their quaternary structures.

PKSI containing large enzyme have multiple functional domains act only one time during the biosynthesis, and types II comprised many single-module proteins with different enzymatic actions for polyketide production. The PKSIII enzyme has single active site that employs to form the final product. It does not include an acyl-carrier protein (ACPacyl-carrier protein) domain and acts as a homodimer. The PKSIII are related to plants also there in bacteria and fungi.

The modules of polyketide synthase have three domains: β-ketosynthase (KS), acyl-transferase (AT), and ACP domains. The first, KS domain attaches to malonyl-CoA extender unit with acetyl-CoA starter molecule. The second, acyl-transferase domains carry accurate substrate onto the enzyme. Ultimately, third, ACP domain is responsible for the proper movement of substrates and products between the different active sites of the enzyme. These domains are used in the elongation of the polyketide chain at each catalytic step.

The elongated polyketide chain further undergoes β-keto processing arbitrate by β-ketoreductase (KR), dehydratase (DH), and trans-acting enoyl (ER) domains (Schwarzer and Marahiel 2001; Staunton and Weissman 2001). Further, fungal PKSs divided into non-reducing (NR) and highly reduced (HR) PKS, on the basis of availability of these domains like when KR or DH domains will present (Cox and Simpson 2009). Mechanism of polyketide biosynthesis has been summarized in Fig. 12.6 (Hranueli et al. 2001; Sandhu et al. 2014a).

12.7 Collection and Isolation Techniques for Endophytic Fungi

The isolation of endophytic fungal strains from the plants and their parts (leaves, bark, roots, fruits, flowers, stems, etc.) is the sources for the isolation of endophytes. The plant samples must be collected in a sterilized polyethylene bags always and

processed within a proper time after sampling. Generally, fresh and clean plant materials should be used for the isolation of endophytes to decrease the chances of contagion. The plant parts must wash in running tap water to eliminate the dirt and debris (Petrini 1986; Radu and Kqueen 2003). After proper washing, explants will be further processed via surface disinfection under aseptic conditions. Surface disinfection is an essential method by which the exterior surface of the explants is disinfected to ensure that all isolated fungi are endophytic (Schulz et al. 1993). General route for isolation and purification of bioactive metabolites from endophytic fungi is summarized in Fig. 12.7.

After washing plant parts, small pieces of 2–4 mm should cut with the help of sterilized blade or by using cork borer and placed in sterilized water for 1–2 min and dip into sodium hypo-chlorite solution (4%) for 2 min following in 70% ethanol for 1 min for disinfection purposes. The samples are rinsed with sterilized water and dried out on a sterilized filter paper later by. Extremely sterilized conditions should be maintained during isolation of endophytic fungi from the plant's parts. After disinfection of the plant's parts, they are placed on prepared Potato Dextrose Agar plate (PDA) supplemented with an antibiotic to inhibit the growth of bacteria and incubated at $26 \pm 1^{\circ}C$ for 6–7 days for hyphal growth observation. Pure colonies of endophytic fungi appeared from the edge of the plant segments can be placed on another PDA plates. Pure cultures are to be maintained on the PDA slant without supplementing any antibiotic (Rubini et al. 2005). Some other method can also be used for isolation of endophytic fungi from the explants as depicted in Table 12.1.

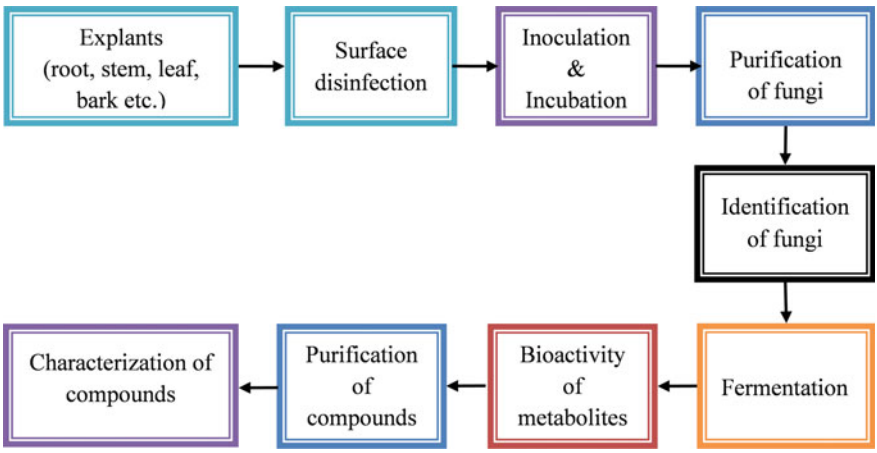


Fig. 12.7 General route for isolation and purification of bioactive metabolites from endophytic fungi

Table 12.1 Show methods of isolation of endophytic fungi

Washing	Rinse with ethanol solution	Surface disinfection	Rinsed with ethanol solution	Rinsed in sterile distilled water	Incubation days, temperature	References
Running tap water (RTW)	70%, 1–2 min	2 min in NaOCl	70%	Twice	6–7, 26 ± 1°C	Rubini etal. (2005)
Water & detergent and the explant dried on the sterile filter paper	70%, 1 min	15%, 1 min in hydrogen peroxide solution	70%, 1 min.	Twice	5–7, 27°C	Guo et al. (2000)
RTW	75%, 1 min	6%, 3 or 5 In NaOCl	75%, 0.5 Min	Three times	5–10, 25°C	Raviraja et al. (1996)
RTW (1 h)	70%, 1 min	2 min in 4% NaOCl	70%, 1 Min	Twice	7 days, 26 ± 1°C	Sandhu et al. (2014b)

12.8 Identification of Endophytic Fungi

Morphological characters still remain to define features for many fungal groups even though some characters can have one or more alternative characteristics. The morphological characteristics of a fungus are often too limited for unquestionable identifications. Molecular systematic of fungi has recently increased the understanding of the taxonomic groupings and evolutionary histories within different groups of fungi. Therefore, in the present era, both morphological and molecular techniques in couple are using for the proper identification and characterization of the fungi. The morphological characterization of fungi carried out by documenting the colony, color, growth rate, texture shape, size of spore, etc., (Agarwal and Hasija 1980; Domasch et al. 2007; Shan et al. 2012).

12.9 Molecular Identification of the Fungi

For molecular characterization of endophytic microorganisms, the total genomic DNA is to be isolated from the organism by using various DNA extraction methods like CTAB and LETS (Lithium chloride EDTA Tris HCL) methods (Sandhu 2010). In the LETS method, a loop full of conidia inoculate in 100 mL conical flask containing Potato Dextrose Broth (PDB) and incubate for 5–7 days in a fungal incubator at 26 ± 1°C. Thereafter, the mycelia are to be harvested, washed with

distilled water, lyophilized by liquid nitrogen, and crushed in a motor-pastel by adding 0.7 ml extraction buffer (LETS [0.1 M LiCl, 10 Mm EDTA, 10 Mm HCL], pH 8 and 0.5% SDS). Following to this, it is to be centrifuged and 1 mL (PCI) phenol: chloroform: iso-amyl alcohol (25:24:1) added, vortexed for 1 min at medium speed. Further centrifugation is to be carried out at 5000 rpm for 5 min so as to get aqueous layer and transferred to the other sterilized tube. After, it is must to add 1 mL 100% chilled ethanol and put on dry ice for 15 min and spin for 10–15 min in a micro-centrifuge at 4 °C, remove supernatant and dry the pellet. The dry pellet is then placed in TAE buffer or nuclease-free water for future use and stored in the refrigerator. For confirmation of isolated DNA, agarose gel electrophoresis can be performed as displayed in Fig. 12.7.

