4
Microbiology Monographs

Series Editor: Alexander Steinbüchel

Predatory Prokaryotes

Biology, Ecology and Evolution

Volume Editor: Edouard Jurkevitch

With 53 Figures, 1 in Color

Springer

Volume Editor:

Dr. Edouard Jurkevitch
Department of Plant Pathology and Microbiology
Faculty of Agricultural, Food and Environmental
Quality Sciences
76100 Rehovot
Israel
e-mail: jurkevi@agri.huji.ac.il

Series Editor:

Professor Dr. Alexander Steinbüchel
Institut für Molekulare Mikrobiologie und Biotechnologie
Westfälische Wilhelms-Universität
Corrensstraße 3
48149 Münster
Germany
e-mail: steinbu@uni-muenster.de

Library of Congress Control Number: 2006931569

ISSN 1862-5576
ISBN-10 3-540-38577-0 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-38577-6 Springer Berlin Heidelberg New York
DOI 10.1007/978-3-540-38582-0

Editor: Dr. Christina Eckey, Heidelberg
Desk Editor: Dr. Jutta Lindenborn, Heidelberg
Cover Design: WMXDesign GmbH, Heidelberg
Typesetting and Production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig

Printed on acid-free paper 02/3100 YL – 5 4 3 2 1 0

Preface

Few biologists, including microbiologists, are aware of the existence of predatory bacteria. However, the reaction of these scientists when they learn about them, whether through casual talks or through exposure at meetings, is invariably: "cool!!" In modern dialect, this is really a good score.

The aim of this monograph is to increase the awareness of the biologists at large about the great possibilities that predation between microbes offer for research and teaching. One can also view this aim within a trend we fully support and think should be strengthened: microbial systems are excellent models for studying and discussing basic ecological and general biological concepts. We won't deliberate over the reasons, as excellent and up-to-date reviews are available, but we hope that a short description of the chapters contained in this book will convince the reader that bacterial predators, their ecology, and their biology at large form an especially appealing field.

While the variety of known predatory bacteria may already appear to be substantial, we are convinced that only the surface of this diversity has been scratched. Moreover, one should not take the term "known" at face value as most of these bacterial predators have only been anecdotally described. Bacterial predators may even be much more widespread than presently accounted for owing to the fact that only a tiny fraction of bacteria occurring in natural habitats can be cultivated in the laboratory. The discovery of new bacterial predators cannot be performed solely by relying upon culture-independent approaches as no one particular molecular signature will account for all predatory phenotypes. The exploration of the diversity of the voracious smalls should also be based on observation, enrichment or isolation whenever possible. The study of this diversity and of its phylogenetic roots can bring forward evolutionary insights that are pertinent to seemingly unrelated fields such as biological polymers degradation and the evolution of the eukaryotic cell. This is treated in the chapter by Edouard Jurkevitch and Yaacov Davidov.

The dynamics of trophic interactions between bacteria is very much a black box. However, they can be addressed in a systematic way, thanks to the inherent possibilities of manipulating variables in microbial systems in an efficient, precise, and reproducible manner. Such studies can bear a large impact on our understanding of the central role of predation in ecology. Microbial predatory systems can be utilized to test essential ecological questions pertaining

to predation, such as the role of spatial structure, the presence of decoy or of multiple species (prey or predator) on predator-prey interactions, and how predation may lead to speciation. In order to address such questions, mathematical frameworks can be developed to define the variables to be tested. Michael Wilkinson presents in his chapter such models in a clear and readily understandable manner.

Klaus Jürgens's chapter provides a thorough background on predation in the microbial world at large and emerging "rules" of bacterial predation. His chapter addresses the impact of predation in microbial systems at various scales, from bacteriophages to metazoans. Bacterial predation is further analyzed in the larger context of trophic cascades and ecological networks. Protists are treated in depth and the larger body of knowledge in this domain is used as a background against which the lesser understanding of predation in the prokaryotic realm is evaluated. This knowledge and understanding is an important resource to draw upon for comparison and inspiration.

Laboratory work with *Bdellovibrio* and like organisms (BALOs), the bacteria most studied for their predatory behavior, presents challenges to the investigator. As wild type strains can only grow in the presence of a host, isolation and enumeration of BALOs from natural samples are not straightforward tasks. Susan Koval addresses these issues as well as other important subjects related to the analysis of isolates in her chapter. The chapter also covers culture-independent approaches, such as fluorescent in situ hybridization as they are applied to BALOs. This chapter should prove of great help to the microbiologist wishing to engage in work with predatory bacteria as well as to the more experienced "BALOlogist."

BALOs are ubiquitously found in nature or at least in most of the environments in which they were looked for. Since oceans cover about 70% of the Earth's surface, the marine milieu is the largest of all environments and also the one that Henry Williams has been studying for three decades. Together with Silvia Piñeiro, he presents a very comprehensive and in-depth review of the ecology of BALOs in aquatic as well as in terrestrial habitats. They ask what may be the central, yet unanswered questions in BALO ecology, i.e. what is the impact of BALOs in bacterial mortality, which bacterial groups constitue prey organisms and how are environmental processes affected. While these are true challenges for the microbial ecologist, the authors suggest that the tools being developed and implemented in BALO research will greatly enhance our ability to answer these basic questions.

Among the most powerful instruments in the biologist's toolbox are genomics and bioinformatics. John Tudor and Michael McCann reexamined the first published BALO genome, that of *Bdellovibrio bacteriovorus* type strain 100, a terrestrial bacterium. They present us new analyses of chemotactic, regulatory, and sensory circuits of the predator, as well as a reevaluation of the amino acid biosynthetic capabilities of this organism. They also provide the first comparative analysis of different BALO genomes using the published

genome data available on the marine BALO *Bacteriovorax marinus* SJ and on the cyst-forming *Bdellovibrio* sp. strain W. These, along with data from proteomic studies are examined within the frame of the unusual life cycle of BALOs, shedding new light on the predators' developmental phases.

Eckard Strauch, Sebastian Beck, and Bernd Appel describe the peculiar biochemistry of BALO cell walls with the presence of sphingolipids in *Bacteriovorax stolpii*, a sugar-linked LPS, and a new family of outer membrane proteins. They address the importance of chemotaxis, locomotion, and attachment appendages in the predatory process and review the literature pertaining to intracellular regulatory signals. They link BALO biochemistry to the life cycle and to the ecology of the predators and examine the potential uses of BALOs or derived compounds as therapeutic agents, weighing the potential uses against the potential hurdles.

We hope the readers of this book will marvel at the intricacies of the biology of microbial predators and at how much of the natural sciences are "packed" in these small cells. We expect it will keep in them the feeling that these tiny predators are indeed "really cool."

We would like to again thank the contributing authors for their dedication to this project and for their enthusiasm from its very beginning.

Finally, I (Edouard Jurkevitch) would like to thank my wife Einat and my children Yaniv, Yoav and Maya for the smiles on their faces when I "talk bdellovibrio" with them, and my mother and late father for the sweet gift of education.

Rehovot and Münster, June 2006

Edouard Jurkevitch
Alexander Steinbüchel

Contents

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_051/Published online: 11 October 2006

A Brief History of Short Bacteria: A Chronicle of *Bdellovibrio* (and Like Organisms) Research

Edouard Jurkevitch

Department of Plant Pathology and Microbiology,
and the Otto Warburg Center for Agricultural Biotechnology,
Faculty of Agricultural, Food and Environmental Quality Sciences,
The Hebrew University of Jerusalem, 76100 Rehovot, Israel
jurkevi@agri.huji.ac.il

Abstract Like many good things in science (and in life at large, starting with evolutionary processes), the obligate predatory bacteria *Bdellovibrio* and like organisms (BALOs) were discovered by chance. These fascinating creatures have since been studied by (not too) many great scientists. As the community studying these organisms has never been too large, small changes in its number of scientists have had a large impact on the advancement of this field. A historical perspective of BALO research is presented here.

1 Introduction

Predation is pervasive at all levels of life and maybe as old as life, or cellular life, itself (Maynard Smith and Szathmáry 1995; Bengston 2002). From the tiniest viruses that parasitize and finally lyze their bacterial hosts to the largest of the sharks, it is found in all walks of life, and possibly in all environments.

Predators kill their prey. Predation is a significant cause of mortality, an important evolutionary force, driving the selection of escape strategies in prey and of effectiveness in predators. Predation is such a basic tenet of life that it is strongly embedded in the human psyche; it has been used to describe economic processes (predatory pricing), asocial, criminal behaviors (sexually violent predator), or political developments (predatory democracy).

This central role played by predation in nature is reflected in the great interest it is generating among the scientific community. The scientific literature abounds with articles and books dealing with this matter but a simple search through a few databases reveals that the amount of work performed on predation in the various fields of ecology differ greatly.

Table 1 Numbers of entries in the PubMed and GoogleScholar databases relevant to predation in predator and prey systems of vertebrates, arthropods, and microorganisms

Search engine	Keywords						
	Predation + mammals	Mammals	Predation + arthropods	Arthropods	Predation + Bacteria	Predation + Bacteria (-protozoa, protozoan)	Bacteria
PubMed	1127	11 328 519	952	165 401	207		969 292
Google Scholar	21 100	451 000	7370	59 200	10 500	261	1 170 000

As seen from Table 1, no rule can be formulated as to the relationship of the number of studies centered on predation and the size of the subject organisms, be they predator or prey. However, one thing appears to be clear: a dearth of work on predatory interactions within the prokaryotic realm.

Predation between bacteria has been known for a long time (Beebe 1941, and probably earlier) but the described interactions were of a facultative nature. Mostly, myxobacterial systems have served as "role models" for this type of interaction. However, because of the peculiar and fascinating social behavior exhibited by these bacteria, predation usually took the back seat of research priorities in these systems. Nevertheless, the lytic activities of myxobacteria and other facultative predators have been thoroughly investigated and some data pertaining to the ecological significance of predation by these organisms is available (see chapter by Jurkevitch and Davidov in this volume).

Another class of predatory bacteria are the obligate predators. Although this book is not solely dedicated to these organisms, they form its central theme. I shall therefore present a short history of the discovery and development of the research centered on *Bdellovibrio*, or according to present designation, the *Bdellovibrio* and like organisms (BALOs).

2 Historical Perspective

In 1963, Moshe Shilo, from the Hebrew University of Jerusalem was spending a sabbatical in Berkeley at Roger Stanier's laboratory, working on endotoxic properties of bacterial lipopolysaccharides. During the very same year, Heinz Stolp was also in California as a postdoctoral fellow, staying with Mortimer Starr in UC Davis.

A year earlier, Stolp had described small, fast-swimming gram negative bacteria, obligate predators of other gram negative cells (Stolp and Petzold 1962). At that time, Heinz Stolp (today a professor Emeritus of the Uni-

versity of Bayreuth) was working in Berlin at the Institut für Bakteriologie, developing lyzotyping methods for pseudomonads. In a particular experiment designed to isolate bacteriophages of the phytopathogen *Pseudomonas syringae* pv. *phaseolicola* from a soil suspension, he ran short of filters, and instead used sintered glass filters. The following day, no lytic plaques were apparent in the top agar, so the plates should have been discarded. However, they were not, and when reexamined two days later, plaques had developed (also see Stolp 1973). Then, "just because the belated generation of the plaque spoke against the existence of phage activity, the cause of this lysis was further inspected" (Stolp 1968). What Heinz Stolp saw were rapidly moving, tiny bacteria that attached to the substrate cell, and finally, lyzed them. Hence, they were named *Bdellovibrio bacteriovorus*, the name describing the morphology and the supposed way of life of the bacteria; they were curved and seemed to stick to their prey and to absorb the prey cell content, reminiscent of a leech ("bdella" in Greek). The term was coined by Robert E. Buchanan, a noted taxonomist and Professor at Iowa State College of Agriculture and Mechanic Art. Had the required filters been available, their cut-off size (0.2 μm) would not have enabled the *Bdellovibrio* cells (0.25 – 0.5 × 0.75–2 μm) to pass, but the sintered glass (1.35 μm) allowed their passage. Moreover, had the negative plates been discarded ...*Dans les champs de l'observation le hasard ne favorise que les esprits préparés* (In the fields of observation, chance only favors the prepared mind – Louis Pasteur, lecturing in at the Université de Lille, December 7, 1854).

Back to Davis, 1963. Stolp and Starr thoroughly investigated the newly discovered organism, describing its morphology, providing first insights into the dynamics of predation and isolating saprophytic host-independent mutants. They remarked that isolates vary in prey (always gram negative) range, that prey bacteria surviving predation do not appear to be mutants, and that since *Bdellovibrio* could be recovered in many natural habitats, it probably was an integral component of the microbial flora (Stolp and Starr 1963). These results are as pertinent today as when they were first published. However, *Bdellovibrio* was thought to remain extracellular and was therefore called an ectoparasite. The term ectoparasite rather than exoparasite was used to distinguish it from a parasite that does not require a continuous contact with the prey. Starr and Baigent (1966) later described host penetration and the intraperiplasmic nature of the predator.

Moshe Shilo went on a visit to Davis and "met the bdellovibrios". From his correspondence, he seems to have been fascinated, and rapidly started to work on the subject along with Barbara Bruff, a student in Stanier's lab. By the summer of 1963, they had developed an efficient protocol for the recovery of host-independent mutants, which were then used to demonstrate the presence of enzymatic activities able to lyze dead prey cells (Shilo and Bruff 1965). Excited about these new and peculiar bacteria, Shilo wrote from Berkeley to Mazal Varon, who had just terminated her M.Sc., and proposed that she

takes up this project and study *Bdellovibrio* as a part of her Ph.D program in his laboratory in Jerusalem. Although microbial ecology was his main field of research, Shilo had an all-encompassing interest in microbiology, as reflected in his work with *Bdellovibrio*. Within the next 15 years or so, Shilo and Varon (first as a student, and then as a researcher), together and independently, contributed enormously to the field. They, and this is not a exhaustive listing, studied the attachment and penetration of *Bdellovibrio* to its prey (Varon and Shilo 1968, 1969a), followed the dynamics of the predatory interaction and deduced mathematical models (Varon and Shilo 1969b; Varon and Ziegler 1978; Varon et al. 1984), and examined various aspects of the physiology of invaded prey cells and of host-independent mutants (Varon and Seijffers 1975; Eksztejn and Varon 1977). They isolated phages active against bdellovibrios (Varon and Levisohn 1972), developed protocols for isolation of the predator from the environment (Varon and Shilo 1970), evaluated the impact of pollutants on predation (Varon and Shilo 1981), addressed the peculiar requirements of marine bdellovibrios (Marbach et al. 1976), and showed that predation can select for a resistant, slower growing prey, leading to the coexistence of the wild type and the mutant in the presence of the predator (Varon 1979). Work on *Bdellovibrio* at the Hebrew University came to a stop when Varon moved to Tel-Aviv University in 1982. I feel lucky that since 1998, I have been able to revive this line of research at the Hebrew University.

In 1969, Sydney Rittenberg, from the department of Microbiology at UCLA, came to Shilo's laboratory for a sabbatical and was infected by Shilo's enthusiasm for *Bdellovibrio*. He spent a year in Jerusalem (Rittenberg and Shilo 1970) and initiated a long and fruitful research on bdellovibrio, almost exclusively publishing on this subject until his retirement in the mid-1980s. The bounds between the Shilo and the Rittenberg groups were tight, with cooperation enduring for years, as Shilo went for a sabbatical at Rittenberg's lab in 1975 and Varon in 1979. Rittenberg and his students (A. Matin, R. Hespell, M. Thomashow, to mention only a few) made landmark discoveries, revealing the extraordinary physiological adaptations of *Bdellovibrio bacteriovorus* as a predator able to built itself an intracellular "cozy niche" within its prey, in which it could devour the latter with amazing efficiency. After leaving Rittenberg's group, Robert (Bob) Hespell continued studying the metabolism of intraperiplasmically growing bdellovibrios and of starving attack cells. Rittenberg's and Hespell's findings are still the reference for understanding the latest data originating from genome and biochemical analyses (for an in depth description see chapters by Tudor and McCann, and by Strauss et al in this volume).