12.9.1 Agarose Gel Electrophoresis

Agarose gel electrophoresis is a most effective method for separating DNA molecules/fragments from the mixture sample of varying sizes. The percentages of agarose gel used for separation of genomic DNA depend upon its size, generally 0.8–1.0% agarose used for separation. The buffer used in agarose gel electrophoresis is TAE or TBE. TBE has better buffering capacity, i.e., gel can run faster or longer without overheating but TAE is cheaper and better for isolation of DNA/fragment (Shan et al. 2012).

The quantification of the isolated DNA can be obtained by UV-VIS spectrophotometer. For detection of desired gene and molecular sequencing of the fungal DNA, band can be purified, and DNA fragment can be amplified by suitable primers using PCR. Table 12.2 showed the general preparation of the sample for PCR (Cui et al. 2016). AmplifiedDNA band of fungi in agarose gel electrophoresis (Fig. 12.7).

Table 12.2 PCR mixture for 20 μL sample

PCR mixture	Stock concentration	Volume (μL)
Nuclease-free water	10X	10.75
$MgCl_2$	1.5 mM	2.0
dNTPs	2.5 mM	2.0
Forward primers	10 pmol/μL	2.0
Reverse primers	10 pmol/μL	2.0
Taq polymerase	5 units/μL	0.25
DNA templates	50 ng	1.0
	Total	20.0

12.10 General Parameters Uses for Maximum Production of Bioactive Compounds

Various favorable physical and chemical factors like temperature, incubation period, pH, carbon and nitrogen sources, and salinity concentration may play determining role in the maximum production of antibacterial secondary metabolites from the microorganisms. Therefore, different parameters can be used for the maximum production of antibacterial bioactive compounds by the fungal strain.

12.10.1 Growth Media

To evaluate the suitable media for maximum production of bioactive compounds by fungal strain can be cultivated into different media (natural, semi-synthetic, synthetic), viz., Sabouraud's dextrose broth (SDB), Richard's broth (RB), Potato dextrose broth (PDB), Czapek dox broth (CB), Muller and Hinton broth (M&HB), Malt extract broth (MEB), and Asthana and Hawkers broth (A&H) (Zain et al. 2009; Kiranmayi et al. 2011).

12.10.2 Incubation Period

It is also very necessary to determine incubation period that provides information when the productions of bioactive compounds are initiated and when it is stopped. For this growth, media is prepared in clean and dry flasks and poured the media in flasks and autoclave at 121°C. After autoclaving, the flasks are inoculating with fungal culture and incubating at 26 ± 1°C for specific incubation. On the 1st day of incubation, the crude broth of one flask is to be tested for the presence of the bioactive compound. In the case of antibacterial activity, the agar well diffusion method or disc diffusion method can be used to scrutinize the antibacterial activity of the bioactive compounds against the test bacterial strains by measuring zone of inhibition (Egorov 1995; Sandey et al. 2015).

12.10.3 Biomass Production

For the observation of biomass accumulations, drying the mycelia mat from the 1st day of incubation by filtering through pre-weighed filter paper to remove the medium. The filter paper along with mycelium is air-dried followed by drying in an oven at 60 ± 1°C till constant weight is obtained and expressed as mg/mL (Sandhu 1989; Sandhu et al. 2014a, b).

12.10.4 Carbon and Nitrogen Sources

Carbon and nitrogen sources play important role in the production of novel bioactive compounds from the microorganisms. Therefore, different carbon source like glucose, sucrose, mannitol, lactose, mannose, fructose, dextrose, maltose, etc., are used for optimization of maximum production of bioactive metabolites. Similarly, nitrogen sources like asparagines, yeast extract, glycine, peptone, tryptone, potassium nitrate, ammonium nitrate, ammonium chloride sodium nitrate, etc., affect the growth and synthesis of bioactive compounds from the endophytic fungi (Singh and Mukhopadhyay 2012).

12.10.5 pH

pH plays an important role in fungal growth and metabolites production. It exerts an indirect effect on cellular metabolism through a change in chemical environment. As the fungi grow, the pH of the growth media is altered, therefore; it is very difficult to study hydrogen ion concentration in the environment of the fungi. However, pH affects the enzyme activity, mineral availability, and membrane function of the cell (Rubini et al. 2005). Hydrogen ion concentration influences the enzymatic action in fungi by modifying surface area and permeability by facilitating or preventing the entry of various substances like vitamins, organic acids, and mineral into the fungal cell. In order to obtain optimal results, steady pH is needed during fermentation. Similarly, the incubation temperature also influences growth and development of any microorganism, and it affects the physiology and synthesis of various bioactive metabolites (Lilly and Barnett 1951). For maximum production of bioactive metabolites from the microorganism, it is very important to provide suitable temperature or incubating then in a suitable external factor for their growth development and production of useful secondary metabolites.

12.10.6 Salinity Concentration

The effect of NaCl concentration on endophytic fungal growth and metabolite production was enumerated by incubating fungal strain in a different range of NaCl concentrations (1% to 10%/L) in basal medium amended with carbon and nitrogen source, respectively, at the same time keeping rest of the conditions at optimum level (Merlin et al. 2013). The bioactive compound production for each NaCl concentration can be estimated to its optimum.

12.10.7 Shelf Life of the Bioactive Compounds

During the optimization, the shelf life of the bioactive compounds can also be observed by storing CFCF at 4-5°C for a specific duration of time (24 h, 1 week, 1–12 months, respectively). Following appropriate storage period metabolites from vial could be taken and evaluated for the bioactivity.

12.11 Large-Scale Production of Bioactive Metabolites from Endophytic Fungi

After optimization, all the necessary parameters required for maximum antibacterial production on large scale from the endophytic fungi are carried out in the bioreactor. Every physical and chemical parameter, like temperature pH, media, carbon and nitrogen sources, dissolved oxygen, is optimized in the bioreactor. After the proper calibration of the fermentor, specific medium (SDB) is to be poured into the fermentation vessel carbon and nitrogen source and other auxiliary factors such as temperature, DO_2, an antifoaming agent, pH (Haider et al. 2009). The fermentation medium inoculated with conidial suspension of the fungus that is to be prepared by washing the mycelia discs of 6–7 days old culture in sterile distilled water, and fermentation must carry out for requiring specific days.

After fermentation, broth should be filtered with the help of Whatman filter paper no. 1 and centrifuged in a cooling centrifuge to remove the cell debris. The separated supernatant further can be selected for purification of bioactive compound by using standard techniques like solvent-solvent extraction, column chromatography, and thin layer chromatography (TLC), HPLC, NMR, LC-MS for separation and purification of bioactive compounds (Yin et al. 2009; Kumar et al. 2013; Jouda et al. 2016; Zhang et al. 2016).