In parallel, Samuel Conti at the University of Kentucky was also interested in *Bdellovibrio*. His group provided the first detailed ultrastructural analysis of the interaction between the micropredator and its prey, showing by electron microscopy how the outer membrane is breached and a penetration pore formed (Burnham et al. 1968). More outstanding electron microscopy was

performed by Dinah Abram and colleagues, who described the possible existence of pilus-like structures at the proximal, penetrating pole of the invading cell, and its association with the cytoplasmic membrane of the prey (Abram et al. 1974). Conti's interest in *Bdellovibrio* was profound and his group investigated the role of chemotaxis in predation (Lamarre et al. 1977; Straley and Conti 1977; Straley et al. 1979), isolated the first bacteriophages of *Bdellovibrio* (Hashimoto et al. 1970), and discovered the presence of sphingolipids in the cell wall of *B. stolpii* (Steiner et al. 1973). With John Tudor, they investigated the peculiar *Bdellovibrio* strain W that becomes encysted within its prey (Tudor and Conti 1977a,b, 1978). Tudor went on studying strain W (Tudor 1980; Tudor and Bende 1986; Tudor and DiGiuseppe 1988) and today he takes part in the ongoing analysis of its genome (see chapter by Tudor and McCann in this volume). Tudor's never ending curiosity about BALOs started in the mid-1960s when, in his words "I first became enamored with the bdellovibrios when I was in graduate school for my master's degree. As part of an electron microscopy course, I chose to do a project using *B. bacteriovorus*. I have been fascinated by these smallest of creatures ever since, and chose to work with S.F. Conti at the University of Kentucky for my doctoral studies mainly because he had one of the few established labs working on the bdellovibrios". The field was rapidly moving forward, with dedicated sessions (such as a roundtable at the ASM 1970 general meeting) and "it was always such fun in those early days to interact at national conferences with graduate students and post-docs from the Rittenberg lab" and "people like Sam Conti and Syd Rittenberg had a profound influence on the direction of my career, and encouraged me greatly in my pursuits with the bdellovibrios… Now, nearly 40 years since I first peered into the microscope to see these most interesting critters, I find myself drawn to them as much as ever, looking forward with great anticipation to being able to understand them a little better".

The contribution of scientists in the former Soviet Union is noteworthy. Of about 300 publications (Fig. 1) on BALOs, 75 emanated from Soviet groups, 50 appearing between 1970 and 1981. Two scientists, Albina Afigenova and V. Lambina worked diligently on the subject, leading a long-term research program from the 1970s into the 1990s and contributing about 35 publications. Sadly, this project was terminated, and since then few works have emanated from Russia. The various groups studied the distribution, taxonomy, predation dynamics, and other ecological subjects pertaining to BALOs, as well as their physiology and biochemistry. They isolated *Micavibrio* (Lambina et al. 1982, 1983), micropredators that while resembling *Bdellovibrio*, were recently shown to be phylogenetically unrelated to these bacteria (see chapter by Jurkevitch and Davidov in this volume). Unfortunately, only a fraction of these publications are available in English.

During the late 1960s and early 1970s, Antonina Guélin, from the Station Biologique in Roscoff, France also actively pursued research on (mainly marine) bdellovibrios, partly in collaboration with the Russian group. She

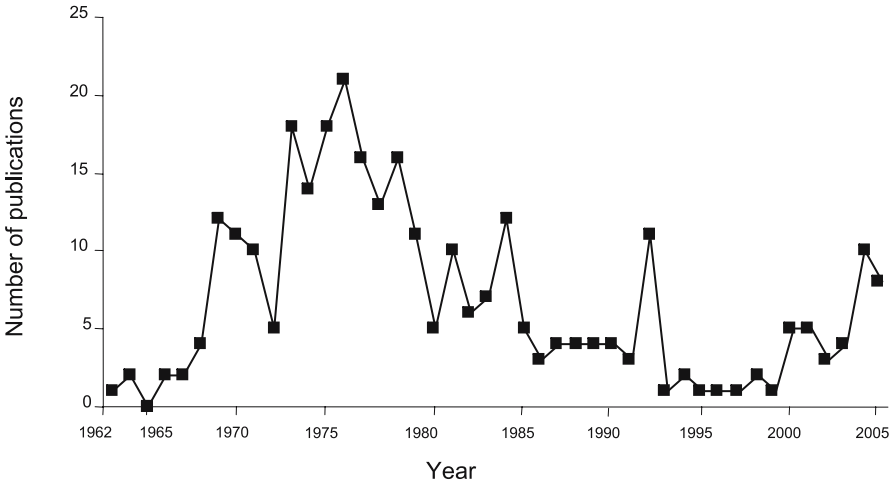


Fig. 1 Yearly fluctuations in the number of publications on *Bdellovibrio* and like organisms. Google Scholar and Medline were searched for the term "bdellovibrio" in the publication's title. The greatest number for each year is shown. Doctoral and Master theses are included

isolated one of the three reported cyst-forming strains of *Bdellovibrio* (the other two are strain W and a strain from Russia). Regrettably, only strain W is still available.

The interest in marine BALOs rose rapidly as the understanding of these bacterial predators increased. Daniel (1969), Shilo, and Varon (Marbach et al. 1976, 1978) were among the first to isolate, characterize and define the conditions required for work with these organisms. But today, if research on marine BALOs could be identified with a particular person, it is Henry Williams. Since a chapter by him in this volume is devoted to this aspect, it would be redundant to mention his contributions (over 20 publications on BALOs) and I refer the reader to his chapter. It is, however, appropriate to share Henry Williams' view of the field: "I was first introduced to the *Bdellovibrio* in graduate school during a seminar presentation by a fellow graduate student in the late 1960s, just a few years following the first report describing these unique predatory bacteria. My intrigue and excitement for the organisms began almost immediately and over time I developed a true passion for them, devoting most of my career to research on the *Bdellovibrio* and like organisms with a primary interest in their ecology in saltwater ecosystems, but also their potential as biological control agents in other venues. However, while my focus was strictly on ecology, my graduate students (Marcie Baer) postdoc fellows (Silvia Pineiro, Kimberly Walker) and collaborators (Jacques Ravel and Russell Hill) dragged me kicking and screaming down the phylogeny path. This paid off as we could show that saltwater BALOs are sufficiently different from the freshwater strains to warrant a separate classification. While now

at Florida A&M, my efforts to maintain support to study the BALOs over the years were made difficult in the early years because I was on the faculty at a dental school. Reviewers would question why this research was being done in a dental school and did not think serious work on aquatic organism could be done in such an environment. I would however use the justification with tongue in cheek that a toothbrush was used to brush oyster shells to remove surface biofilms in our studies on the association of BALOs with surfaces in natural ecosystems...".

Williams also mentions that "funding to support research on the *Bdellovibrio* has always been difficult to come by" because "program managers at funding agencies and reviewers of proposals would question the significance of research on the BALOs since such few investigators were submitting research proposals for the study of the organisms".

Indeed, the interest in researching predatory prokaryotes predators has been an oscillating matter. BALO research reached its peak in the 1970s to rapidly decrease during the 1980s (Fig. 1). The trend was felt from the beginning of the decade, and was a concern for leading scientists such as Rittenberg (Fig. 2 – in this letter, one can also see the satisfaction of reviving a BALO culture!). After an all-time low in the 1990s, interest in BALOs seems to steadily increase, and there is room for cautious optimism. A main reason for these ups and downs, besides the above mentioned difficulties in securing re-

DEPARTMENT OF MICROBIOLOGY
COLLEGE OF LETTERS AND SCIENCE
LOS ANGELES, CALIFORNIA 90024

20 January 1981

Dr. Mazal Varon
Division of Microbial and
Molecular Ecology
Institute of Life Sciences
The Hebrew University
Jerusalem, Israel

Dear Mazal:

Your letter of December 29 and the cultures arrived simultaneously last Friday. By now it is certain that BM4 has grown out and is doing nicely. Since this is the one we were after, we are much relieved. As yet I cannot give you information on BM4 str^r or BM1. They may or may not be revived, and even if they do not, we really do not need them. They were today - all 4000 or 1,

From your comments about the rest of your cultures, I am sort of assuming that Moshe is going out of the bdellovibrio business, which I hope is not the case. It was lonesome enough with only a few of us working seriously in the field, and unless some new blood shows up, there will be no futhur activity once I run out of steam, which may not be too long from now. I am glad that you sent stocks to the ATCC.

Fig. 2 Excerpt of a letter by Sydney Rittenberg to Mazal Varon, January 1981

search funds, was, as both Varon and Williams pointed out to me, because of the inherent complexity involved in working with two-membered cultures of predator and prey. It is somehow ironic that the interest in the field dwindled as molecular biological tools, which can help overcome some of the basic problems of mixed cultures, were becoming available and were even applied to the study of BALOs (Cotter and Thomashow 1992a,b). Fortunately, one can sense that this trend is reversing, and research on predatory bacteria is rising again as new groups are now entering the field, and genome projects have been and are being pursued. This book may also stand witness to this trend: it summarizes the latest developments in the field and hopefully, will contribute to its strengthening.

Acknowledgements My deepest gratitude goes to Mazal Varon, John Tudor, and Henry Williams for their help in gathering information and for their personal insights. I would like to warmly thank Heinz Stolp for providing unique material. I am grateful to Rafael Springmann for translations from German.

References

Abram D, Castro e Melo J, Chou D (1974) Penetration of *Bdellovibrio bacteriovorus* into host cells. J Bacteriol 118:663–680

Beebe JM (1941) Studies on the myxobacteria. 2. The role of myxobacteria as bacterial parasites. Iowa State J Sci 15:319–337

Bengston S (2002) Origins and early evolution of predation. Paleontol Soc P 8:289–318

Burnham JC, Hashimoto T, Conti SF (1968) Electron microscopic observations on the penetration of *Bdellovibrio bacteriovorus* into gram-negative bacterial hosts. J Bacteriol 96:1366–1381

Cotter T, Thomashow MF (1992a) Identification of a *Bdellovibrio bacteriovorus* genetic locus, *hit*, associated with the host-independent phenotype. J Bacteriol 174:6018–6024

Cotter TW, Thomashow MF (1992b) Identification of a *Bdellovibrio bacteriovorus* genetic locus, *hit*, associated with the host-independent phenotype. J Bacteriol 174:6018–6024

Daniel S (1969) Etude de l'influence de *Bdellovibrio bacteriovorus* dans l'auto-épuration marine. Rev Int Océanogr 15–16:61–102

Ekstejn M, Varon M (1977) Elongation and cell division in *Bdellovibrio bacteriovorus*. Arch Microbiol 114:175–181

Hashimoto T, Diedrich DL, Conti SF (1970) Isolation of a bacteriophage for *Bdellovibrio bacteriovorus*. J Virol 5:97–98

LaMarre AG, Straley SC, Conti SF (1977) Chemotaxis toward amino acids by *Bdellovibrio bacteriovorus*. J Bacteriol 131:201–207

Lambina VA, Afinogenova AV, Romai Penabad S, Konovalona SM, Pushkareva AP (1982) *Micavibrio admirandus* gen. et sp. nov. Mikrobiologiya 51:114–117

Lambina VA, Afinogenova AV, Romay Penabad S, Konovalona SM, Andreev LV (1983) A new species of exoparasitic bacteria from the genus *Micavibrio*. Mikrobiologiya 53:777–780

Marbach A, Varon M, Shilo M (1976) Properties of marine bdellovibrio. Microb Ecol 2:284–295

Maynard-Smith J, Szathamary E (1995) The major transitions in evolution. Freeman, New York

Rittenberg SC, Shilo M (1970) Early host damage in the infection cycle of *Bdellovibrio bacteriovorus*. J Bacteriol 102:149–160
Steiner S, Conti SF, Lester RL (1973) Occurrence of phosphonosphingolipids in *Bdellovibrio bacteriovorus* strain UKi2. J Bacteriol 1199–1211
Stolp H, Pertzold H (1962) Untersuchungen uber einen obligat parasitischen Mikroorganismus mit lytischer Aktivitat fur *Pseudomonas*-Bakterien. Phytopathol Z 45:364–390
Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* gen. et sp., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek 29:217–248
Stolp H (1969) Am 11. Dezember 1968 jährte sich zum 125. Male der Geburtstag von Robert Koch 843–1910. Bundesgesundheitsblatt 2:S17–21
Stolp H (1973) The bdellovibrios: bacterial parasites of bacteria. Ann Rev Phytopathol 11:53–76
Starr MP, Baigent NL (1966) Parasitic interaction of *Bdellovibrio bacteriovorus* with other bacteria. J Bacteriol 9:2006–2017
Shilo M, Bruff B (1965) Lysis of Gram negative bacteria by host-independent ectoparasitic *Bdellovibrio bacteriovorus* isolates. J Gen Microbiol 40:317–328
Straley SC, Conti SF (1977) Chemotaxis of *Bdellovibrio bacteriovorus* toward prey. J Bacteriol 132:628–640
Straley SC, LaMarre AG, Lawrence LJ, Conti SF (1979) Chemotaxis of *Bdellovibrio bacteriovorus* toward pure compounds. J Bacteriol 140:634–642
Tudor JJ (1980) Chemical analysis of the outer cyst wall and inclusion material of *Bdellovibrio* bdellocysts. Curr Microbiol 4:251–256
Tudor JJ, Bende S (1986) The outer cyst wall of bdellocysts is made *de novo* and not from pre-formed units from the prey wall. Curr Microbiol 13:185–189
Tudor JJ, Conti SF (1977) Characterization of bdellocysts of *Bdellovibrio* sp. J Bacteriol 131:314–322
Tudor JJ, Conti SF (1977b) Ultrastructural changes during encystment and germination of *Bdellovibrio* sp. J Bacteriol 131:323–330
Tudor JJ, Conti SF (1978) Characterization of germination and activation of *Bdellovibrio* bdellocysts. J Bacteriol 133:130–138
Tudor JJ, DiGiuseppe JA (1988) The effect of penicillin on encystment of *Bdellovibrio* sp. strain W. Arch Microbiol 149:422–426
Varon M (1979) Selection of predation-resistant bacteria in continuous culture. Nature 277:386–388
Varon M, Fine M, Stein A (1984) The maintenance of *Bdellovibrio* at low prey density. Microb Ecol 10:95–98
Varon M, Levinsohn R (1972) Three-membered parasitic system: a bacteriophage, *Bdellovibrio bacteriovorus*, and *Escherichia coli*. J Virol 9:519–525
Varon M, Seijffers J (1975) Symbiosis-independent and symbiosis-incompetent mutants of *Bdellovibrio bacteriovorus* 109J. J Bacteriol 124:1191–1197
Varon M, Shilo M (1968) Interaction of *Bdellovibrio bacteriovorus* and host bacteria: I. Kinetic studies of attachment and invasion of *Escherichia coli* B by *Bdellovibrio bacteriovorus*. J Bacteriol 95:744–753
Varon M, Shilo M (1969a) Attachment of *Bdellovibrio bacteriovorus* to cell wall mutants of *Salmonella* spp and *Escherichia coli*. J Bacteriol 97:977–979
Varon M, Shilo M (1970) Methods for separation of *Bdellovibrio* from mixed bacterial population by filtration through Millipore filters or by gradient differential centrifugation. Rev Intern Océanogr Med 18–19:145–152
Varon M, Shilo M (1981) Inhibition of the predatory activity of *Bdellovibrio* by various environmental pollutants. Microb Ecol 7:107–111

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_052/Published online: 27 October 2006

Phylogenetic Diversity and Evolution of Predatory Prokaryotes

Edouard Jurkevitch (✉) · Yaacov Davidov

Department of Plant Pathology and Microbiology,
and the Otto Warburg Center for Agricultural Biotechnology,
Faculty of Agricultural, Food and Environmental Quality Sciences,
The Hebrew University of Jerusalem, 76100 Rehovot, Israel
jurkevi@agri.huji.ac.il

Abstract Predation is one of the commonest types of interaction in the living world. Its roots appear to be ancient, and it may have first occurred early in the evolution of life forms. Predators have evolved many times in the animal realm, and this also seems to be the case within the prokaryotes. Although still rather limited, our knowledge of obligate and non-obligate bacterial predators suggest that they are common in many bacterial phyla, as well as in the environment. In this work, we survey and describe the known bacterial predators according to their phylogenetic affiliation. A hallmark of many bacterial predators is their ability to degrade the polymeric structures of their bacterial preys. An additional characteristic of known obligate predators is a small cell size. We use such distinguishing features to put forward hypotheses relating to the origin of predation in prokaryotes and to the impact of predation on the evolution of the eukaryotic cell.

1 Introduction

Predation is a common mode of interaction between organisms across the scales of the realm of life. If bacteriophages are included, a continuum of predators exists from submicron to (tens) of meters in size, spanning about eight orders of magnitude. At the lower end of the scale—viruses and bacteria—predators are usually smaller than their prey, while at the other levels, the opposite is the rule. The boundary stands at the prokaryote–eukaryote divide, as protozoa are usually larger than their bacterial prey. The reason underlying this difference in predatory modes between eukaryotes and prokaryotes may stem from the ability of the former, and the inability of the latter to engulf particles. Obviously, a cell that is unable to phagocytose is not able to "swallow" an object, let alone if this object happens to be larger than it. Although size matters, strategies and mechanisms are crucial to the outcome of the interaction, as described below.