12.12 Role of Endophytic Fungi

In the current scenario, attention on plant study has augmented over the world, and an enormous number of plants are known to have the potential for pharmaceutical value. Pharmaceutical biology perceives plants as a unique source of potentially precious remedial compounds. Plants appear to be a pool of innumerable numbers of endophytic organisms like bacteria, actinobacteria, and fungi that play an imperative role in the production of a variety of bioactive compounds for the treatment of variety of diseases (Jalgaonwala et al. 2011; Premjanu and Jayanthy 2012). Throughout the long era of mutual interactions between the endophytes and the host plant, a friendly association was steadily set up among the organisms. The host plants may provide benefits of nourishment and habitation to endophytic fungi,

whereas, in return, endophytic fungi produce a number of secondary metabolites that give protection to host plants from external biotic and abiotic factors. Some endophytic fungi could be used as a vector for incorporation of foreign genes into host due to their host specificity (Clay 1988).

Endophytic fungi are valuable resource of novel bioactive compoundsalkaloids which include alkaloids, amines, amides, indole derivatives, isocoumarin derivatives, pyrrolizidines, steroids, terpenoids, sesquiterpenes, diterpenes, quinines, lignans, peptides, phenolic acids, aliphatic compounds, chlorinated metabolites benzopyranones, chinones, cytochalasines, depsipeptides, enniatines, furandiones, isocumarines, peptides, polyketones, flavonoids, phenyl propanoids, phenols and quinols, etc., (Tan and Zou 2001; Gunatilaka 2006; Tenguria et al. 2011). Therefore, the novel bioactive compound isolating from endophytic fungi can be used in the field of agriculture and pharmaceutical industries etc.

12.12.1 Endophytic Fungi Metabolites as Antibacterial Agents

Recently, among the microorganisms, fungi have been accepted as one of best resources for new active bioactive compounds (Samuel et al. 2011). Penicillin was the first and most important discovery which provides to have an effective action against Gram positive bacteria (Demain and Sanchez 2009). The crude extract of *Aspergillus ochraceus* and *Penicillium citrinum* showed wide spectral antibacterial properties, inhibiting developing germs, especially *Pseudomonas aeruginosa*. The hypericin (Fig. 12.8), a naphthodianthrone derived compound, and Emodin ($C_{15}H_{10}O_5$) thought to be the main precursor for synthesis of hypericin, in an endophytic fungus isolated from medicinal plant, have an antimicrobial activity against a number of bacteria and fungi, like *Staphylococcus* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteric*, *Escherichia coli* and fungal organisms *Aspergillus niger* and *Candida albicans* (Kusari et al. 2012).

Three steroids, namely 5α,8α-epidioxyergosta-6, ergosta-5,7, 22-trienol, 22-dien-3β-ol, ergosta-7, 22-dien-3β,5α,6β-triol, and one triterpenoid helvolic acid, were separated from *Pichia guilliermondii* an endophytic fungal strain from *Paris polyphylla var. Yunnanensis* showing the strongest antibacterial activity against all test bacteria (Jianglin et al. 2010).

12.12.2 Endophytic Fungi Metabolites as Antiviral Agents

Another interesting aspect is the utilization of secondary metabolite from endophytic fungi for inhibiting the growth of viruses. It is evident that the possible isolation of antiviral compounds from endophytes is under progress, though some

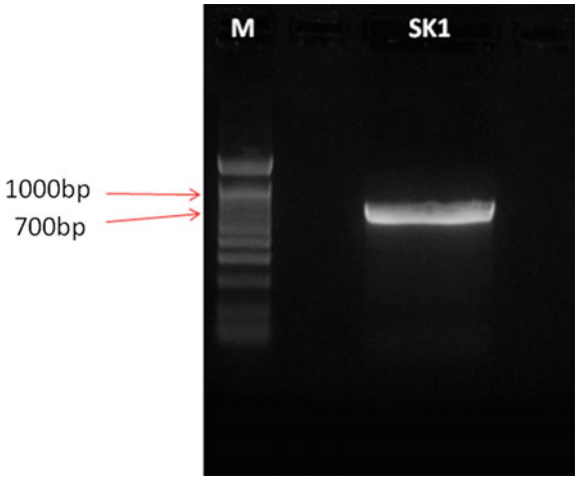


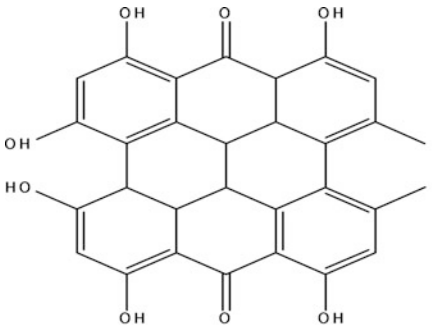
Fig. 12.8 Amplified DNA band of fungi in agarose gel electrophoresis

promising bioactive compounds have been discovered. The emergence of resistance and multi-resistance against accessible medicine, adverse effect and high price tag of current therapies, as well as HIV/AIDS epidemic and AIDS-associated opportunistic infection, such as cytomegalovirus and polyoma virus, made the development of novel antiviral drugs a central priority. Cytonic acid A and B are accounts as human cytomegalovirus protease inhibitors isolated from the endophytic fungus *Cytonaema* sp. obtained from *Quercus* sp. (Guo et al. 2008). During the course of experimentation by Fukami et al. (2000), on fungus *Trichoderma atroviride* FKI-3849, they find two new anti-influenza viral agents wickerol A and B diterpene compounds with a novel fused 6-5-6-6 ring skeleton.

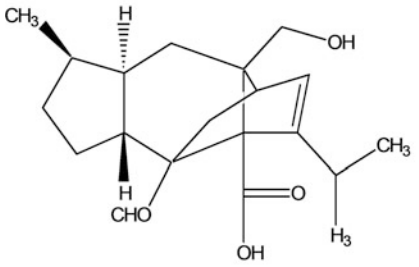
The antiviral compound wickerol A isolated from *T. atroviride* FKI-3737 fungi (Obuchi et al. 1990) showed an effective antiviral action against the A/H1N1 flu virus (A/PR/8/34 and A/WSN/33 strains) and provides an opportunity of being lead compounds to make easy development of novel anti-influenza, antiviral drugs with novel structure. The fungal strain *Pestalotiopsis theae is* obtained from an unidentified tree from Jianfeng mountain, and Chinese were capable of producing Pestalotheol C (Fig. 12.9) with anti-HIV properties (Li et al. 2008). Chemical structure of antiviral compound is isolated from endophytic fungi (Fig. 12.10).