In fact, an escape strategy to protozoal predatory pressure is observed as the size distribution of bacterial cells exposed to grazing is altered (see Jurgens, 2006, in this volume). As it is advantageous for a swallowing predator to be larger than its prey, a positive feedback loop is created (Bengston 2002). Therefore, predatory eukaryotes would most necessarily prey on cells smaller than themselves. In contrast, for cells unable to phagocytose such as prokaryotes, efficient predation would almost dictate the opposite: a small cell size and therefore, predation upon larger prey cells. A small predatory cell could more efficiently adhere to the surface of a large prey. Moreover, and possibly more significant for an obligate predator, it could be advantageous to prey upon a cell larger than your own as this could provide enough supplies for replication and growth at once. A relatively large prey could provide an ample nutrient and energy source for each successful predatory interaction, yielding a number of progeny cells. Moreover, a small-sized predator could more easily gain access to the interior of its host. Another interesting feature of some prokaryotic predators is their high motility. If motility is a plus for scavengers, it is obviously an advantage for predators. It seems that a small cell size could enable rapid and very active motility (Starr and Seidler 1971). However, larger organisms are usually faster than smaller ones (Bonner 1993), but paradoxically small bacterial predators, such as *Bdellovibrio* and like organisms (BALOs) are also the fastest swimmers (Stolp 1967). Therefore, opting for a small size would seem a likely strategy for predatory bacteria, at least for obligate predators.

It has been argued that prokaryotes are limited to small sizes because they do not possess a real cytoskeleton (Zlatanova 1997) (although its origin may be traced to bacteria (van den Ent et al. 2001; Doolittle and York 2002)), they cannot fuel the metabolic demands of a large cell as diffusion limits the uptake of nutrients and their internal distribution as well as waste dis-

posal (Koch 1996). While these may place constraints on the evolution of large prokaryotic cells, the discovery of "gigantic" prokaryotes such as *Thiomargarita namibiensus* (Schulz 1999) and *Epulopiscium fishelsoni* (Angert et al. 1993; Clements and Bullivant 1991) demonstrates that prokaryotes may reach sizes well above the "common" bacterial size. Theoretical consideration shows that in fact, diffusion may sustain cells even larger than the largest known prokaryotes (Koch 1996). While prokaryotic cells do not grow to be as large as most eukaryotic cells, one may ask why aren't large bacteria more common. A frequently invoked advantage for a large cell size is protection from protozoan predation. While a large body would protect from predation by protozoa, could it make the organism more exposed to bacterial predation? Could that be a reason for the dearth of such large bacteria? Or has our sampling been biased by our inability to culture most prokaryotes (Rappé and Giovannoni 2003) and a propensity to mainly sample accessible habitats? *T. namibiensus* and *E. fishelsoni* have been found in sulfurous marine sediments in the sea-floor off the coast of Namibia, and in the intestinal tract of surgeonfish, respectively. It would be interesting to test for the presence of bacterial and protozoan predators in the environments supporting these large prokaryotes.

Predatory bacteria are phylogenetically diverse and ubiquitous in terrestrial and aquatic environments and appear to form a part of their microbial fabric (Baer et al. 2000; Snyder et al. 2002; Davidov and Jurkevitch 2004, and this work). Moreover, culture-based and culture-independent analyses of extreme or "exotic" environments reveal that predatory bacteria are also to be found there: 16S rDNA sequences or isolates related to *Bdellovibrio* and like organisms (BALOs) were retrieved from arsenite-oxidizing biofilms, from hot-spring travertine depositions, arctic marine sediments and hyper-saline waters (Davidov and Jurkevitch 2004; Pineiro et al. 2004), and predatory interactions between bacteria have been documented in anaerobic layers in sulfurous lakes (Esteve et al. 1983; Guerrero et al. 1986). Predatory bacteria exhibit very different phenotypes and often amazing physiological adaptations. They also probably make use of different predatory strategies. They represent an untapped resource for the microbial ecologist as well as for microbiologists at large.

Predation is a major ecological force, shaping the structure of communities, driving diversity and evolution of life histories (Stanley 1973; Day et al. 2002), and as such is a central subject for ecological research. Microbial models are very useful for testing basic questions of ecological importance as they can be controlled, tracked, manipulated and replicated much more easily than most biological systems (Jessup 2004), enabling experimental verification that otherwise may be very difficult to achieve. For example, understanding the evolution of a predator–prey interaction requires a description of the potential dynamics of one or more traits in one or both species through time (Abrams 2000). This may be difficult to implement experimentally but

microbial models can offer great opportunities to test such systems (Yoshida et al. 2003). Other theoretical features of predator–prey interactions, can be tested such as (among many others) the role of spatial structure for explaining coexistence (Schrag and Mittler 1996; Bohannan et al. 2002), fitness costs of resistance to predation (Lenski 1988; Bohannan et al. 2002) and the link between productivity and food chain length (Kaunzinger 1998). However, few studies have used purely bacterial components as predator and prey to test theoretical hypotheses (Varon 1978, 1979).

In this work, we shall try to show that while the data on predatory bacteria is not immense, predation is a common mode of feeding within prokaryotes, and that by investigating it, hypotheses pertinent to the evolution of certain extant life forms can be proposed.

2
Predatory Bacteria: a Phylogenetic Perspective

As our primary focus in this work is the description of bacterial predators in an evolutionary perspective, micropredators will be mostly treated on a phylogenetic basis. We shall start with a description of phylogenetically defined bacterial predators along taxonomic lines. We shall focus on bacterial species, in which predatory behavior has been demonstrated, i.e. the ability to grow on prey cells as the sole source of nutrients, including both obligate and facultative predators.

Until recently, the obligate predators described as *Bdellovibrio* and like organisms (BALOs), while forming different families, were only found within the *δ-proteobacteria* and were historically treated together. However, new findings indicate that obligate predators can be found in different proteobacterial classes: *Micavibrio* spp. belong to the *α-proteobacteria*. Since this work's organization is based on a phylogenetic classification and the term BALO has hitherto only been used to designate *δ-proteobacteria* predators, *Micavibrio* will be treated here under the *α-proteobacteria*. However, we propose that obligate predators such as *Micavibrio*, the mode of action, morphology and behavior of which resemble the hitherto describes BALOs should also be covered by this general term. Furthermore, we propose to add a prefix to the term BALO that would indicate the phylogenetic affiliation of the organism. *Micavibrio* would therefore be *a*-BALOs, and the other known obligate bacteria predators, *d*-BALOs.

2.1
Alpha Proteobacteria

Ensifer adhaerens. *Ensifer adhaerens* is an aerobic rod-like Gram-negative soil bacterium ($0.7–1.1 \times 1.0–1.9\ \mu m$) occurring singly or in pairs. It attaches

endwise, in a picket fence-like fashion to its prey (Fig. 1A) which are various living Gram-positive and Gram-negative bacteria but it is not an obligate predator (Casida 1982). The prey and the predator are connected through an electron dense material that seems to emanate from the predator (Fig. 1B). Prey lysis occurs in the absence of other available resources (Casida 1982). *E. adhaerens* divides by budding at one cell pole followed by asymmetrical polar growth and binary fission (Casida 1982; Fig. 1C). *E. adhaerens* exhibiting a predatory behavior towards *Micrococcus luteus* and Gram negative cells as well were found in the four soils in which they were searched for (Casida 1980). In soils enriched with *M. luteus*, both the population of *E. adhaerens* and of a *Streptoverticillium*-like predatory bacterium increased simultaneously as they preyed upon the added bacteria. A *Myxococcus*-like predator also able to utilize *M. luteus*, developed later (Casida 1980). *E. adhaerens* was able to lyze both these predators in soil (Casida 1980; Germida 1983). However, under laboratory conditions, *E. adhaerens* could not lyze the *Streptoverticillium*-like strain and was itself destroyed by the *Myxococcus*-like predator (Germida 1983).

Recently, polyphasic analyses including genetic and phenetic characters showed that strains of non-symbiotic rhizobia, some isolated from nodules, were closely related to the type strain *E. adherens* 7A (Willems 2003). Unfortunately, it is not known whether these other strains are capable of predatory behavior. On the basis of this relatedness, it was proposed that the nomenclature be changed to *Sinorhizobium adhearens* in spite of the fact that *Ensifer* should take prevalence (Young 2003).

When a symbiotic plasmid from *Rhizobium tropici* was introduced into a predatory *E. adhaerens* strain, the latter was able to form nitrogen-fixing nodules in *Phaseolus vulgaris* (bean) and *Leucaena leucocephala* (Rogel 2001). Similarly, introduction of a plasmid from *Rhizobium phaseoli* into *Agrobacterium tumefaciens* endowed the plant pathogen with nodule-forming and nitrogen-fixing capacities in *P. vulgaris* and *L. leucocephala*

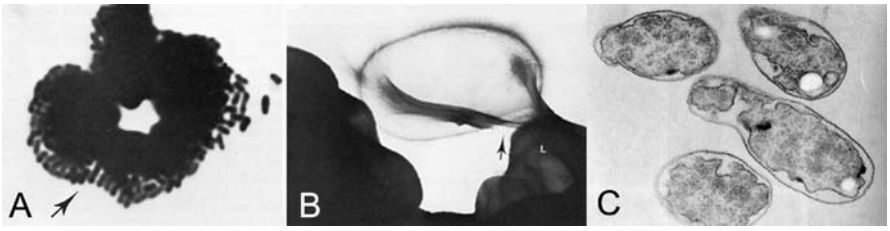


Fig. 1 **A** Attachment of *Ensifer adhaerens* strain A cells (*arrow*) onto *Micrococcus luteus* prey in a picket fence manner (×3123). **B** Electron dense connective material appears between an *E. adhaerens* cell and the darkly stained *Micrococcus luteus* prey (*arrow*) (×50 900). **C** Bud formation and growth *E. adhaerens* strain A (×31 450). (Casida LE 1982, Int J Syst Bacteriol 32:339–345, by permission)

(Martinez et al. 1987). A genome comparison between *A. tumefaciens* and *Sinorhizobium meliloti* suggested a recent evolutionary divergence (Wood et al. 2001). Conceptually, it is tempting to link predation with symbiosis and pathogenesis because these behaviors share common needs such as recognition of a host, attachment, and eventually penetration. *E. adhaerens*, a little-studied organism can serve as a good model, and the taxonomic identity of *E. adhaerens* with *S. meliloti* calls for further investigations of this link: Is *E. adhaerens* ancestral, are predatory activities outstanding and solely found in a few non-symbiotic strains or are they expressed in symbiotic rhizobia? We can suggest that a closer look at this species, the determination of the presence or the absence of predatory capabilities in *E. adhaerens* (*S. adhearens*) strains and other rhizobia, and genome-wide comparison between strains could lead to interesting and surprising insights into rhizobial ecology.

Micavibrio. *Micavibrio* are obligate predatory bacteria. Two species were described, *M. admirantus* (Lambina et al. 1982), isolated using *Stenotrophomonas maltophila* as a prey and *M. aeruginosavorus* (Lambina et al. 1983), isolated on *Pseudomonas aeruginosa*. Both were isolated from sewage works. They were not able to prey upon any of the 55 other prey cell types tested, although they did utilize almost all of the different strains of the species that were used for isolating them. These bacteria are small (0.25–0.4 × 0.5–1 μm), they possess a single, non-sheathed flagellum of 15 nm in diameter, with a regular wavelength (in opposition to other d-BALOs that have a sheath flagellum with a damping wavelength form, see below) (Fig. 2A). *Micavibrio* prey in an epibiotic manner and do not penetrate the prey's inner compartments.

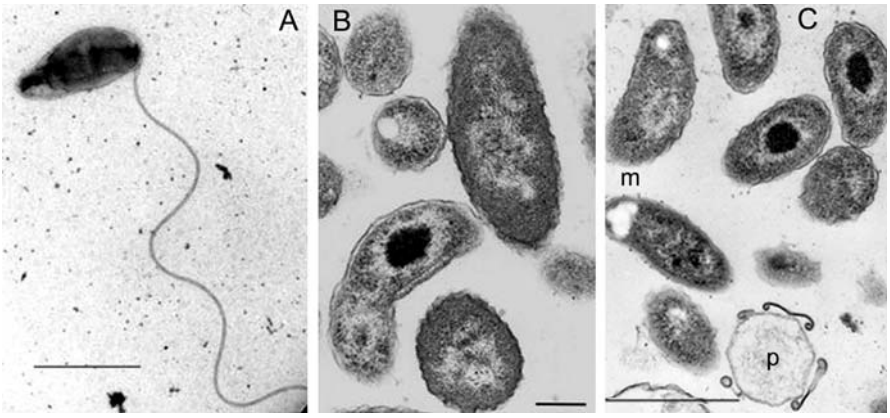


Fig. 2 **A** A *Micavibrio* sp. predator. The *scale bar* represents 1 μm. **B** A dividing *Micavibrio* cell attached to a *Pseudomonas corrugata* prey. The *scale bar* represents 0.5 μm. **C** *Micavibrio* cells (m) and an empty prey (p). Note the electron-lucent zones in the predator and the scroll-like structures in the prey. The *scale bar* represents 1 μm. (Pictures by Susan Koval)

They also divide by binary fission (Fig. 2B). As seen in other BALOs and in *Daptobacter* (see below) a few mesosomes surrounding electron-lucent ovale granules are present in each cell (Fig. 2B,C). Destruction of the prey results in many ghost cells, the cell wall of which often exhibit scroll-like structures (Fig. 2C).

Recently, we isolated six isolates of plaque-forming bacteria from a salt-laden soil in Northern Israel using *Pseudomonas corrugata* as a prey. These six isolates were morphologically very similar to *Micavibrio*. Phylogenetic analysis of these strains and of *M. aeruginosavorus* revealed that they were part of a single, deep branching cluster within the *α-proteobacteria* (Davidov et al. 2006b). As described in the earlier reports, no host-independent derivatives could be obtained from *Micavibrio* strains on a rich medium.

Although *Micavibrio* and the other *Bdellovibrio* and like organisms do not belong to the same proteobacterial class and some morphological features differ between them, they show some interesting similarities at the biochemical level: with both predators, an increase in material absorbing at wavelengths of 260 and 280 nm and originating from the prey occurs during the first stages of the interaction (Afinogenova et al. 1986). Moreover, enzymes of the glycolytic pathways and tricarboxylic acid cycle enzymes although present, are weakly active (Afinogenova et al. 1986).

2.2 Beta Proteobacteria

Cupriavidus necator. *Cupriavidus necator* forms short Gram negative rods ($0.7 - 0.9 \times 0.9 - 1.3\ \mu m$), and belongs to the *Burkholderiales*. According to Vandamme and Coenye (2004), *Wautersia eutropha* (formerly *Ralstonia eutropha*) is a later synonym for the genus *Cupriavidus*. The type strain is *C. necator* LMG 8453^T, corresponding to strain N-1^T, a non-obligate soil predator isolated by Casida (1987).

This non-obligate predatory bacterium was isolated from soils and was shown to prey upon a large range of Gram positive and Gram negative bacteria, such as *Agromyces ramosus, Arthrobacter globiformis, Azotobacter vinelandii, Bacillus subtilis, B. thurigiensis, Ensifer adhaerens, Escherichia coli, Micrococcus luteus, Staphylococcus aureus* and *Streptomyces* spp. (Makkar and Casida Jr 1987). Within this list, other facultative predators are also included (Zeph and Casida 1986 and see below). *C. necator*, like other bacteria belonging to this genus was not only able to sustain high concentrations of copper, but these high concentrations were actually required in order to initiate growth. *C. necator* produced a peptidic copper-related factor that was needed for growth initiation but was at the same time inhibitory to other facultative predators (Casida 1987; Casida 1988). In contrast, a magnesium-related factor, also produced by *C. necator* elicited a positive response in other predators (Byrd et al. 1985; Casida 1987).

Aristabacter necator. *Aristabacter necator* 679-2 is among a number of facultative bacterial predators that were retrieved from soil, based on resistance to high copper concentrations (Casida 1987, 1988, 1992). It was isolated from a small patch of soil and could not be detected elsewhere. Analysis of its 16S rRNA sequence revealed that it belonged to the *β-Proteobacteria*. A 16S rRNA phylogeny showed that *A. necator* was not related to any known bacterium, with all its closest relatives diverging by more than 10% (Cain et al. 2003). Strain 679-2 attached to its prey in a picket-fence mode, and was able to track groups of prey cells. It also exhibited inhibitory activities on a broad range of bacteria, as well as against some fungi (Casida 1992). Moreover, *A. necator* 679-2 was highly competitive in soil, and was able to survive and destroy other microorganisms in-situ. On the basis of this high potency it was proposed that *A. necator* stood at the top of the hierarchy of non-obligate bacterial predators in soil (Casida 1992). Its general and strong antimicrobial activity could be traced to the three compounds pyrrolnitrin (an antibiotic), maculosin (a molecule with herbicidal activities) and banegasine (Cain et al. 2003). The last two compounds, while lacking activity alone, exhibited synergy when combined. Predatory bacteria may form an interesting source of potent new biochemicals, as such compounds may be of use in their warfare against other microorganisms (Cain et al. 2003).