12.12.3 Endophytic Fungi Metabolites as Anticancer Agents

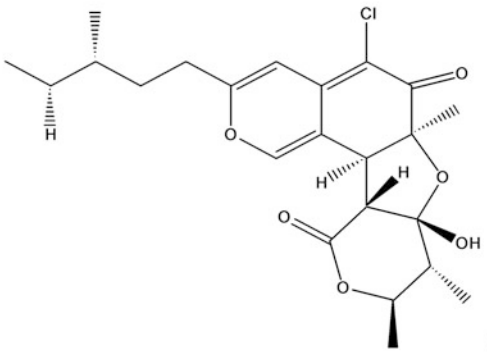
Cancer is a group of diseases describes by uncontrolled growth of a cell that loses the properties of density and anchorage dependant in the case of a tumor, contact inhibition, and failed to go for apoptosis that causes death in an organism (Pimentel et al. 2010). Evidence is present about anticancer secondary metabolites isolated from endophytic fungal isolates and could be a substitutional approach for



Hypericin



Soradcirin



Chaetomugilin D

Fig. 12.9 Chemical structure of some antibacterial compounds isolated from endophytic fungi

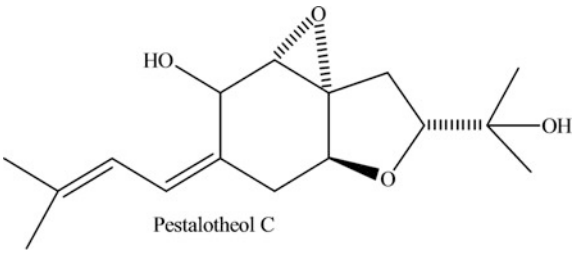


Fig. 12.10 Chemical structure of antiviral compound isolated from endophytic fungi

improvement of novel drugs from plants, microorganisms, and marine sources (Firakova et al. 2007). The anticancer activity of bioactive compounds obtained from endophytes has been investigated (Qi et al. 2009). The first anticancer agent produced by endophytes was Taxol (Fig. 12.10) and its derivatives. Taxol is a highly functionalized diterpenoid, isolated from yew *Taxus* species (Bacon and White 1994). The novel bioactive metabolites' Taxol provoke polymerization of microtubule during the progression of cell division (Tan and Zou 2001).

The anticancer drugs isolated from endophytic fungi are Camptothecin have potent anti-neoplastic agent separate from *Camptotheca acuminata* Decaisne (Nyssaceae) from China (Wall et al. 1966). For the synthesis of anticancer drugs, topotecan, and irinotecan, Camptothecin and 10-hydroxycamptothecin are two major precursors (Uma et al. 2008). Another compound Secalonic acid D, a mycotoxin belong to class ergochrome, also has strong anticancer activity isolated from a mangrove endophytic fungal strain (Bills et al. 1996; Qi et al. 2009). Chemical structure of anticancer compounds of endophytic fungi origin has been summarized in Fig. 12.11.

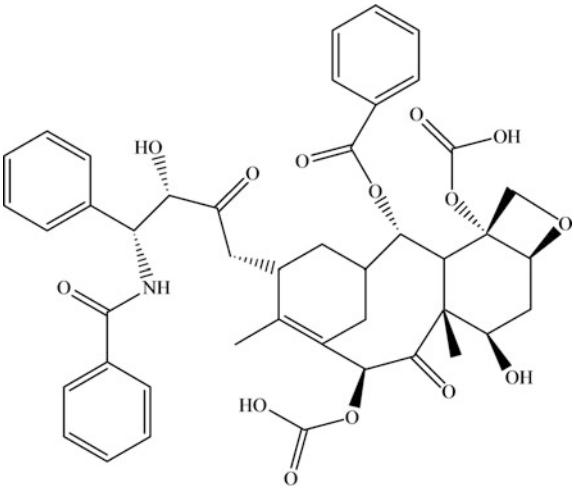


Fig. 12.11 Chemical structure of anticancer compounds isolated from endophytic fungi

12.12.4 Endophytic Fungi Metabolites as Antifungal Agents

The endophytic fungi provide a wide diversity of antifungal metabolic compound which plays an important role against a number of pathogenic fungi. Altomare et al. (2000) and co-worker isolated two alpha pyrones antifungal compounds named as fusapyrone and deoxyfusapyrone from *Fusarium semitectum* of high potential against a number of pathogenic or mycotoxogenic filamentous fungi like *Alternaria alternata, Aspergillus flavus, Botrytis cinerea, Cladosporum cucumerinum, Phoma tracheiphila,* and *Penicilllium verrucosum. Candida albicans*, *Cryptococcus neoformans,* and *Aspergillus fumigatus* are the major pathogenic fungi which cause disease in human beings. *Streptomyces* sp. produces bioactive compound polyenes which have a broad spectrum activity against *Aspergillus* sp., *Candida* sp., etc., (Hay 2003). Amphotericin B, nystatin, and natamycin are main polyenes which are extensively used for the cure of diseases like coccidiodal meningitis, cutaneous dermatophytes, and histoplasmosis and in the treatment of mycotic disease (Gupte et al. 2002; Iznaga et al. 2004; Gohel et al. 2006).

Recently, Wu et al. (2015) isolated the two new antifungal and cytotoxic component (4S,6S)-6-[(1S,2R)-1, 2-dihydroxybutyl]- 4-hydroxy-4-methoxytetrahydro-2H-pyran-2-one (1), (6S,2E)-6-hydroxy-3-methoxy-5-oxodec-2-enoic acid (2), and other three compounds, LL-P880 (3), LL-P880 (4), and Ergosta-5,7,22-trien-3b-ol (5) from the secondary metabolites of *Dendrobium officinale*. The results of the investigation indicated compounds 1-4 display prominent antifungal properties against the tested microbes which comprise *Cryptococcus neoformans, Candida albicans, Aspergillus fumigatus* and *Trichophyton rubrum*.

12.12.5 Endophytic Fungi Metabolites as Anti-diabetic Agents

Diabetes mellitus (DM) or simply diabetes is a very common disorder in the present situation due to blemish in insulin secretion and action of this hormone produced by the beta cell of the liver. The deficiency of insulin, in turn, causes a high level of sugar in the blood (hyperglycemia) that affects the metabolism of carbohydrate, fat, and protein. Severe diabetic snags such as retinopathy, neuropathy, nephropathy, cardiovascular complications, and ulceration occur during diabetics. Thus, diabetes covers a wide range of heterogeneous diseases (Bastaki 2005). Therefore, a large number of medicine and drugs are prepared from different biological sources to control this type of chronic disease. Endophytic microbe's ability to produce bioactive compounds in common with its host plants is an opportunity to get source material anti-diabetic drugs from them (Dompeipen et al. 2011).

The α-glucosidation inhibitors isolated from the endophytic fungi are mainly widespread oral agents used to decrease postprandial hyperglycemia (Hanefeld and Schaper 2007). However, some natural products isolated from a range of medicinal

plants and microorganisms have potential as α-glucosidase inhibitors (Suthindhiran et al. 2009; Elya et al. 2012). Similarly, isolation and characterization of α-glucosidase anti-diabetic bioactive compound of endophytic fungi from *Swietenia macrophylla* were done by Ramadanis et al. (2012). In African forest, a non-peptide fungal metabolite was isolated from *Pseudomassaria* sp. These compounds act as insulin and not get destroyed in the digestive tract; therefore, it can be taken orally and gave significant result in two-mouse model by lowering of blood glucose which leads to the development of new remedies for the treatment of diabetes (Zhang et al. 2006).