2.3 Gamma Proteobacteria

Stenotrophomonas maltophilia. A bacterium identified as *Stenotrophomonas maltophilia* and able to inhibit *Chlorobium* spp. was isolated from a freshwater sulfurous lake in Northern Spain (Nogales 1997). Although no direct contact between the two organisms was seen, *Chlorobium* was lyzed, resulting in empty ghost cells. Lysis occurred in a mineral medium used to grow the phototrophic bacteria and in the absence of exogenous carbon sources. *Stenotrophomonas maltophilia* acted upon the phototrophs through a diffusible signal and leaks from dead or injured cells may have been used as resources for growth. However, cell lysis probably provided the bulk of the nutrients needed for the growth of *S. maltophilia*. In that sense it can be considered predatory. *S. maltophilia* was able to grow as a pure culture; hence it may act as a facultative predator in nature.

Lysobacter. Within the *γ-proteobacteria*, the genus *Lysobacter*, family *Lysobacteriales*, belongs to the xanthomonads (Reichenbach 2001). These gliding bacteria can grow very long cells and filaments (up to 70 μm) but most cells are usually of common sizes for bacteria ($0.4 - 0.6 \times 2 - 5$ μm). *Lysobacter* produces a lot of slime and by using gliding motility the colonies can swarm on solid media. Lysobacters are soil and fresh water, sewage, reservoir, lake and river dwellers. On the basis of their overall morphology, the term

"myxobacter" has been used in the past to describe certain *Lysobacter* strains, generating confusion as to their accurate taxonomic placement.

Many cyanobacteria, other Gram negative, as well as Gram positive bacteria including actinomycetes, green algae, fungi and nematodes were shown to fall victim to the lytic activities of members of the *Lysobacteriales* (Shilo 1970; Stewart and Brown 1971; Daft et al. 1973). As such, *Lysobacter* can be isolated using bacteria (such as *Arthrobacter*) as a sole nutrient source in agar (Reichenbach 2001 based on Ensign and Wolfe 1965). Some *Lysobacter* strains are also active against plant pathogens (Folman et al. 2003; Kobayashi 2005). Although not stringent, different *Lysobacter* isolates exhibited different prey ranges. More so, strains of a particular cyanobacterial species varied in their sensitivity to lysis by a specific *Lysobacter* strain (Daft and Stewart 1971).

Lysobacter may lyze their prey using a "wolfpack" strategy, which requires a high density of predators to ensure that the concentrations of the lyzing factors remain high enough to act upon the prey cell wall (Dworkin 1999). However, cell to cell contact seems to also be the rule, and membrane-bound enzymes may be implicated in prey lysis (Shilo 1970; Daft and Stewart 1971, 1973; Kobayashi 2005). Attachment occurs when one cell pole binds to the prey cell, often close to the cross-septa of the filaments, ending up perpendicular to the prey (Daft and Stewart 1973, Figure 3A). This would fit an epibiotic mode of predation (Martin 2002), reflected in the capacity of a single *Lysobacter* cell to lyze a *Nostoc* cell in 20 minutes (Shilo 1970, Fig. 3A). Prey search may involve an aerotactic response by which the sensing of an increasing oxygen gradient leads towards photosynthetic prey cells (Reichenbach 2001). Interestingly, the same parameter (oxygen concentration) but in reverse, may be used by *Bdellovibrio bacteriovorus* to locate its prey in environments slightly depleted in oxygen by the respiratory activity of the latter (Rendulic et al. 2004). In opposition to *Bdellovibrio* and like organisms, no defined structure can be seen at the attachment site of *Lysobacter* to the prey. Lysis occurs rapidly after attachment (Gillespie and Cook 1965), with the peptidoglycan layer being the first structure to be attacked by the predator (Reichenbach 2001). Lyzed cells contained the remains of internal membranous complexes and the layers from the cell wall may form scroll-like structures (Fig. 3B). Similar scroll-like structures can also be observed in *Pseudomonas corrugata* prey lyzed by *Micavibrio* (Fig. 2C).

Lysobacter spp. are a source of enzymes for the biotechnology industry as they produce a wide array of extracellular biopolymer-degrading enzymes such as nucleases (von Tigerstrom 1980), chitinases (Christensen and Cook 1978), proteases (Epstein and Wensink 1988; Wright et al. 1998), glucanases (Palumbo 2003), lipases (Folman et al. 2003), as well as antibiotic compounds (Christensen and Cook 1978; Kato et al. 1997), and a muramidase and two peptidases endowed with bacteriolytic activities (Sitkin et al. 2003; Stepnaya et al. 2004). These latter enzymes are positively charged and interact with a negatively charged extracellular polysaccharide, forming lysoamidase,

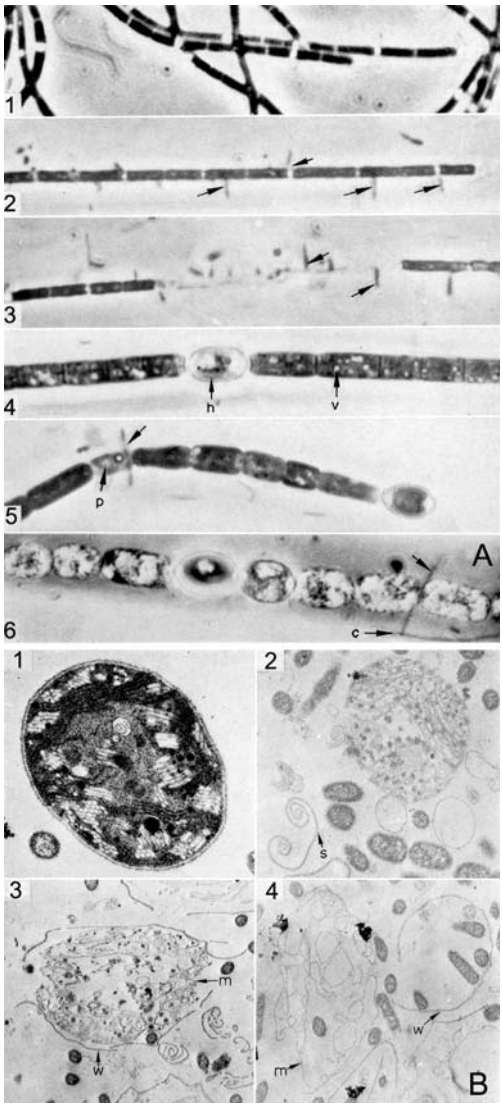


Fig. 3 **A** *1*. Healthy filaments of *Oscillatoria redekei*; *2*. Polar attachment of *Lysobacter* CP-1 (*arrow*) along filaments of *O. redekei*; *3*. Lyzed cells of an *O. redekei* filament. The bacterial predator is still attached; *4*. A healthy filament of *Aphanizomenon flos-aquae* with heterocyst (h) and gas vacuoles (v) in vegetative cells.; *5*. Attachment of CP-1 (*arrow*) and a partially lyzed cell (p) of *A. flos-aquae*. *6*. Attachment of two CP-1 cells (*arrow*) near a cross-wall in *Anabaena flos-aquae*. Note bacteria attached end-on to each other. **B** *Lysobacter* preying on *Microcystis* sp. *1*. A healthy cell of *Mycrocystis* sp.; *2*. Lyzed cell with a typical scroll structure (s) of *Mycrocystic* sp.; *3*. Lyzed cell of *Mycrocystis* sp. showing disorganized membranes (m) and multiple breaks in the cell walls (w); *4*. Distended internal membranes (m) and persistent cell walls (w) of *Mycrocystis* sp. (Daft MK, Steward W 1973, New Phytol 72:799-808, by permission)

a complex able to lyze the peptidoglycan of many Gram positive but of only a few Gram negative bacteria (Begunova 2004). However, in Gram negative cells with a compromised lypopolysaccharide layer, lysoamidase was active against the peptidoglycan (Begunova 2004).

Some strains of *Lysobacter* were isolated following cyanobacterial and algal blooms. They were able to multiply to high extents by preying on both populations (Shilo 1970). It is therefore possible that *Lysobacter* plays a role in the control or the modulation of certain microbial populations (Reichenbach 2001).

2.4 Delta Proteobacteria

Myxobacteria. Myxobacteria are gliding, relatively large rod cells ($0.6 - 1.2 \times 3 - 15\ \mu m$). Myxobacteria's most striking characteristic is the formation of swarms and their multicellular life-style which has been and still is the focus of much research. We shall restrict ourselves to the predatory aspects of the life-style of myxobacteria. Most myxobacteria are proteolytic and exhibit bacteriolytic activities, feeding among other substrates, on dead and live bacterial cells alike (Singh 1947; Margalith 1962; Shimkets 1990). The spectrum of bacteria lyzed by myxobacteria is rather large, but Gram positive cells appear to be more sensitive to myxobacterial bacteriolysis than Gram negative cells (Shimkets 1990). However, cyanobacteria were efficiently preyed upon by aquatic *Myxococcus* sp. (Burnham et al. 1981). Even though they are prevalent in soils, with densities reaching 5×10^5 cells per gram (Singh 1947), the role of myxobacteria in controlling bacterial populations is not known. Their gliding motility permits myxobacteria to efficiently find substrates, especially in the soil environment (Reichenbach 1999). Contact with the substrate may also enhance the efficiency of the degradative process by securing higher local concentrations of excreted extracellular, and of membrane-bound lytic enzymes. Contact between the myxococcal swarm and prey cells appears to trigger predation, while chemotaxis toward prey colonies may not be involved in that process. When an *M. xanthus* swarm encountered an *E. coli* colony, the swarming behavior of the predator was altered: the *Myxococcus* swarm cells remained in the prey colony until its lysis was complete (McBride and Zusman 1996).

A great variety of lytic enzymes, including lipases, nucleases and polysaccharidases are produced by myxobacteria (Dworkin 1996). Like other bacterial predators, many of the cell-lyzing activities of myxobacteria target the peptidoglycan, and amidases, glucosamidases and a variety of peptidases can be obtained in the culture supernatant (Sudo 1972; Dworkin 1996). The genome of *M. xanthus* is presently under analysis and should yield more data on its hydrolytic potential (http://www.tigr.org/tdb/mdb/mdbinprogress.html). BALOs and myxobacteria both belong to the *δ-proteo-*

bacteria. Therefore, a comparison of BALO and myxobacterial genomes and more specifically of their hydrolytic enzyme complements may yield interesting information on their predatory behavior and on its origin. Some of the hydrolytic enzymes of myxobacteria were shown to be active even when adsorbed to clay minerals suggesting they may have "ecological significance" (Haska 1981).

As mentioned above, Gram negative cells are lesser prey for the myxobacteria. The outer membrane may shield diderms by preventing access of hydrolytic enzymes to the peptidoglycan substrate but this substrate may still be sensitive to degradation by the hydrolytic enzymes of myxobacteria. Damaging the outer shield of Gram negative cells resulted in sensitization to myxococcal bacteriolytic activities, as found with *Lysobacter* (Begunova 2004). It is remarkable that the type of interaction between *Lysobacter* (which were often mistaken for myxobacteria) with their prey, their range of bacteriolytic activities, and their ability to degrade purified peptidoglycan of Gram negative bacteria and those of myxobacteria resemble each other so much. The lack of sequence data still prevents the elucidation of the origins of these properties, be it in lateral gene transfer or convergent evolution.

Most myxobacteria produce antibiotics (Reichenbach et al. 1988), a large fraction of which have not been characterized. Although it is doubtful that antibiotics could be produced at levels high enough to play a direct, significant role in prey lysis, one may speculate that under natural conditions some of these compounds may compromise the integrity of the outer membrane, facilitating the access of peptidoglycan-degrading enzymes to their substrate.

The predatory activities of *M. xanthus* and *M. fulvus* strains were studied in relation to cyanobacterial population control (Burnham et al. 1981; Burnham 1984; Daft et al. 1985). These myxococci formed colonial spherules in which the peripherally located mycoccocal cells concentrated the cyanobacteria within the spherule's core and caused their lysis: First, myxococci cells attached to a number of cyanobacterial trichomes, ensnared an increasing number of these, then multiplied to form an encapsulating cell mass within which prey degradation occurred (Burnham et al. 1981, Fig. 4A,B). The mechanisms underlying this orchestrated attack are not known. Similarly to *Lysobacter*, the cyanobacterial-lyzing myxococci exhibited varying prey ranges. However, while *Lysobacter* could not lyze cyanobacteria when the predator's inoculum was less than 10^6 cell ml^{-1}, as few as 50 *Myxococcus* cells per 100 ml and 10^7 prey cells ml^{-1} were sufficient to start a lytic cycle (Burnham et al. 1984). Nevertheless, natural blooms could not be controlled by myxobacteria, because of an insufficient supply of inorganic nutrients (Fraleigh and Burnham 1988).

Myxobacteria were shown to require a density threshold to grow on casein-containing medium (Rosenberg et al. 1977). This type of experiment led to their categorization as predators opting for a wolfpack strategy (Dworkin 1999). Nevertheless, a low level of predators, as described above, would not

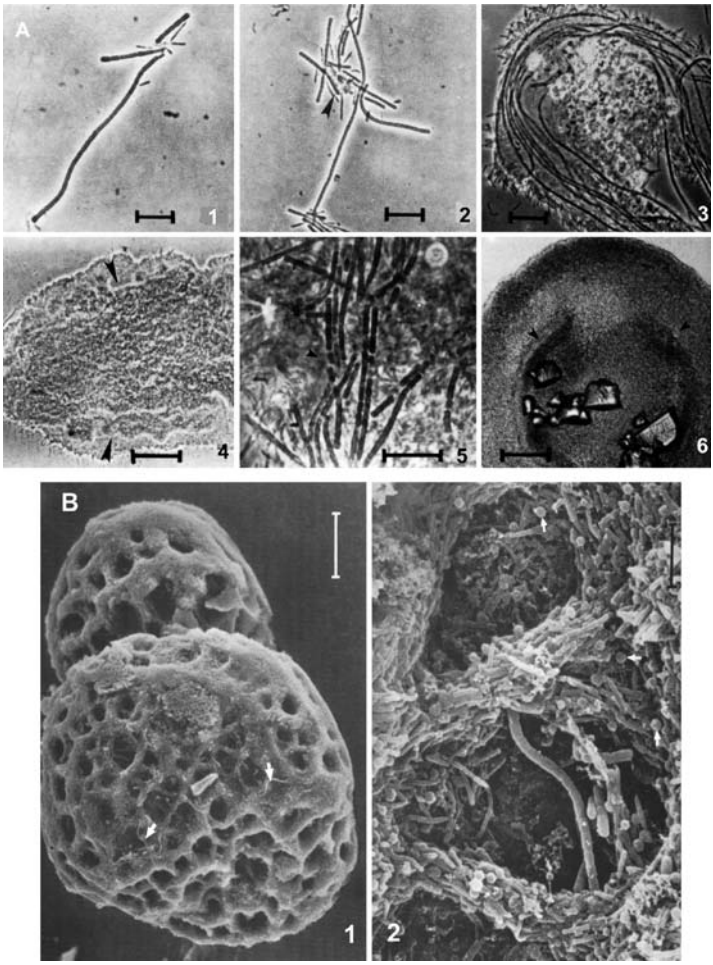


Fig. 4 *Myxococcus* spp. preying upon cyanobacteria. **A** *1*. and *2*. Development of *M. xanthus* PCO2 colonies in the presence of 10^7 *P. luridum* cells.ml^{-1}, 1% myxococcal inoculum, 17 h interaction. Note microfloccula formation. Cyanobacterial lysis is indicated by an *arrow*. The *scale bars* represents 10 μm; *3*. One hour interaction, 50% myxococcal inoculum. The *scale bar* represents 10 μm; *4*. Ninety hour interaction, 1% myxococcal inoculum. The boundary between the myxococcus's periphery and the cyanobacterial core is marked by *arrows*. The *scale bar* represents 100 μm; *5*. The core region of the colony shown in (4); *6*. Six day interaction, 1% myxococcal inoculum. The spherule was placed under a coverslip and gently compressed to reveal the internal morphology. Note the large crystals commonly observed in the core of such colonies. The *scale bar* represents 50 μm. **B** *1*. SEM of two mature colonies of *M. xanthus* PCO2 and *P. luridum* taken after 5 days of interactive culture. *Arrows* indicate the ensnared filamentous cyanobacteria. The *scale bar* represents 50 μm; *2*. A higher magnification SEM of the surface of the spherule. Note the presence of myxospores (*arrows*) along the edges of the outer layers of the spherule. The *scale bar* represents 5 μm. (Burnham J, Collart S, Highison B 1981 Arch Microbiol 129:285–294, by permission)

suffice to form an efficient "group". The wolfpack term has been used to depict an interaction based on a large quantity of predators. The "prey-trap mechanism" described above actually acts more wolfpack-like than stated in the definition: it traps the cyanobacterial prey with a scout force that attacks and weakens it, then new recruits (mostly obtained by way of cell division) force the prey into a constrained, surrounded space in which it is finally devoured. One has to keep in mind that bacterial predators may exhibit or combine various predatory strategies that might vary between closely related organisms, according to the type of substrate or to their environment.

Bdellovibrio and like organisms of the δ-proteobacteria (d-BALOs). The obligate predators *Bdellovibrio* spp., *Bacteriovorax* spp. and *Peridibacter* spp., all belong to the δ-proteobacteria and have been clustered under the name "*Bdellovibrio* and like organisms". However, *Micavibrio*, a clade of α-proteobacteria that should also be covered by the term BALO, were recently characterized. Therefore, we will use the term d-BALOs when *Bdellovibrio* spp., *Bacteriovorax* spp. and *Peridibacter* spp. are specifically addressed. "BALO" will be used when no specific affiliation is known.

Bdellovibrio and like organisms of the δ-proteobacteria, the most-studied group of predatory bacteria, were serendipitously discovered in 1962 by Heinz Stolp, while he pursued the isolation of soil bacteriophages (Stolp and Pertzold 1962, see introductory part of this volume).

d-BALOs are small ($0.25–0.5 \times 0.75–2\ \mu m$), rod or vibrio-shaped, highly motile Gram-negative bacteria (Figure 5A). d-BALOs possess a single sheathed, polar flagellum $\sim$ 28 nm in diameter, with an internal core $\sim$ 14 nm in diameter (Thomashow and Rittenberg 1985b). This flagellum exhibits a typical damping wave pattern (Thomashow and Rittenberg 1985a, Fig. 5A). d-BALOs are obligate predators of Gram negative bacteria, i.e. they are totally dependent upon other bacteria for their multiplication. These constitute their nutrient basis for growth, and cell to cell interactions are required for replication. The typical life cycle is composed of a free-swimming, attack phase and of prey-dependent stages (Fig. 5A). In almost all d-BALO strains, the predatory cell penetrates into the periplasm of its host, although a few exceptions are known (Shemesh et al. 2003, Fig. 5B). Under laboratory conditions, host independent mutants that require a rich medium for growth can be isolated from wild-type strains (Seidler 1969b; Varon et al. 1974; Barel and Jurkevitch 2001, Fig. 5C). For detailed descriptions of the life stage of wild-type d-BALOs and of host-independent mutants, their physiology and biochemistry, see the work by Tudor and McCann, and Strauch et al. in this volume.

BALOs are common in natural and manmade habitats. They are found in bulk soil (Stolp and Starr 1963), in the rhizosphere (Elsherif and Grossman 1996; Jurkevitch et al. 2000), in all sorts of water bodies —rivers (Fry and Staples 1974, 1976), the brackish environment of estuaries (Williams et al. 1982; Williams 1988; Rice et al. 1998), the open sea (Marbach and Shilo 1978; Williams 1987; Sanchez-Amat and Torrella 1989; Pan et al. 1997), hy-

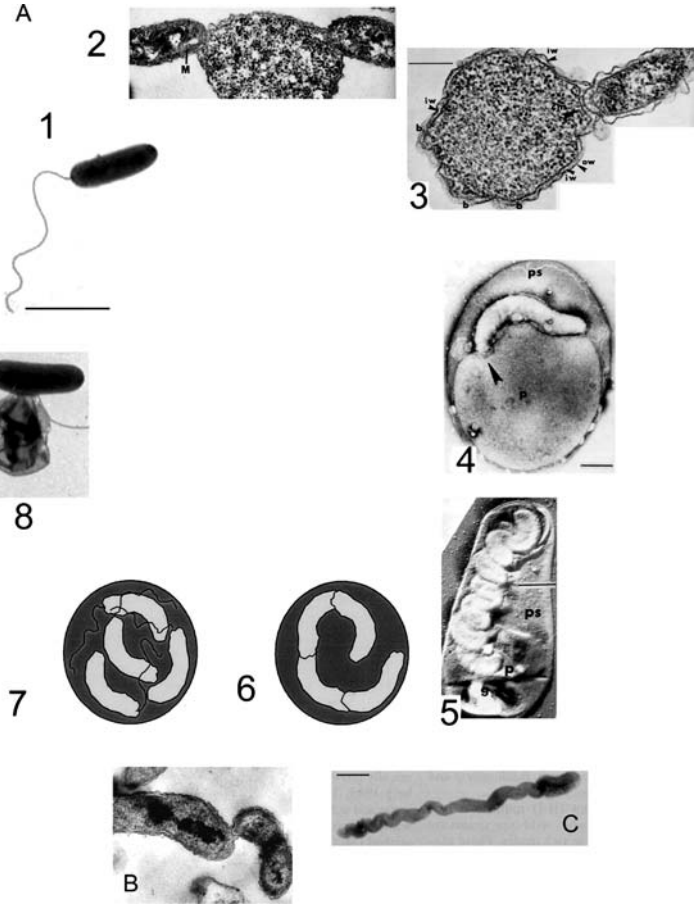


Fig. 5 **A** The life cycle of BALOs: *1*. An attack phase *Bdellovibrio bacteriovorus* 109J cell. Note the sheathed flagellum and its damping wave form. The *scale bar* represents 1 μm. *2*. Concomitant attachment of two *B. bacteriovorus* predators onto an *E. coli* prey. A mesosome (M) and an electron-lucent zone are visible. The *scale bar* represents 0.2 μm. *3*. Irreversible attachment and penetration. Note the fimbriae-like projections at the proximal, penetrating pole of the predator. cm: prey cytoplasmic membrane. The *scale bar* represents 0.2 μm. *4*. Establishment of the predator in the prey's periplasm and death of the prey. The predator is in contact with the prey's cytoplasm. ps: periplasm. p: prey's protoplast. The *scale bar* represents 0.2 μm. *5*. The intraperiplasmic BALO cell grows in a filamentous form while DNA replication occurs. g: granule. The *scale bar* represents 1 μm. *6*. and *7*. Fragmentation of the predator into progeny and synthesis of a single flagellum per progeny. *8*. Lysis of the prey ghost envelope and release of progeny cells. The *scale bar* represents 1 μm. (Abram D, Melo CJ, Chou D (1974) J Bacteriol 118:663–680; Barel G et al. (2005) J Bacteriol 187:329–335; Burnham JC, Hashimoto T, Conti SF (1968) J Bacteriol 96:1366–1381, by permission). **B** The epibiotic predator strain *Bdellovibrio* JSS preying upon a *Caulobacter crescentus* prey (Koval SJ (2001) Bacteriol 183—cover illustration. By permission). **C** A host independent mutant cell of *Bdellovibrio bacteriovorus* 109J (Barel and Jurkevitch (2001) Arch Microbiol 176:211–216, by permission)

persaline waters (Sanchez-Amat and Torrella 1989; Pineiro et al. 2004), at the various stages of treatment in water treatment plants (Dias and Baht 1965; Staples and Fry 1973; Afinogenova et al. 1981)—in crab gills (Kelley and Williams 1992), in hen and mammals feces (Schwudke et al. 2001), in extreme environments (Davidov and Jurkevitch 2004 and below), in biofilms (Kelley et al. 1997), and associated with biotic and abiotic surfaces (Kelley et al. 1997). Intriguingly, short sequences clustering within the *Bdellovibrionaceae* and within the *Bacteriovoracaceae* were obtained from clinical blood samples (GenBank accession numbers AY886552, AY886636, AY886663, AY886665, AY886691, AY886717, AY886727, AY886741, Davidov and Jurkevitch, unpublished data). Some phylogenetically undefined bacterial predators were classified as "*Bdellovibrio*" but this affiliation cannot be confirmed. This includes a predator of cyanobacterial symbionts of sponges (Wilkinson 1979) and a predator of *Chlorella vulgaris* (Coder and Starr 1978), later renamed *Vampirovibrio* (Gromov and Mamkayeva 1980).

The number of BALO propagules detected in environmental samples using a standard double-layer isolation procedure (see work by Koval in this volume) is usually low, ranging from tens to tens of thousands of plaque-forming units per gram or milliliter of sample. Accordingly, the number of BALO-related 16S rRNA sequences originating from environmental studies and found in databases is also low (to date, less than one hundred). The paucity of such sequences in the databases may stem from PCR biases when domain-wide 16S rDNA primers are used for amplifying environmental DNA, as BALOs do not form dominant populations. The recent development and application of specific 16S rDNA primers targeting the various groups within the BALOs promises to expand our ability to assess their diversity, without reliance on cultivation (Herschkovitz et al. 2005; Davidov et al. 2006a).

BALO strains are usually able to utilize various Gram negative prey (Stolp and Starr 1963; Jurkevitch et al. 2000). Some strains were reported to prey upon a single species (Shemesh et al. 2003), although this should be treated cautiously: most bacteria are not amenable to cultivation, therefore most prey may go unidentified. To date, the preys used to isolate and characterize BALOs were almost exclusively from the *Proteobacteria* phylum (mainly *Escherichia coli, Pseudomonas* spp. and *Erwinia* spp. for terrestrial and freshwater habitats and *Vibrio parahaemolyticus* for marine habitats) and the range of BALO strains isolated may therefore be accordingly restricted. Moreover, at least for terrestrial habitats, the commonly used preys are not relevant for soils, where the prevailing bacterial groups are different, but unfortunately, often not culturable, or difficult to grow. In a few such attempts to isolate predators using autochthonous bacteria as prey, it was shown that most Gram negative bacteria isolated from the Great Salt Lake and from the Chesapeake Bay were susceptible to BALOs isolated from the same habitat (Pineiro et al. 2004; Rice et al. 1998). Remarkably, a few of these strains belonged to the Flavobacteria, and other strains could not be identified (Rice et al. 1998). In

conclusion, a definition of BALOs as generalist rather than specialist predators may be better suited, since probably most (if not all) BALOs are able to use a range of prey in nature.

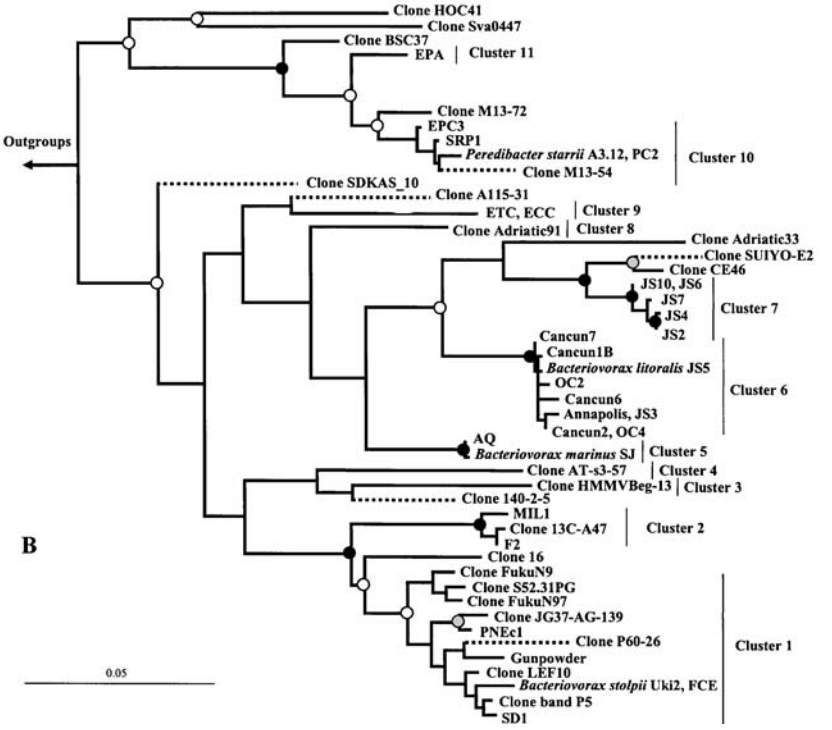
The unique bacteria isolated by Stolp and Pertzold (1962) were soon defined as the new genus *Bdellovibrio*, specie *bacteriovorus* (Stolp and Starr 1963). On the basis of whole genome DNA–DNA hybridization tests, GC contents, rRNA homology, enzyme migration patterns and genome estimation sizes, two new species, *Bdellovibrio starrii* and *Bdellovibrio stolpii* were later proposed (Seidler et al. 1972). Although BALOs exhibited high variability in their GC ratio, fatty-acid profiles, serological reactions and partial 16S rRNA sequences, and DNA–DNA hybridizations could yield very low values between certain strains (Seidler et al. 1972; Kramer and Westergaard 1977; Torrella et al. 1978; Hespell et al. 1984; Donze et al. 1991), d-BALOs were kept under a single genus for almost four decades. Recent molecular studies, for the most part based on 16S rRNA analyses drastically changed this: *Bdellovibrio stolpii* and *Bdellovibrio starrii* were reclassified as the new genera *Bacteriovorax* (Baer et al. 2000) and *Peredibacter* (Davidov and Jurkevitch 2004), respectively. Two new species, *Bacteriovorax marinus* and *Bacteriovorax litoralis* were defined (Baer et al. 2004). Except for *Micavibrio*, known BALOs are affiliated within the *δ-proteobacteria* (Woese 1987). However, they do not form a monophyletic group but rather two very heterogenic clades (Davidov and Jurkevitch 2004). These were reclassified into the two families *Bdellovibrionaceae* and *Bacteriovoracaceae* (Davidov and Jurkevitch 2004), together forming the *Bdellovibrionales* order.

The phylogenetic diversity of d-BALOs summarized here is based on all the 16S rRNA available sequence data gathered from isolated strains and environmental clones (Baer et al. 2000; Jurkevitch et al. 2000; Schwudke et al. 2001; Snyder et al. 2002; Davidov and Jurkevitch 2004; Pineiro et al. 2004; Song 2004). Phylogenetic analyses suggested the existence of 11 stable monophyletic clusters within each of the *Bdellovibrionaceae* and *Bacteriovoracaceae* (Davidov and Jurkevitch 2004, Fig. 6A,B). Clusters were separated by at least 3% divergence, and therefore represent putative species (Stackebrandt and Goebel 1994). Some of these clusters together formed stable monophyletic groups. As the distances between these groups were above 6%, they may be defined as different genera (Vandamme et al. 1996; Ludwig 1998). Within the next section we'll provide some detail on the structure of the *Bdellovibrionaceae* and *Bacteriovoracaceae* families.

Bdellovibrionaceae. Isolates and clones of the *Bdellovibrionaceae* originated from soil, roots, fresh water, sewage, and vertebrata feces, but none were from marine environments. Eleven stable monophyletic clusters were included within this clade (Fig. 6A).

The largest cluster (cluster 1) comprised the majority of the isolates, from all the habitats described above. Also included were *B. bacteriovorus* strains 100^T and 109J, the most studied *Bdellovibrionales*. Both 16S rRNA se-


Outgroups
Clone Oral CA006
R4
TRA2
Cluster 11
Clone PHOS-HC31
Clone mv13.13
Clone SM1G01
Clone SM1H08
Cluster 10
Clone BPC2_F12
Clone HC20
Cluster 9
MPA
Cluster 8
Clone D10
Clone A1
Clone 300A-H04
Clone C12-11
Cluster 7
BEP2, BRP4, Ec13
Cluster 6
W
Cluster 5
Clone WB-66
Clone TRK42
ETA, MTA
R0
SRA9
Cluster 4
Clone PHOS-HE81
JSS
Cluster 3
Clone R6-126
HEA, SRE14, SJE
Clone Sta2-55
Clone 6i39
NASA
Lanham
Cluster 2
BG278
BG282
BG279
BG287
Hefner
100, 109J, 127, E, 6-5-S, 109D, Tu-113, Ox92, Viet, CHI
SRE7
2484Se2
ARL1
L
ARL12
114
BG260
BG273
Cluster 1
A
0.05


Clone HOC41
Clone Sva0447
Clone BSC37
EPA
Cluster 11
Clone M13-72
EPC3
SRP1
Peredibacter starrii A3.12, PC2
Clone M13-54
Cluster 10
Outgroups
Clone SDKAS_10
Clone A115-31
ETC, ECC
Cluster 9
Clone Adriatic91
Cluster 8
Clone Adriatic33
Clone SUIYO-E2
Clone CE46
JS10, JS6
JS7
JS4
JS2
Cluster 7
Cancun7
Cancun1B
Bacteriovorax litoralis JS5
OC2
Cancun6
Annapolis, JS3
Cancun2, OC4
Cluster 6
AQ
Bacteriovorax marinus SJ
Cluster 5
Clone AT-s3-57
Cluster 4
Clone HMMVBeg-13
Cluster 3
Clone 140-2-5
MIL1
Clone 13C-A47
F2
Cluster 2
B
Clone 16
Clone FukuN9
Clone S52.31PG
Clone FukuN97
Clone JG37-AG-139
PNEc1
Clone P60-26
Gunpowder
Clone LEF10
Bacteriovorax stolpii Uki2, FCE
Clone band P5
SD1
Cluster 1
0.05

quence analysis and whole genome DNA–DNA hybridization data (Baer et al. 2000; Davidov and Jurkevitch 2004, Fig. 6A) were indicative of a high internal diversity, suggesting the existence of more than one species within the group. Nonetheless, no environmental clone from the databases belonged to this clade, implying that the protocol (or preys used) employed to retrieve BALOs may introduce an isolation bias that does not reflect their natural distribution.