12.12.6 Endophytic Fungi Metabolites as Antioxidant Agents

Endophytic fungi play an important role to produce valuable antioxidant bioactive compounds. Theantana et al. (2011) isolated thirty-nine fungi from five Thai medicinal plants, and these fungi produced phenolic compounds. Phenolic compounds are very important antioxidant compounds and having very high reducing power. From the thirty-nine fungi, *Eupenicillium shearii* CMU18 showed the maximum amount of phenolic compound, $ABTS^{+}$ radical scavenging effect and have very high reductional potential and lipid peroxidation inhibition activity in rat liver tissue. The *Paraconiothyrium* sp. was isolated from the leaves of *Rheedia brasiliensis* showed good antioxidant properties. The crude extract of *Paraconiothyrium* sp. has the competence to prevent cell growth of human keratinocytes immortalized and also acts against psoriasis by reducing free radical (Carvalho et al. 2012).

12.12.7 Endophytic Fungi Metabolites as Insecticidal Agents

Endophytic fungi play a significant role in the formation of insecticidal compounds which are very effective against a number of insects-pest causing serious crop damage. An endophytic fungus *Nodulisporis* sp. isolated from *Bonita daphnoides* which produce noduliosporic acid and indole diterpenes which exhibit potential insecticidal activities against the caterpillars of blowfly (Demain 2000). In another study, *Muscodar vitigenus* secluded from *Paullina paullinioides which* produce naphthalene acts as a strong insect repellant. Two new biopesticide compounds also isolated from endophytic fungus *Gaultheria procumbens* 5-hydroxy-2-(1-hydroxy-5-methyl-4-hexenyl) benzofuran and 5-hydroxy-2-(1-oxo-5-methyl-4-hexenyl) benzofuran. These compounds exhibit high toxicity against spruce budworm and its

larvae (Findlay et al. 1997). *M. vitigenus* shows an insect inhibitor and also showed insect repellant activity against the wheat stem sawfly (Daisy et al. 2002).

Recently, *Claviceps purpure* and *Claviceps chaetomium* have been isolated from *Achnatherum inebriansin* in China which shows the evidence for insecticidal action against cotton aphis (Zhang et al. 2010). Earlier, Miles et al. (1998) isolated endophytic fungi from *Neotyphodium* sp. that produces N-formilonine and a paxiline analogous in the host *Echinopogum ovatus*. These bioactive elements contained removing action against *Listronotus bonariensis* and other insects. Endophytic fungi such as *Fusarium oxysporum* protect tomatoes from root knot disease caused by Meloidogyne incognata (Hallman and Sikora 1994) and endophytic fungi isolated from banana plant of Central America control the burrowing nematode Rhadopholus similis (Pocasangre et al. 2000). In another study, terpenes isolated from *Copaifera* sp. also showed in vitro antiparasitic and synergic activity (Izumi et al. 2012). Two insecticidal Azadirachtins A and B extracted from the endophytic fungi *Penicillium* (Eupenicillium) *parvum* from the neem plant (*Azadirachta indica*) showed insecticidal activity (Kusari et al. 2012).

12.13 Conclusions

In the present situation, there is an urge to investigate bioactive compounds from the natural sources for the treatment of ailments and work also against multi-drug resistance microbes. Therefore, several alternative strategies have been adapted to isolate the bioactive compounds from the natural sources. Indeed, in recent years, there are great achievements in the production of metabolically active compounds from endophytic fungi. These organisms have tremendous sources of metabolically active compounds that may be used in pharmaceutical, medical, agriculture, and industries. Endophytic fungus offers a broad variety of secondary metabolites with their unique structures like flavonoids, terpenoids, alkaloids, phenolic acid, etc. Such bioactive metabolites find wide range of application against infectious diseases, autoimmune, enteric, cardiovascular, and other diseases. With all the aspects of the Phyto-biology, the mutual relationship between endophytic fungi and their hosts may be investigated. Scientists are able to obtain more information about host-plant relationships which will be very valuable in the exploration of novel bioactive compounds for sustainable management of environment.

References

Aggarwal GP, Hasija SK (1980) Microorganism in the laboratory. In: A laboratory guide of mycology, microbiology and plant pathology. Ravi printers, Jabalpur India

Aharwal RP, Kumar S, Sandhu SS (2016) Endophytic mycoflora as a source of biotherapeutic compounds for disease treatment. J App Pharm Sci 6(10):242–254

Altomare C, Perrone G, Zonno MC, Evidente A, Pengue R, Fanti F et al (2000) Biological characterization of fusapyrone and deoxyfusapyrone two bioactive secondary metabolites of *Fusarium semitectum*. J Nat Prod 63(8):1131–1135

Bacon CW, White JF (1994) Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton

Bastaki S (2005) Review: diabetes mellitus Mappersons and its treatment. Int J Diab Metabol 13:111–134

Bills GF (1996) Isolation and analysis of endophytic fungal communities from woody plants. In: Erdlin SC, Carris LM (eds) Endophytic fungi in grasses and woody plants. APS Press, St Paul

Bram RJ, Hung DT, Martin PK, Schreiber SL, Crabtree GR (1993) Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcineurin binding and cellular location. Mol Cell Biol 13:4760–4769

Bultman TL, Murphy JC (2000) Do fungal endophytes mediate wound-induced resistance? microbial endophytes. Marcel Dekker, New York, pp 421–452

Cabral D, Cafaro MJ, Saidman B, Lugo M, Reddy PV, White JF Jr (1999) Evidence supporting the occurrence of a new species of endophyte in some South American grasses. Mycologia 91:315–325

Carroll GC (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69:2–9

Carvalho PLND, Oliveira PA, Gois-Ruiz ALT, Alencar SMD, Pfenning LH, Carvalho JED et al (2012) Paraconiothyrium sp. P83F4/1: antioxidant and antiproliferative activities an endophytic fungus associated with *Rheedia brasiliensis* plant. Int J Biotech Well Indus 1:172–176

Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69(1):10–16

Cox RJ, Simpson TJ (2009) Complex enzymes in microbial natural product biosynthesis, part B: Polyketides, aminocoumarins and carbohydrates. Chapter 3: Fungal type I polyketide synthases. Methods Enzymol 459:49–98

Cronan JE, Thomas J (2009) Bacterial fatty acid synthesis and its relationships with polyketide synthetic pathways. Methods Enzymol 459:395–433

Cui J, Guo T, Chao J, Wang M (2016) Potential of endophytic fungi *Phialocephala fortinii* Rac56 in *Rhodiola* plant to produce Salidroside and *p*-Tyrosol. Molecule 21(502):2–13

Daisy BH, Strobel GA, Castillo U, Ezra D, Sears J, Weaver DK (2002) Naphthalene an insect repellent is produced by *Muscodor vitigenus* a novel endophytic fungus. Microbiol 148:3737–3741

De Bary HA (1884) Vergleichende Morphologie und Biologie der Pilze Mycetozoen und Bacterien. Verlag von Wilhelm Engelmann, Leipzig

Demain AL (2000) Microbial natural products: a past with a future. In: Wrigley SK, Hayes MA, Thomas R, Chrystal EJT, Nicholson N (eds) Biodiversity: new leads for pharmaceutical and agrochemical industries. The Royal Society of Chemistry, Cambridge, UK

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. The J Antibiot 62:5–16

Deshmukh S, Huckelhoven R, Schafer P (2006) The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc Nat Acad Sci 49:18450–18457

Dewick PM (1997) Medicinal natural products: a biosynthetic approach. Wiley, New York

Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2(2):303–336

Dompeipen EJ, Srikandace Y, Suharso WP, Cahyana H, Simanjuntak P (2011) Potential endohytic microbes' selection for anti-diabetic bioactive compounds production. Asia J Biochem 6 (6):465–471

Domsch KH, Gamas W, Anderson TH (1980) Compendium of soil fungi. Academic press, New York, pp 168–169

Domsch KH, Gams W, Anderson TH (2007). Compendium of soil fungi. (2nd eds) IHW—Verlag, Eching

Egorov N (1995) Microorganisms- antagonists and biological methods for evaluation of antibiotic activity. Vissha shkola, Moskva, p 200

Elya B, Katrin, Munim A, Yuliastuti W, Bangun A, Kurnia SE (2012) Screening of α-glucosidase inhibitory activity from some plants of apocynaceae, clusiaceae, euphorbiaceae,and rubiaceae. J Bio med Biotechnol, 1–6

Findlay JA, Buthelezi S, Li G, Seveck M, Miller JD (1997) Insect toxins from an endophytic fungus from Wintergreen. J Nat Prod 60:1214–1215

Finking R, Marahiel MA (2004) Biosynthesis of nonribosomal peptides. Annu Rev Microbiol 58:453–488

Firakova S, Sturdikova M, Muckova M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia 62(3):251–257

Fischbach MA, Walsh CT (2006) Assembly-line enzymology for polyketide and nonribosomal peptide antibiotics: Logic, machinery, and mechanisms. Chem Rev 106:3468–3496

Fitriani A, Herdiansyah SA (2016) Detection of Nonribosomal peptide synthetase (NRPS) genes on bacterial endophytes from *Vetiveria zizanioides* L. and *Ageratum conyzoides* L. Int J Pharm Sci Rev Res 36(1):124–128

Freeman EM (1904) The seed fungus of *Lolium temulentum* L. Phil Trans R Soc Lond (Biol) 196:1–27

Fukami A, Nakamura T, Kim YP, Shiomi K, Hayashi M, Nagai T et al (2000) A new anti-influenza virus antibiotic-10-norparvulenone from *Microsphaeropsis* sp. FO-5050. J Antibiotics 53:1215–1218

Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS (2006) Bio-prospecting and antifungal potential of chitinolytic microorganisms. Afric J Biotechnol 5:54–72

Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek 108(2):267–289

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):505–526

Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* (Palmae) using morphological and molecular techniques. New Phytol 147:617–630

Guo B, Wang Y, Sun X, Tang K (2008) Bioactive Natural Products from endophytes: a review. Appl Biochem Microbiol 44:136–142

Gupte M, Kulkarni P, Ganguli BN (2002) Antifungal antibiotics. Appl Microbiol Biotechnol 58:46–57

Haider M, Hamzah AH, Ali HG (2009) Physiological regulation of protease and antibiotics in *Penicillium* sp. using submerged and solid state fermentation techniques. J Engi Sci Technol 4 (1):81–89

Hallman J, Sikora RA (1994) Influence of *Fusarium oxysporum*, a mutualistic fungal endophyte on *Meloidogyne incognita* infection of tomato. J Plant Disease Prot 101:475–481

Hanefeld M, Schaper F (2007) The role of Alpha glucosidase inhibitors (acarbose). In: Mogensen CE (ed) Pharmacotherapy of diabetes: new developments improving life and prognosis for diabetic patient. Springer Science-Business Media, New York

Hanson JR (2003) The classes of natural product and their isolation. In: Natural products: the secondary metabolites. The Royal Society of Chemistry

Hay RJ (2003) Antifungal drugs used for systemic mycoses. Dermatol Clin 21:577–587

Hertweck C, Luzhetskyy A, Rebets Y, Bechthold A (2007) Type II polyketide synthases: gaining a deeper insight into enzymatic teamwork. Nat Prod Rep 24:162–190

Hirsch G, Braun U (1992) Communities of parasitic microfungi. In: Winterhoff W (ed) Handbook of vegetative science, Volume 19. Fungi in vegetation science. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 225–250

Hoffmeister D, Keller NP (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep 24:393–416

Hranueli D, Peric N, Borovicka B (2001) Molecular biology of polyketide biosynthesis. Food Biotechnol 39(3):203–213

Iznaga Y, Lemus M, González L, Garmendía L, Nadal L, Vallin C (2004) Antifungal activity of Actinomycetes from Cuban soils. Phytother Res 18:494–496

Izumi E, Ueda-Nakamura T, Veiga VF Jr, Pinto AC, Nakamura CV (2012) Terpenes from Copaifera demonstrated in vitro antiparasitic and synergic activity. J Med Chem 55(7):2994–3001

Jalgaonwala RE, Vishwas B, Raghunath M, Mahajan T (2011) A review: Natural products from plant associated endophytic fungi. J Microbiol Biotechnol Res 1(2):21–32

Ji HF, Li XJ, Zhang HY (2009) Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Rep 10:194–200

Jianglin Y, Mou Y, Shan T, Li L, Zhou L, Wang M et al (2010) Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla* var. *yunnanensis*. Molecules 15:7961–7970

Jouda JB, Fopossib JLD, Mbazoaa CD, Wandji J (2016) Antibacterial activity of the major compound of an endophytic fungus isolated from *Garcinia preussii*. J Appl Pharma Sci 6 (6):026–029

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism from biochemistry to genomics. Nat Rev Microbiol 3:937–947

Kiranmayi MU, Sudhakar P, Sreenivasulu K, Vijayalakshmi M (2011) Optimization of culturing conditions for improved production of bioactive metabolites by *Pseudonocardia* sp. VUK-10. Mycobiol 39(3):174–181

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus *Fusarium oxysporum* isolated from *Catharanthus roseus*. PloS One, 16;8(9):e71805

Kusari S, Spiteller M (2012) Metabolomics of endophytic fungi producing associated plant secondary metabolites: progress, challenges and opportunities. In: Roessner U (ed) Metabolomics. Rijeka, Croatia, InTech, pp 241–266

Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012) An endophytic fungus from *Azadirachta indica* A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28(3):1287–1294

Li E, Jiang L, Guo L, Zhang H, Che Y (2008) Pestalachlorides A-C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. Bioorg Med Chem 16:7894–7899

Lilly VG, Barnett HL (1951) Physiology of fungi. Mc Graw Hill Book Co. Inc., New York, Toronto, London

Manitto P, Sammes PG (1981) Biosynthesis of natural products. Ellis Horwood Ltd. Mao XB, Eksriwong T, Chauvatcharin S and Zhong JJ (2005). Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*. Process Biochem, 40(5):1667–1672

Merlin JN, Nimal IVS, Christhudas KP, Agastian P (2013) Optimization of growth and bioactive metabolite production from *Fusarium solani*. Asia J Pharma Clin Res 6(3):98–103

Miles CO, Mena ME, Jacobs SWL, Garthwaite I, Lane GA, Prestidge RA et al (1998) Endophytic fungi in indigenous Australasian grasses associated with toxicity to livestock. Appl Env Microbiol 64:601–606

Obuchi T, Kondoh H, Omura S, Yang JS, Liang XT (1990) Armillaric acid, a new antibiotic produced by *Armillaria mellea*. Plants Med 56:198–201

Owen NL, Hundley N (2004) Endophytes - The chemical synthesizers inside plants. Sci Prog 87 (2):79–99

Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Fokkema NJ, van den Heuvel J (eds) Microbiology of phyllosphere. Cambridge University Press, Cambridge, UK.

Pimentel MR, Molina G, Dionısio AP, Junior RM, Pastore GM (2010) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Intract, 1–11

Pocasangre L, Sikora RA, Vilich V, Schuster RP (2000) Survey of banana endophytic fungi from Central America and screening for biological control of the burrowing nematode (*Radopholus simili*). Info Musa 9:3–5

Premjanu N, Jayanthy C (2012) Endophytic fungi a repository of bioactive compounds: a review. Int J Institutional Pharm Life Sci 2(1):135–162

Qi SH, Xu Y, Xiong HR, Qian PY, Zhang S (2009) Antifouling and antibacterial compounds from a marine fungus *Cladosporium* sp. F14. World J Microbio Biotechnol 25:399–406

Radu S, Kqueen CY (2003) Preliminary screening of endophytic fungi from medicinal plants in Malaysia for antimicrobial and antitumour activity. Malaysia J Med Sci 93:23–33

Rai MK, Verma A (2005) Arbuscular mycorrhiza-like Biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. Elec J Biotechnol 8:1–4

Rai MK, Acharya D, Singh A, Varma A (2001) Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania sonmifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza 11:123–128

Rakshith D, Sreedharamurthy S (2010) Endophytic fungi: 'Trapped' or 'hidden' store houses of bioactive compounds within plants. A Review. J Pharm Res 3(12):2986–2989

Ramadanis R, Soemiati A, Munim A (2012) Isolation and glucosidase inhibitory activity of endophytic fungi from Mahogany (*Swietenia macrophylla* King) seeds. Int J Med Aroma Plants 2(3):447–452

Raviraja NS, Sridhar KR, Barlocher F (1996) Endophytic aquatic hyphomycetes of roots of plantation crops and ferns from India. Sydowia 48:152–160

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F et al (2008) Stress tolerance in plants via habitat adapted symbiosis. ISME J 2(4):404–416

Rubini MR, Silva-Ribeiro RT, Pomella AWV, Maki CS, Araujo WL, Santos DR et al (2005) Diversity of endophytic fungal community of Cacao (*Teobroma cacao* L.) and biological control of *Crinipellis perniciosa* causal agent of witches broom disease. Int J Biol Sci 1:24–33

Samuel P, Prince L, Prabakaran A (2011) Antibacterial Activity of marine derived fungi collected from South East Coast of Tamilnadu. India. J Microbiol Biotechnol Res 1(4):86–94

Sandey K, Aharwal RP, Kumar S, Sandhu SS (2015) Production and optimization of antibacterial metabolites from endophytic fungi *Nigrospora* sp.ML#3. J Appl Pharm Sci, 5(11):031–037

Sandhu SS (1989) Epizootiological studies on *Beauveria bassiana* parasitizing *Heliothis armigera* Hub. Pod borer of gram (*Cicer arietinum* Linn.) PhD thesis, R.D. University, Jabalpur (M.P.) India

Sandhu SS (2010) Recombinanat DNA technology. I.K. International Publishing house Pvt. Ltd., New Delhi India

Sandhu SS, Aharwal RP, Kumar S (2014a) Isolation and antibacterial property of endophytic fungi isolated from Indian medicinal plant *Calotropis procera* (Linn.) R. Br. World J Pharm Pharm Sci 3(5):678–691

Sandhu SS, Kumar S, Aharwal RP, Shukla H, Rajak RC (2014b) Endophytic fungi: as a source of antimicrobials bioactive compounds. World J Pharma Pharma Sci 3(2):1179–1797

Sauer M (2002) Estimating polyketide metabolic potential among nonsporulating fungal endophytes of *Vaccinium macrocarpon*. Mycol Res 106:460–470

Schardl CL (2001) Epichloë festucae and related mutualistic symbionts of grasses. Fungal Gen Biol 33:69–82

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbiosis of grasses with seed-borne fungal endophytes. Ann Rev Plant Biol 55:315–340

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109(6):661–686

Schulz B, Wanke U, Draeger S, Aust HJ (1993) Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol Res 97:1447–1450

Schumann J, Hertweck C (2006) Advances in cloning, functional analysis and heterologous expression of fungal polyketide synthase genes. J Biotechnol 124(4):690–703

Schwarzer D, Marahiel MA (2001) Multimodular biocatalysts for natural product assembly. Naturwissenschaften 88:93–101

Shan T, Lou J, Gao S, Zhou Y, Sun W, Luo C, Zhou L (2012) Antibacterial activity of the endophytic fungi from a traditional Chinese herb *Paris polyphylla* var. *chinensis*. Afr. J Microbiol 6(14):3440–3446

Singh AK, Mukhopadhyay M (2012) Overview of fungal lipase: a review. Appl Biochem Biotechn 166:486–520

Smith S, Tsai SC (2007) The type I fatty acid and polyketide synthases: a tale of two megasynthases. Nat Prod Rep 24:1041–1072

Stack D, Neville C, Doyle S (2007) Non-ribosomal peptide synthesis in *Aspergillus fumigatus* and other fungi. Microbiol 153:1297–1306

Staunton J, Weissman KJ (2001) Polyketide biosynthesis: a millennium review. Nat Prod Rep 18:380–416

Stone K, Bacon EW, White F (2000) An Overview of endophytic microbes: endophytism defined. In: Bacon EW, White F (eds) Microbial endophytes. Marcel Dekker Inc., New York, Basel, pp 3–29

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mole Biol Rev 67:491–502

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167 (12):1009–1017

Suthindhiran KR, Jayasri MA, Kannabiran K (2009) A–Glucosidase and α–amylase inhibitory activity of *Micromonospora* sp. VITSDK3 (EU551238). Int J Integrative Biol 6(3):115–120

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Taylor J, Hyde K, Jones E (1999) Endophytic fungi associated with the temperate palm, *Trachycarpus fortune*, within and outside its natural geographic range. New Phytol 142:335–346

Tenguria RK, Khan FN, Quereshi S (2011) Endophytes - Mines of pharmacological therapeutics. World J Sci Tech 1(5):127–149

Theantana T, Kanjanapothi D, Lumyong S (2011) *in vitro* inhibition of lipid peroxidation and the antioxidant system of endophytic fungi from Thai Medicinal Plants. Chiang Mai J Sci 39 (3):429–444

Uma SR, Ramesha BT, Ravikanth G, Rajesh PG, Vasudeva R, Ganeshaiah KN (2008) Chemical profiling of *N. nimmoniana* for camptothecin, an important anticancer alkaloid: towards the development of a sustainable production system. In: Ramawat KG, Merillion J (eds) Bioactive molecules and medicinal plants. Springer, Berlin

Verdine GL (1996) The combinatorial chemistry of nature. Nature 7(384):11–13

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A et al (1998) *Piriformospora indica* a new root-colonizing fungus. Mycologia 90:896–903

Vining LC (1990) Functions of secondary metabolites. Rev Microbiol 44:427

Wall ME, Wani MC, Cook CE, Palmer KH, Mcphail AT, Sim GA (1966) Plant antitumor agents; the isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88(16):3888–3890