Strain JSS (cluster 3) was isolated from sewage and preyed upon *Caulobacter crescentus*. Contrarily to all other d-BALOs, it did not penetrate its prey's periplasm, did not form a bdelloplast and divided by binary fission (Fig. 5B). It cannot be determined whether this epibiotic life cycle is an ancestral phenotype or a specific feature of this strain. However, strain HEA, its closest relative, exhibited a "standard" periplasmic behavior, suggesting that the shift between the two phenotypes might not imply profound changes in the predatory arsenal. Alternatively, JSS may prey differently upon other prey in nature.

Although prey ranges are usually not linked to phylogenetic affiliations, the strains constituting clusters 4 and 11, retrieved from six different soil samples, were all isolated using *Agrobacterium tumefaciens* as a prey. No explanation can be provided for this particular feature. Members of the seven remaining clades exhibited varied features: strain W is the sole member of cluster 5 and the only available d-BALO isolate known to form a bdellocyst resting stage (Hoeniger et al. 1972). All the members of cluster 7 are environmental clones obtained from rocks contaminated with crude oil (C12-11), groundwater contaminated with high levels of nitric acid bearing uranium waste (300A-H04), or from a subsurface microbial community (A1 and D10). The unique clone constituting cluster 9 originated from an arsenite oxidizing biofilm. The unclassified clone BPC2_F12 originated from subalpine stream sediments. Two environmental clones obtained from hot spring travertine depositions at 55 °C, formed cluster 10. These two sequences showed clear signs of unusually rapid evolution (Davidov and Jurkevitch 2004). Clone mv13.13 was identified during an analysis of methanotrophic communities using $^{13}CH_4$ for stable isotope probing (SIP) (Hutchens et al. 2004). Three

◀ **Fig. 6** Phylogenetic 16S rRNA tree of the *Bdellovibrionaceae* (**A**) and of the *Bacteriovoracaceae* (**B**) lineages. The tree is based on maximum-likelihood (FastDNAml) analysis, using a 50% conservation filter. Branch points supported by parsimony bootstrap values (1000 replicates) of > 90%, > 75% and > 50% are indicated by *filled black*, *filled gray* and *open circles*, respectively, while branch points without circles were not resolved (bootstrap values of < 50%). Partial sequences ($\leq$ 600 bp), which did not provide sufficient information for an unequivocal phylogenetic position are indicated by *dash lines*. *Scale bar* indicates 5% (**A**) or 10% (**B**) estimated sequence divergence. *Bacteriovoracaceae* strains Uki2^T, A3.12^T and ETC were used as outgroups for treeing the *Bdellovibrionaceae* lineage while *Bdellovibrionaceae* strains 100^T, HEA and TRA2 were used as outgroups for treeing the *Bacteriovoracaceae* lineage. (Davidov Y, Jurkevitch E 2004 Int J Syst Evolution Microbiol 54:1439–1452, by permission)

additional $^{13}CH_4$ labeled clones, including one clone from another independent DNA–SIP experiment (Morris et al. 2002), were most closely related to *Bdellovibrionaceae* but these short sequences and their outlying phylogenetic position did not allow their certain classification as true *Bdellovibrionaceae* (not shown). It should be added that phylogenetically undefined BALOs preying on *Methylomonas* sp. were isolated from the broth of an abnormal fermentation in a methanol pilot plant (Lin and Wang 1983). The occurrence of BALOs in such remarkable habitats shows that these bacteria inhabit quite extreme environments. It is reasonable to speculate that these clones represent "real" predatory bacteria, active within these habitats, as the most divergent cluster of the tree is constituted of isolated, phenotypically defined predatory bacteria (cluster 11). In contrast, the outlying oral clone CA006 although clearly clustering with the *Bdellovibrionaceae* lineage cannot be confidently assigned the d-BALOs. The large divergence (> 6.5%) exhibited between clusters 2 plus 3, 10 and 11 and the other *Bdellovibrionaceae* supports their definition as new genera.

Bacteriovoracaceae. At least four groups and 11 clusters representing different putative genera and species, respectively, can be identified within the *Bacteriovoracaceae* family (Davidov and Jurkevitch 2004, Fig. 6B). Isolates and clones forming the two groups A3.12^T and Uki2^T (Davidov and Jurkevitch 2004, Fig. 6B), originated from soil, fresh water or sewage samples but not from marine environments. Isolates and clones from the other groups were of marine origin, except for the ETC cluster. The organisms constituting this cluster were isolated from a highly saline soil. Some of the "marine" clones were found in corals (BM89, DS1, BbB4), including white (A115-31) and black (140-2-5) band-diseased corals, marine sponge (Hoc41), deep sea hydrothermal sites (SUIYO-E2, AT-s3-57), methanotrophic communities (HMMVBeg-13), and arctic marine sediments (Sva0447).

16S rRNA-based phylogenies suggested that *Bdellovibrionaceae* and *Bacteriovoracaceae* clustered best within the *δ-proteobacteria* (Woese 1987). However, they did not form a monophyletic group and the phylogenetic distance between them is very large (> 20%) (Davidov and Jurkevitch 2004). Neither of these groups formed a stable clade with any other δ-proteobacterial group. 16S rRNA secondary structure analysis showed that *Bdellovibrionaceae* contain motifs atypical of the *δ-proteobacteria*, suggesting it is ancestral to *Bacteriovoracaceae* (Schwudke et al. 2001; Davidov and Jurkevitch 2004). As no other group clustered between these two families, and given their morphological similarity and unusual life cycle, it is reasonable to hypothesize that most of their shared unique characters originated from the same common predator ancestor.

Using this phylogenetic information it is now possible to design oligonucleotides that specifically target groups of d-BALOs. A *Bdellovibrio*-directed 16S rRNA probe is presented by Koval in another part of this volume. We have developed a series of 16S rDNA primers that specifically target the *Bdellovib-*

rionaceae, the *Bacteriovoracaceae* excluding *Peridibacter, Peridibacter*, and also the α-proteobacterial predator *Micavibrio*. Sequences that were closely or distantly related to any known BALO were retrieved from soil DNA, thereby demonstrating that the application of targeted culture-independent strategies will significantly increase the known diversity of BALOs (Davidov et al. 2006a).

2.5 Chloroflexi

Herpetosiphon species are aerobic, chemo-organotrophic, filamentous bacteria that are Gram-negative but do not have a typical Gram negative cell wall (Reichenbach 2001). They form filaments that reach great lengths of 300 to 1200 μm, and they can glide on surfaces. They are commonly encountered in aerobic environments such as soil, decaying organic matter, freshwater and activated sludge. Although they are part of the *Chloroflexi* clade, they are non-photosynthetic. Not all *Herpetosiphon* isolates were capable of lyzing bacterial cells, and sludge organisms did not lyze bacteria at all (Reichenbach 2001). The strains able to do so lyzed dead as well as living bacterial colonies. *Herpetosiphon* strains may differ in prey range, with preyed-upon bacteria including both Gram negative (including *Myxococcus* spp.) and Gram positive cells (but not spores) (Quinn and Skerman 1980). The ability to produce a capsule around the cells apparently provided prey cells with some protection against predation (Quinn and Skerman 1980), in contrast to what was observed with *Bdellovibrio* predators that attack capsulated bacteria (Koval and Bayer 1997). On Petri dishes, the predatory sequence included the penetration of the prey colony through sheer physical pressure by swarms of *Herpetosiphon* cells, followed by cell proliferation as the *Herpetosiphon* filaments grew, forming a great cell mass at the margins of the colony (Fig. 7). This mode of action is reminiscent of a wolfpack-like strategy (Dworkin 1999) combined with a trap strategy. Lysis of prey cells occurred beyond the colony edge, enabling the filaments to advance toward the center of the colony thus indicating that extracellular, diffusing bacteriolytic enzymes were produced. The commonness of *Herpetosiphon* in freshwater and their predatory ability suggests they may have an influence on lake microbial communities (Quinn and Skerman 1980).

2.6 Cytophagaceae

A number of *Cytophaga* strains were isolated from a lake in Quebec at the onset of the decline of cyanobacterial blooms (Rashidan 2001). The predators differed in prey range, one of them solely preying upon *Anabena-flos-aquae* cultures, another on various *Synechococcus* species as well as on *Anacystic*

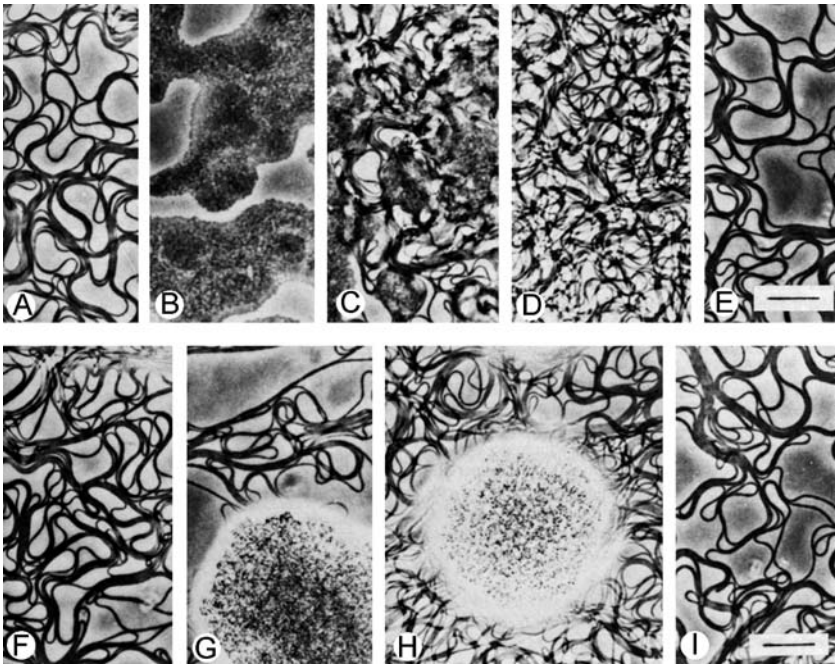


Fig. 7 Growth of *Herpetosiphon* UQM 1753. Before (**A**), during (**C**, **D**) and after (**E**) traversing a confluent streak of *Chromatium violaceum* UQM 51 (**B**) inoculated 48 h prior to the *Herpetosiphon*; **C**. Early attack on host cells; The *scale bar* represents 25 μm; **D**. Total digestion with intense filament production. The *scale bar* represents 25 μm. Before (**F**), during (**G**, **H**) and after (**I**) traversing a streak of *Micrococcus luteus* UQM 117 inoculated 48 h prior to the *Herpetosiphon*; **G**. Initial entry of filaments between colonies of *M. luteus*. **H**. Colonies are totally surrounded but remain undigested. The *scale bar* represents 25 μm. (Quinn R, Skerman V (1980) Curr Microbiol 4:57–62, by permission)

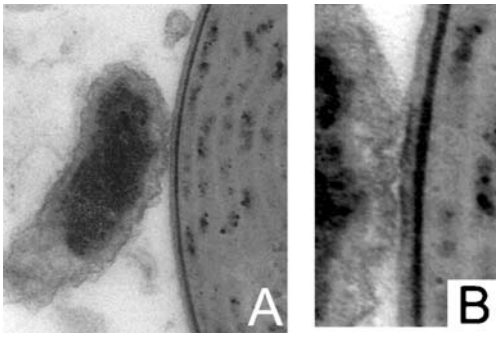


Fig. 8 **A** Electron micrographs showing attachment of *Cytophaga* strain C1 to a cell of *Anabaena flos-aquae* (×80 000). **B** Expanded view of attachment site where changes in electron density of the cell wall are evident (×280 000). (Rashidan KK, Bird DF (2001) Microbial Ecology 41:97–105, by permission)

nidulans. These facultative predators required cell to cell contact in order to lyze their prey (Fig. 8A). The prey's cell wall exhibited changes at the point of contact (Fig. 8B). No extracellular lytic factor could be isolated (Rashidan 2001), suggesting that the bacteriolytic activities were bound to the predator's surface. Other predatory *Cytophaga* were reported to lyze diatoms (Chan et al. 1997) or other marine phytoplankton (Imai et al. 1993).

2.7 Gram Positives

Streptoverticillium. A filamentous, facultatively predatory Gram positive bacterium resembling *Streptoverticillium* was isolated from a number of soils along with *Ensifer adhaerens* (Casida 1980). It efficiently preyed upon *Micrococcus luteus* by lyzing their colonies. This bacterium sought hosts by extending a slender filament from colony to colony. Lysis of the *M. luteus* cells may have been due to a diffusible factor and not necessarily to direct contact between the predator and its prey.

Agromyces ramosus. *Agromyces ramosus*, another facultative predator belonging to the *Actinomycetes* is a common soil inhabitant (Casida 1983). Some strains were capable of attacking and destroying various Gram negative and Gram positive bacteria, including *Azotobacter vinelandii*, *Rhizobium leguminosarum*, *Sinorhizobium meliloti*, and *Agrobacterium tumefaciens*, as well as yeast cells (Casida 1983) in a process that seemed to involve chemotaxis (Byrd et al. 1985). Prey cell destruction required that both predator and prey be in close vicinity, with the prey entwined by or in contact with the predator. No diffusible factor could be detected. This facultative predator could be consumed by another facultative bacterial predator, *Cupriavidus necator*.

2.8 Archea

Although apparently not predatory in the sense that the attacked cells are not killed in the process, the interaction between two archea species provides a fascinating glimpse at the evolution of bacterial parasitism. The first and hitherto uniquely described case of parasitism in *Archea* is the association of *Nanoarchaeum equitans* that represents the new archeal phylum Nanoarchaeota, with *Ignicoccus* sp. (Huber 2002). *N. equitans* cells are spherical (0.4 μm in diameter). They grow attached to the surface of their specific archeal host (Fig. 9) under anaerobic conditions at 90 °C and in the presence of S, H_2 and CO_2. *N. equitans* bears a small genome (less than 0.5 Mbp) which lacks genes for lipid, cofactor, amino acid, or nucleotide biosynthesis (Waters 2003). These, and possibly energy, must be acquired from the host. Probably forming part of the substrate-supply mechanism for *N. equitans*, many vesicles are formed in the cytoplasmic membrane of the *Ignicoccus* host (Hu-

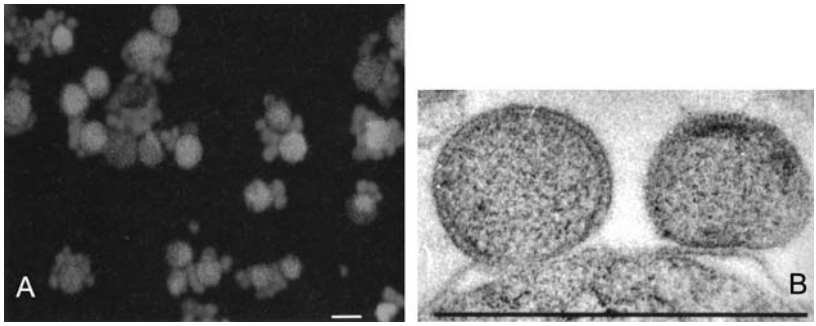


Fig. 9 Electron microscopy and fluorescence microscopy of the *Nanoarchaeum equitans–Ignicoccus* sp. coculture. **A** Confocal laser scanning micrograph after hybridization with a CY3-labeled probe 515mcR (*Nanoarchaeum*, *gray*) and a rhodamine-green-labeled probe CREN499R (*Ignicoccus*, *white*). **B** Ultrathin section of two cells of *Nanoarchaeum* attached to the outer membrane of *Ignicoccus*. *Scale bar* in both panels = 1.0 μm. (Stetter K, by permission)

ber et al. 2000). As seen below, vesicular extrusion may be a rather common mechanism for acquiring substrate from parasitized or preyed-upon cells. Phylogenetic analysis showed that although *N. equitans* diverged relatively early in the archeal lineage, it is not primitive and its genome appears to be stable (Waters 2003).

2.9 Phylogenetically Undefined Predators

A number of obligate and non-obligate predatory bacteria have been observed or isolated from various environments. They differed greatly in the type of prey they were interacting with, in their morphologies and modes of predation.

Vampirococcus* and *Daptobacter. Blooms of photosynthetic bacteria in sulfurous lakes in Spain provided the grounds for the observation, characterization and isolation of various predators of *Chromatium* spp.

Increasing numbers of an epibiotic bacterium preying upon *Chromatium minus* cells were found with increasing depth, suggesting that this organism was an opportunistic, anaerobic scavenger, taking advantage of environmental conditions detrimental to *Chromatium* (Esteve et al. 1983; Guerrero et al. 1986). These aptly named *Vampirococcus* cells appeared as little spheres (0.6 μm in diameter), ovals or slightly curved rods (0.3×0.6 μm) attached onto the surface of *Chromatium* (Esteve et al. 1983). *Vampirococcus* was not motile, therefore could probably not find its prey by chemotaxis. Nevertheless it seemed to be an obligate predator. Binary cell fission was only observed in cells attached to their prey. At the attachment site, a conspicuous, electron-opaque structure was observed (Fig. 10).

Daptobacter, a rod-like (0.5×1.5 μm) Gram negative bacterium was also observed to prey upon *Chromatium* spp. *Daptobacter* attached to the prey, breached the membranes, penetrated and degraded the cytoplasm, to finally divide within the emptied prey cell (Guerrero et al. 1986, Fig. 11A). This was dubbed a "direct-invasion" strategy (Martin 2002) or a "diacytotic" strategy (Moulder 1985). This bacterium was motile, and facultatively anaerobic.

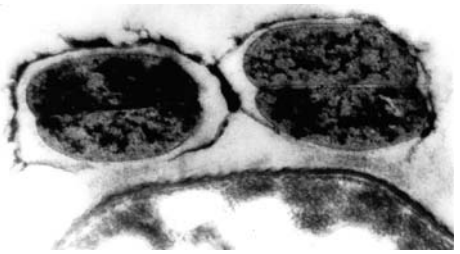


Fig. 10 *Vampirococcus* cells attached to *Chromatium*. There is a cross wall in the dividing cells. The attachment structure is made of dense material that spans between the *Vampirococcus* and the *Chromatium* cells. The outer membrane of the predator is breached where the dense material appears. (Kindly supplied by I. Esteve)

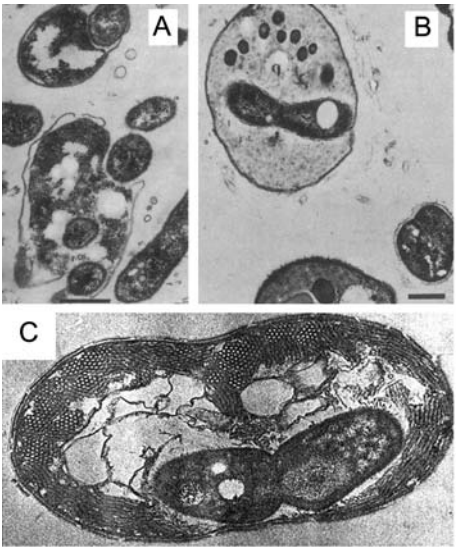


Fig. 11 *Daptobacter* penetrates both membranes of the prey cell walls and reproduces in their degrading cytoplasm. **A** Three *Daptobacter* cells are clearly associated with the degradation of a single prey cell's cytoplasm. **B** A *Daptobacter* cell dividing in the partially degraded cytoplasm of a *Chromatium* cell. A *Vampirococcus* cell can be seen on the lower right. **C** A *Daptobacter*-like cell in a *Chromatium* cell. The *scale bar* represent 0.5 μm. (Guerrero R (1991), by permission)

Electron-lucent zones were apparent at the cell poles (Guerrero 1987). While *Daptobacter* did not prey upon various heterotrophic bacteria or upon *Rhodospirillaceae*, it did utilize a number of *Chromotium* spp. and *Thiocapsa* spp. as prey. Also, *Daptobacter* was a facultative predator and could be grown axenically in rich media (Guerrero 1987). *Chromatium* cells have been shown to harbor other *Daptobacter*-like bacteria in their cytoplasms but these still remain unidentified (Guerrero 1991, Fig. 11B).

Unfortunately, the phylogeny of these bacteria is unknown. Although *Daptobacter* has been isolated, the strain available with culture collections is not *Daptobacter*.

Predators of Gram positive rods. Small rods (1×0.4 μm) denominated X-pfr, and preying upon *Clostridium perfringens* were isolated from Seine and Arkansas waters (Guelin and Maillet 1978). The predator adhered to the surface of the prey, along which it multiplied until it formed a thin cell layer that covered the prey (Fig. 12A). The destruction of the host seemed to occur through the rupture of its membrane and exudation of the cell content (Fig. 12B). Although the predator was attached epibiotically to its prey during the latter's destruction, X-pfr cells could sometimes be found in emptied prey cells as well (Fig. 12C).

Santek, a predatory bacterium originating from sea waters near Roscoff (France) was observed preying upon *Bacillus megatherium* (Guelin and Maillet 1978). This bacterium (1.2×0.4 μm) had a *Caulobacter*-like morphology (Guelin and Maillet 1978). It attached to its prey through its pedoncule. The latter's cytoplasm was lyzed but later on, Santek cells also lyzed, for an unknown reason (Fig. 13).

Parasites of *Thiotrix* sp. A *Thiotrix* sp. was found growing over larvae of the mayfly *Drunella grandis*, with apparently no deleterious effect upon the fly (Larkin 1990). Electron microscopy revealed that at least three bacterial morphotypes with Gram negative cell structures were multiplying within the *Thiotrix* trichomes. Two of the morphotypes clearly induced destruction

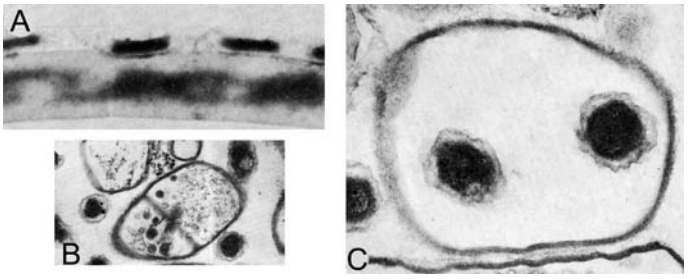


Fig. 12 The predatory bacterium X-pfr preys upon *Clostridium perfringens*. **A** X-pfr adheres and multiplies on the prey surface (×12 000). **B** The host membrane is ruptured, releasing prey cell content (×37 500). **C** X-pfr cells are sometimes found in empty prey cells (×37 500). (Guelin A, Maillet P-L (1978) CR Acad Sc Paris Serie D, by permission)

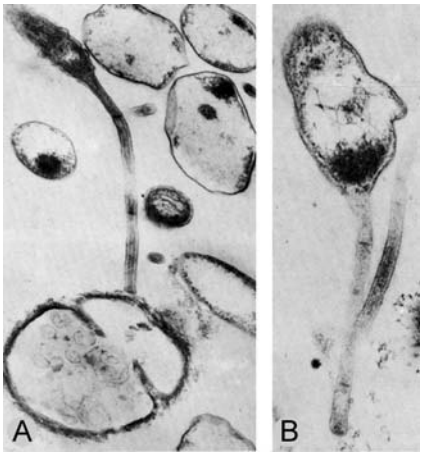


Fig. 13 The Santek predator attached to the prey through its peduncle. **A** The prey's cytoplasm is lyzed (×29 000). **B** The predator, for unknown reasons is later degraded (×37 500). (Guelin A, Maillet P-L 1978 CR Acad Sc Paris Serie D, by permission)

of the host by penetrating its cytoplasm, while a third appeared to settle within the periplasm (Fig. 14). None of these bacteria were defined. Within *Thiothrix*, the two types of "periplasmic" and "diacystotic" predatory strategies are exemplified. Whether these three morphotypes represented different species or morphs of one or two species is unknown.

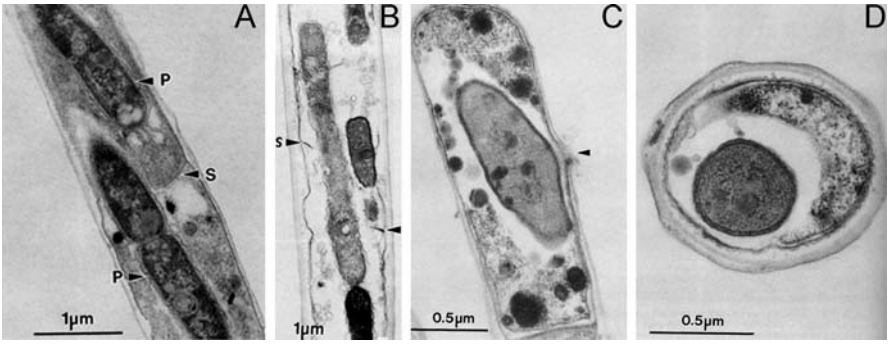


Fig. 14 Infected *Thiothrix* trichomes on a mayflies larvae infected with predatory bacteria. **A** A single cell of morphotype 1 and a dividing cell of the parasite (P) within a *Thiothrix* trichome. Note that the lower parasite is penetrating the septum (S) between two cells of the host. **B** Parasites of morphotype 2. Note the remnants of destroyed septa (S) and the lack of cytoplasm in the host. The ends of this parasite are blunt. **C** Parasite of morphotype 3 within the terminal cell of a *Thiothrix* trichome. The *arrow* indicates a place where a weakness in the cell wall may have been produced and through which the parasite may emerge. **D** Transverse section of a parasite of morphotype 3. It appears that the cytoplasm has been pushed aside and that the parasite is in the periplasmic space. (Larkin JM, Henck MC, and SD Burton 1990 Appl Environ Microbiol 56:357–361, by permission)

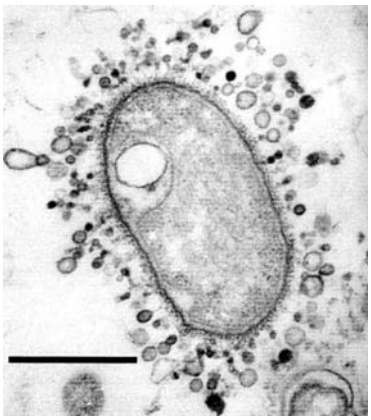


Fig. 15 Thin section of an unidentified Gram-negative bacterium found in a freshwater biofilm in a river. This bacterium possesses a micro-capsule and is liberating a prodigious amount of membrane vesicles. The *scale bar* represents 1 μm. (Beveridge TN 1999 J Bacteriol 181:4725–4733)

Membrane vesicles. Gram negative bacteria constantly produce outer membrane vesicles and discharge them into the environment (Beveridge 1999, Fig. 15). These vesicles contain outer membrane proteins, lipopolysaccharides, phospholipids, and periplasmic contents, including autolysins, which are peptidoglycan hydrolyzing enzymes (Li et al. 1996, 1998). The production of vesicles has been noticed in many instances, including from the natural environment (for a review, see Beveridge 1999). Membrane vesicles were shown to have a lytic action upon Gram positive and Gram negative bacteria (Li et al. 1998). However, only starved cells were attacked, suggesting that the vesicle-producing cell may use this capacity to increase its nutrient basis, in a "remote distance/wolfpack" kind of predation. Vesicles produced by the opportunistic, soil dwelling human pathogen *Pseudomonas aeruginosa* were recently shown to pack quinolic signaling molecules for interspecies communication, along with antibacterial toxins (Marshburn et al. 2005).

3
A Word on Predatory Strategies

Martin (2002) proposed to divide bacterial predation into four basic strategies: Wolfpack, epibiotic, direct invasion and periplasmic. However, it may be difficult to draw clear-cut lines that delineate between these functional predatory strategies. Variations on the themes, utilization of more than one and possibly other strategies are possible and may even be common, as may be suggested by the behavior of some *Myxococcus* and *Lysobacter* strains (see above).

Within the *Bdellovibrio*, strain JSS and a few other strains exhibit an epibiotic behavior (Shemesh et al. 2003) while all other known d-BALO strains are periplasmic. Therefore, their classification as "periplasmic predators" may not reflect the diversity of strategies that are found within this type of predator. Whether these behaviors are solely predator-dependent or prey- or environmental condition-dependent is not known. Furthermore, culture supernatants of various d-BALO strains could lyze heat-killed prey cells (Shilo 1965; Seidler 1969b) and mixing predator with prey under a high predator–prey ratio resulted in prey lysis before penetration (Varon 1968), indicating that predator density may exert some effect upon prey cells. Also, the formation of stable bdelloplasts was increased in diluted cultures of marine d-BALOs, suggesting density-dependent mechanisms are involved in this process (Sanchez-Amat and Torrella 1990). With all other factors kept constant, the concentration and ratio of both predator and prey are crucial parameters in determining predation rates and predator survival (Hespell et al. 1974; Varon 1978).

It should be emphasized that in order to obtain a larger picture of the interaction of a predatory bacterium with its prey, predatory "strategies" cannot be disconnected from the larger ecological context. This includes, among others, aspects such as resource allocation and its influence upon the prey (Fussmann et al. 2000), "environmental" conditions (Wilkinson 2001; Buckling and Rainey 2002; Forde 2004), spatial and temporal influences (Buckling and Rainey 2002; Forde 2004), prey responses (Agrawal 2001; Shemesh and Jurkevitch 2004. Also see the work by Jurgens in this volume) and genetics and evolution (Bohannan et al. 2002; Yoshida et al. 2003). For a detailed discussion of predator–prey dynamics, see the work by Wilkinson in this volume.

4
Predation Between Prokaryotes: An Evolutionary Perspective

4.1
Origins

The study of predatory bacteria almost inevitably leads to the study of the evolution of predation itself.

Prokaryotic organisms first evolved during the Archean era, 3800 millions years ago (Schopf 1993), and probably remained the only cellular life form for more than one billion years, until the first eukaryotic cells developed (Brocks et al. 1999; Nealson and Conrad 1999). During that time, many of the ecological functions performed, and the biochemical diversity exhibited by bacteria were established, and the major bacterial lineages diverged (Hoenigsberg 2002; Sheridan et al. 2003). Whether life at its beginning was

heterotrophic or autotrophic (Maynard-Smith and Szathamary 1995), as it expanded, so did the amount of dead material, offering a niche for the evolution of scavengers that could efficiently degrade and recycle this material. Such degradation entailed the presence of enzymes able to break down polymeric material into lower molecular weight components that could be taken up. As such, these components are more diffusible. So, the closer the substrate to the degrader's uptake system, the lower the amount of compounds lost to the environment (Guerrero 1987). The ability to move onto a surface (by means such as gliding or twitching motility) would also have been advantageous, as the decomposer would stick onto its food and could slide along the surface to find new, pristine spots. This ability probably evolved independently a number of times in the various groups of gliding bacteria, as different mechanisms are involved in this process (McBride 2004). It is interesting that in *Flavobacterium johnsoniae* and *Cytophaga johnsonae*, non-motile mutants fail to utilize chitin (Chang et al. 1984; Kirchman 2002; McBride 2003). It was suggested that in *F. johnsoniae*, gliding evolved from a polysaccharide utilization system (McBride 2004). Could the ability to stick to a surface that is also a substrate for growth lead to the evolution of parasitism or predation?

Bacterial predators and degraders essentially perform the same processes, i.e. they attach onto and then degrade polymeric substrates but with one major difference: predators degrade the cell walls of living cells. At first, access to the nutritious contents of a prey cell would not necessarily require additional functions, as breaching the cell wall may lead to membrane disruption and total cell rupture. It becomes clear that within the prokaryotic realm, such bacterial predators would gain from being smaller than their prey. It therefore can be argued that the evolution of predation, at least between bacteria, may almost be a consequence of their degrading ability. Could that account for the presence of bacteria able to display a predatory behavior in widely different taxonomic groups?