Wilson D (1993) Fungal endophytes: out of sight but should not to be our mind. Oikos 68:379–384

Wu Yougen, Girmy S, Silva VMD, Perry B, Hu X, Tan GT (2015) The role of endophytic fungus in the anticancer activity of *Morrind citrifolia* Linn. (Noni). Evid Based Complement Alternat Med, 1–8

Yin H, Zhao Q, Sun FM, An T (2009) Gentiopicrin-producing endophytic fungus isolated from *Gentiana macrophylla*. Phytomed 16:793–797

Zain ME, Razak AA, El-Sheikh HH, Soliman HG, Khalil AM (2009) Influence of growth medium on diagnostic characters of *Aspergillus* and *Penicillium* species. Afr J Microbiol Res 3(5):280–286

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zhang X, Shi Y, Wang X, Zhang W, Lou K (2010) Isolation, Identification and insecticidal activity of endophyte from *Achnatherum inebrians*. Wei Sheng Wu Xue Bao 50(4):530–536

Zhang H, Sun X, Xu C (2016) Antimicrobial activity of endophytic fungus *Fusarium* sp. isolated from medicinal Honey suckles plant. Archiv Biol Sci Belgrade 68(1):25–30

Chapter 13
Conclusion

Dinesh K. Maheshwari

Abstract Endophytes are intimate associates of plant those help them as plant-growth-promoting rhizobacteria. This chapter exclusively concluded the horizon covered in this book content, exploring the current advancement in biology and biotechnology of endophytes.

Keywords Agroecosytem · Holobiont · Agroeconomy · Plant-endophyte interaction

This book contains current knowledge about endophytic bacteria, fungi and actinobacteria, mycorrhiza and their occurrence, distribution, diversity for the benefits of plants. Their invasion and interaction with crops attained for sustainable agroecosystem. Information is given about lower and higher plant genera for delivering novel endophytes for new drug, or bioactive molecules are derived for agrochemical development.

The major emphasis has been laid down on promising role of endophytes for green technology and genomic analysis to understand endophytic bacteria for evolving knowledge of the plant holobiome. Holobiont (host and its associated micro-organisms) plays a vital role in plant microbe interaction processes. The endophytic microbial communities are closely associated with plant tissues; the associated organisms affect host physiology and performance suggesting co-evolution of both. A small number of taxa, i.e. microbial hub, consist of strongly interconnected taxa having several effects on community, and specific attention is required to understand the function of host-associated microbiomes (Ciancio et al. 2016).

Endophytes being utilized for intensification of sustainable agriculture as eco-friendly natural resources for novel bioactive compounds. Their functional role to mitigate the impact of climate change, particularly in vineyards affected by encroaching desertification and soil salinization described for functional role of host-associated microbes. The plants bear endophytic flora that grows faster than

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2_13

those devoid of endophytes. It is interesting to note that endophytes affect the nutrient and fibre contents in certain plant varieties (Agler et al. 2016).

Ecologically, some endophytic microbes could not be isolated on artificial culture media. This might be due to their obligate parasitic nature to the host tissues. For example, in *Chrysopogon zizanioides,* i.e. vetiver, the beneficial nature of the plant is due to the presence of oil canal in root. The microbial community of vetiver root and its involvement in its biogenesis is reviewed by Del Giudice et al. (2008), but the native microbes associated with plant tissue is yet to be studied. Traditional methods are not universal for the isolation and identification of fungi. For some reasons, many endophytic fungi cannot cultivate and hence remain non-culturable on artificial culture media. For such cases, the metagenomic approach may analyse the endophytes and can provide additional information to determine the microbial community of endophytes. Molecular approaches have been recommended in the identification of non-culturable organisms. Research is to be employed for the identification of endophytic fungi for their 5.8s gene and flanking internal transcribed spaces (ITS_1, and ITS_2) of the rDNA, 18s and 28s rRNA genes.

High-throughput sequencing served as molecular tool to study wide range of mycorrhizal fungi (Dumbrell et al. 2011). Emergence of DNA barcoding system where ITS region is considered as the most widely used DNA barcode molecular identification has some limitation in species distinction (Sun and Gao 2012). A bottleneck understanding of endophytic microbeal-plant interaction is limited. The local environment determines the assembly of root endophytic fungi (Soto-Barajas et al. 2016). This requires a thorough knowledge of microbiology and plant physiology. Modern technique and tools with knowledge of both become a new area for future research.

Endophytes not only a source of potential metabolites but, also play key role in specific microbial processing in improving phyto-extraction efficiency (Štursová et al. 2016). For the production of bioactive compounds, screening of endophyte is essential to identify the marker gene or enzyme because some specific genes involved in the synthesis of bioactive molecule found to be negative for their involvement in biosynthesis as reviewed by Vasundhara et al. (2016).

Harnessing useful endophytic micro-organism for deleterious phyto-pathogen and pest control is evident from the available the literature in different chapters. Application of endophytic microbes may provide a new insight to agroeconomy, if it is simply designed to apply as formulary product. Wider scope is possible if the bioformulation is available for their performances in the field for broad range of crops for the development of microbial inoculant preparations. Product development and application can be derived similar to that of plant growth-promoting bacteria (Maheshwari 2015). More emphasis may now be given on the contribution of secondary metabolites from endophyte production in the success of bioformulations as stated by Morel et al. (2016). This book will be useful for microbiologist, plant pathologist, physiologist, agronomist, environmentalist and those making biotechnological applications of microbial products and consequently for over all significance.

References

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoSBiol 14(1):e1002352

Ciancio A, Pieterse CM, Mercado-Blanco J (2016) Editorial: Harnessing useful rhizosphere microorganisms for pathogen and pest biocontrol. Front Microbiol 7:1620

Del Giudice L, Massardo DR, Pontieri P, Bertea CM, Mombello D, Carata E, De Stefano M (2008) The microbial community of *Vetiver* root and its involvement into essential oil biogenesis. Environ Microbiol 10(10):2824–2841

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 190(3):794–804

Maheshwari DK (2015) Bacterial diversity in sustainable agriculture. Springer, Netherlands

Morel MA, Cagide C, Castro-Sowinski S (2016) The contribution of secondary metabolites in the success of bioformulations. In: Arora NK et al (eds) Bioformulations: for Sustainable Agriculture. Springer, India, pp 235–250

Soto-Barajas MC, Zabalgogeazcoa I, Gómez-Fuertes J, González-Blanco V, Vázquez-de-Aldana BR (2016) Epichloë endophytes affect the nutrient and fiber content of *Lolium perenne* regardless of plant genotype. Plant Soil 1–13

Šturšová M, Bárta J, Šantrůčková H, Baldrian P (2016) Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. FEMS Microbiol Ecol 92(12):fiw185

Sun X, Guo LD (2012) Endophytic fungal diversity: review of traditional and molecular techniques. Mycology 3(1):65–76

Vasundhara M, Kumar A, Reddy MS (2016) Molecular approaches to screen bioactive compounds from endophytic fungi. Front Microbiol doi.org/10.3389/fmicro 2016.017774

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D.K. Maheshwari (ed.), *Endophytes: Biology and Biotechnology*,
Sustainable Development and Biodiversity 15, DOI 10.1007/978-3-319-66541-2