If the hypothesis presented here is acceptable, it seems reasonable to us to assume that predation within prokaryotes has evolved many times, as already proposed by Guerrero (1987), as is the case within the metazoans. Whether the traits endowing predatory abilities can be laterally transferred between different bacterial species or not is not known. The huge increase in data from whole genome sequencing projects will enable comparative analyses that could help answer some of the questions asked: what are the phylogenies of the lytic complement of predatory bacteria? What makes a bacterium a predator? Can these traits spread by lateral gene transfer? Is there a stepwise transition between degraders to non-obligate predators and to obligate predators?

Obligate predatory bacteria do not only rely on their prey for growth, but are also directly dependent upon them for multiplication, blurring the distinction between predator and parasite (and in turn, the one between prey and host). This is especially true for obligate predators like the BALOs that are

defined by an incapability to propagate extra-cellularly (Varon 1969). However, while terminological representation is a problem, fuzzy lines such as those between predation and other interactions are common in natural phenomena (Bengston 2002). The spontaneous occurrence of host independent variants of d-BALOs that retain a predatory behavior (Barel and Jurkevitch 2001) suggests that the transition between a facultative to an obligate predatory life history may only involve a limited adaptive sequence (Cotter and Thomashow 1992; Rendulic et al. 2004). The study of this system may help understand the evolution of such transitions. One could notice that numerous members of the taxonomic groups to which non-obligate predators belong are non predatory (although in most cases no verification of the predatory phenotype has been attempted). Many of these non-obligate predators belong to groups of bacterial scavengers such as the myxobacteria, *Lysobacter* or *Cytophaga*, all endowed with gliding motility and strong degrading capacities, and with the possession of a wealth of extracellular or membrane-bound polymer-degrading enzymes (Reichenbach 2001; Kirchman 2002). Similarly, the obligate predators *Bdellovibrio bacteriovorus*, *Bdellovibrio* strain W and *Bacteriovorax marinus* have among the largest arsenal of hydrolytic enzymes. Notably, sequences homologous to *Myxococcus* genes encoding for twitching motility and adventurous gliding are found, at least in the genome of *Bdellovibrio bacteriovorus* type strain 100 (Rendulic et al. 2004. See the work by Tudor and McCann, in this volume). It is tempting to propose that the adsorptive and the motile capacities, as well as the lytic functions found in heterotrophic degraders stood at the basis of the development of the attachment mechanisms and of the lytic capacities of predatory bacteria.

Attachment to a prey was investigated in d-BALOs and was shown to require two steps, which are independent of protein synthesis (Varon 1968, Fig. 5A). First, there is a reversible event during which the predator appears to scan the prey's surface. Often, the predator will not commit to the second step of irreversible attachment preceding degradation of the prey cell (Shemesh and Jurkevitch 2004). This step appears to involve fimbriae-like projection, from the pole of the predator to the cytoplasmic membrane of the prey (Fig. 5A). Whether the same dynamics are at work with non-obligate predators is not known. It can be argued that in the case of an obligate predator, commitment through irreversible binding to the prey could be a crucial, life-or-death decision and should therefore be tightly controlled.

B. bacteriovorus exhibits an extremely efficient metabolism (Hespell 1978) which is dependent on the timely and gradual degradation of its prey contents (a non-exhaustive list of references would be: Rittenberg 1970, 1983; Thomashow and Rittenberg 1978; Thomashow 1978a,b; Ruby 1984, 1985, 1988; Tudor 1990; Barel et al. 2005). *Micavibrio* spp. appears to act in a rather similar way, as by the end of the interaction ghosts and membrane fragments are all that is left of prey cells (Lambina et al. 1982, 1983). The evolution of such a large panoply of lytic enzymes and of their regulatory mechanisms

enables this exquisite molecular dissection. In that sense, BALOs are highly evolved predators. Although data are missing for non-obligate predators, the lytic processes engendered by the interactions of these predators with their prey may not be as regulated and finely tuned as the ones performed by BALOs because their survival and reproduction are not solely dependent upon the utilization of a prey.

The known genomes of d-BALOs are not small genomes (see Tudor and McCann, in this volume). In contrast, obligate parasites of eukaryotic hosts such as *Chlamydia* (Stephens 2001; Horn et al. 2004; Horn 2004), *Rickettsia* (Ogata et al. 2001) or *Buchneria* (Shigenobu et al. 2000) all have shrunken genomes. The genome of the parasitic archeon *N. equitans* has also undergone a strong reductive process, resulting in a small genome size (Waters 2003). Such reductive processes are not random (Andersson and Kurland 1998) as can be observed in *Sadalis glossinidius* and *Sitophilus oryzae*, relatively recently acquired commensal symbionts of the tsetse fly (Diptera, *Glossina* spp.) and of the rice weevil (Coleoptera). The genomes of these symbionts are being reduced as compared to that of their close relative *Escherichia coli* (Andersson et al. 2003): Functions of carbon compound catabolism, energy metabolism, fatty acid metabolism, and transport are differentially lost between the two symbionts that live under very different ecological constrains, pointing to the role species ecology has on a symbiont's genome (Mira 2002). Similarly, function losses are apparent in the *B. bacteriovorus's* genome with enzymes required for the metabolism of a number of amino acids being absent (Rendulic et al. 2004). On the other hand, genome expansion might have occurred as reflected by the large complement of hydrolytic enzymes present. Comparative genomic analysis of the genomes of *B. marinus* and *Bdellovibrio* strain W, both in the processes of sequencing and annotation might also reveal further differential losses or acquisition of functions which are species-specific adaptations. It should be emphasized that these d-BALOs originated from terrestrial and marine environments. Within these biotopes, they are subjected to very different abiotic and biotic conditions and fluctuations. Moreover, an important difference between d-BALOs and obligate symbionts is that d-BALO cells are not always found within the protective, intracellular milieu of their hosts but must cope with a changing external environment during their attack phase. It also appears that within their hosts, d-BALOs still sense the external milieu, as bdelloplast lysis can be delayed (Sanchez-Amat and Torrella 1990) or a bdellocyst can be formed when conditions are deleterious (Tudor 1977). These examples suggest that prey utilization is not an "automated" mode and that delays in host destruction may occur in response to environmental conditions. These smallest hunters, that earn a living killing other cells, can be viewed as predators or parasites (Jurkevitch 2000). However, can such deleterious relationships evolve, under proper conditions into cooperative systems (Sachs et al. 2004)?

4.2 The Predatory Hypothesis to the Origin of Mitochondria

On the basis of present knowledge, we think that the assumption that predatory interactions between prokaryotes constitute an ancestral trait that has influenced microbial evolution (almost) since cellular life came to be is a reasonable one. This hypothesis (Maynard-Smith and Szathamary 1995; Bengston 2002) also served as a rational basis for proposing that predation could provide a means by which a bacterium could enter another bacterium, forming an endosymbiotic association (Guerrero et al. 1986).

Recent data strongly suggest that all characterized amitochondrial eukaryotes have had mitochondria and that when absent, this resulted from reductive evolution (van der Giezen and Tovar 2005). Moreover, amitochondriate eukaryotes may not form the deepest branches in the *Eukarya* phylogenetic tree (Embly and Hirt 1998; Simpson and Roger 2002; Stechmann and Cavalier-Smith 2002). Therefore, the establishment of the mitochondrial symbiosis might have been a primordial event during the evolution of the eukaryotic cell. A number of hypotheses have been formulated on whom these ancestors were. One such hypothesis, based on phylogenomic analyses, suggests that the eukaryotic genome evolved from a single fusion between an archeal and a bacterial genome (Rivera and Lake 2004). This fusion may have occurred between the mitochondrial ancestor and its host (Martin and Embley 2004). If the host of the protomitochondrial symbiont was a prokaryote, how was cell fusion achieved? Although some rudimentary cytoskeleton exists in prokaryotes, no such cell type has ever been shown to phagocytose another cell. Possibly, phagocytosis cannot occur when the cell is surrounded by a stiff cell wall as the ones prokaryotes possess. About 20 years ago, Guerrero and Margulis (1993, 1991, 1986) proposed that predation could provide a simple explanation for the entry of a prokaryotic proto-endosymbiont into another prokaryotic cell. The latest data provide support for the revival of this hypothesis.

Extant predatory prokaryotes attach to their prey's cell wall, lyze the peptidoglycan, sometimes in an exquisitely regulated manner, and consume their prey while staying on the outer side, or after penetrating the prey's periplasm or its cytoplasm. Could such predatory interactions lead to stable symbioses between the two organisms (Guerrero et al. 1986, 1991; Margulis 1993)? As proposed above, predatory bacteria may have been common at the time Earth became increasingly oxidized, or about 2500 million years ago. This was a catastrophic transition for most anaerobes that could not adapt to higher oxygen levels (Andersson et al. 2003). It has been advanced that an oxygen respiring cell can reduce local oxygen concentration, so the presence of an oxygen-respiring cell in the vicinity of an anaerobe could enable it to survive (Andersson et al. 2003). If the anaerobe would provide substrates to the respiring partner, an enduring association may have developed. If one con-

siders the case that the aerobic partner was a facultative predator, predation may offer a parsimonious explanation for a way by which the aerobic partner could have gained access into the host cell. At the least, cell to cell contact could have been established (epibiotic predator), then further develop. Or, in the case of intracellular predation, the predatory cell could have directly penetrated the host (periplasmic or diacytotic predators). It was shown that under nutrient stress, d-BALOs may stop their growth cycle, and remain in the bdelloplast, apparently without growth and replication (Sanchez-Amat and Torrella 1990) or, as seen in very few strains, as a (bdello) cyst, which also does not replicate (Tudor 1978). As described above, at least one parasite of *Thiothrix* may settle in the periplasm of its host. Such behaviors may constitute a starting point for the development of a stable endosymbiosis. A stable endosymbiosis, i.e. of a prokaryote within another prokaryote was found in mealybugs bacteriocytes. These structures contain γ-proteobacterial endosymbionts within the cytoplasm of a β-proteobacterial host (von Dohlen et al. 2001) but how they were acquired is unknown.

A problem stemming from this hypothesis is that for the evolution of a stable interaction to happen, mechanisms for moderating predation should have been at work. These types of mechanisms may occur through the modulation of predatory aggressiveness in a predator that may also be a saprophyte, i.e. a facultative predator. A high level of available resources may reduce predatory activity (Casida 1988a,b). Along similar lines, host-independent mutants of d-BALOs can still be predatory when grown in the presence of prey in a nutrient-poor medium but will grow saprophytically in a rich medium (Barel and Jurkevitch 2001). Population dynamics and prey responses are important factors to consider: it has been demonstrated that certain regimens of resources and dilution rates can bring about stability in predator–prey interactions (Fussman et al. 2000). Also, in BALO cultures, a fraction of the prey population always survives, and therefore, the prey is not eradicated. While being more resistant to predation, this fraction is not totally immune to it. Consequently, the predator is still able to replicate, potentially enabling co-existence (Shemesh and Jurkevitch 2004). The dynamics of such systems should be taken into account in the model proposed because evolutionary changes in any trait that affects parameters such as predator and prey growth rates, prey carrying capacity etc., depend on these dynamics. In other words, to study the evolution of such traits without incorporating the population dynamics that the ecological interaction implies may not be realistic (Abrams 2000).

Although symbiotic interactions evolve from chance associations between species, symbiotic phenotypes are more likely to originate in highly interactive communities (Law 1991). BALOs can be found in association with biofilms (Kelley et al. 1997) and are able to efficiently consume prey in such structures (Kadouri and O'Toole 2005). Within biofilms, there might be more opportunities for such long-term co-existence to evolve, as exposed above. In a milieu such as stromatolites, or microbial mats, that were common during the Pro-

terozoic Eon (Bengston 2002), the levels of resources may have been high enough to enable stable interactions to become established. Bacterial predators could have co-existed with their prey within these structures, as animals less than a few millimeters in size tend not to disrupt the fabric of modern microbial mats, and so may co-exist with stromatolites (Farmer 1992). Then, there may be mechanisms through which long-term coexistence may be stabilized. Although such conditions intuitively appear to be appropriate for the evolution of a symbiotic interaction, this has to be demonstrated.

It is accepted that the mitochondrial ancestor was an α-proteobacterium (Gray et al. 1999). Recently, α-proteobacterial predators were isolated from a soil in Israel (Davidov et al. 2006b). These obligate predators belong to the *Micavibrio*, a hitherto phylogenetically uncharacterized bacterial predator (Lambina et al. 1982, 1983). The *Micavibrio* cluster is not related to any known, cultured *α-proteobacteria* and forms a deep-branch within this bacterial class. However, although present-day technologies may impair a precise placement of the origin of the mitochondrion, the *Rickettsiales* appear to be the closest extand relatives of this organelle (Emelyanov 2003; Fitzpatrick et al. 2006). All known members of the *Rickettsiales* are obligate intracellular parasites or symbionts of eukaryotes (Yu and Walker 2000). Remarkably, *Ixodes* ticks bear a *Bdellovibrio*-like predator of mitochondria in their ovarian tissues, and these bacteria are closely related to the *Rickettsiales* (Sacchi et al. 2004). Notably, as Kalberg (2004) stated: "If the mitochondria belong to the *Rickettsiales*, then it is tempting to assume that the mitochondrial ancestor had already started on the path of intracellular parasitism that is a common feature of its descendant". This assumption is reinforced by recent analyses pointing to this ancestor as an aerobic motile bacterium with pili and surface proteins for interactions with its host cells, containing an abundance of metabolite transporters including lipid, glycerol and amino acid transporters, and a host dependency for several compounds (Boussau et al. 2004; Gabaldón and Huynen 2003).

Predation constitutes a relatively parsimonious mechanistic explanation for the entry into a host as compared to phygocytosis between prokaryotes. Moreover, the presence of predatory bacteria as deep α-proteobacterial branches, suggestive of a possible ancient origin for predatory behaviors in *α-proteobacteria* and the existence of predators within the *Rickettsiales* help us put forward the hypothesis that predatory bacteria could have been at the origin of the mitochondrial endosymbiosis.

5 Conclusions

Predation appears to be a common trait within the prokaryotes: predators are found in a relatively large number of bacterial classes. While the known dis-

tribution of obligate and facultative predators within the prokaryotic domains may still be rather patchy, groups such as *Bdellovibrio* and like organisms that have been more thoroughly studied encompass a very large diversity of organisms (Baer et al. 2000; Davidov and Jurkevitch 2004; Pineiro et al. 2004), suggesting that much more is to be found "out there". Moreover, exotic or extreme habitats, as they become more widely explored, reveal that populations of prokaryotic predators or of bacterial parasites of bacteria exist under harsh conditions. Even in the most common habitats, such as soils and the marine environment, there surely are myriads of bacterial predators at work, as the still fragmentary knowledge we have on the subject suggests.

To date, predatory behavior between bacteria can only be defined by observation of the interaction between a predator and its prey. As most bacteria are not amendable to standard culture practice, many of these interactions can go unnoticed. Nonetheless, a dedicated observer might discover numerous new such interactions, as exemplified by the collection of non-obligate predators found by Casida (see above). We are convinced that these efforts, combining direct observation, ingenious in-situ enrichment and careful observation (Casida 1969, 1980), modern microscopic techniques including confocal and scanning probe microscopy (Nunez et al. 2003), and cryoelectron tomography, along with original culture approaches (Fry 2000; Sait et al. 2002) and of course, the power of molecular biology will lead to the discovery and characterization of many more bacterial predators.

Predation is an important factor in evolution. It influences the prey's biology (Abrams 2000; Agrawal 2001), and this, in turn may also have important consequences on the predator itself, and on species diversification (Kitchell 1983; Buckling and Rainey 2002). The impact of bacterial predation on bacterial community structure, function and diversity is still not understood but the diversity and ubiquity of bacterial predation suggests that it should be taken into account. Fortunately, the theoretical and experimental tools available today have the potential to provide important data, if only partial.

The study of bacterial predators can greatly contribute to the understanding of earlier events that have shaped the world as we know it today. One major example of a critical role that bacterial predation may have played in evolutionary transitions is the evolution of the mitochondrial endosymbiosis (Guerrero et al. 1986; Davidov et al. 2006b), and see above. Novel genomic and phylogenetic analyses, as well as the isolation of α-proteobacterial predators descending from an early split of this class help bring forward this assumption. We think that other predatory organisms such as *Micavibrio*, that branch deep into the α-proteobacteria can be found. The study of their biology, and phylogenomic analyses should certainly contribute to confirm or invalidate this intriguing possibility. Future research may sieve the right from the wrong in the views that were exposed here.

References

Abrams PA (2000) The evolution of predator–prey interactions: theory and evidence. Ann Rev Ecol Syst 31:79–105

Afinogenova AV, Romai Penabad S, Konovalona SM, Churkina LG, Lambina VA (1981) Comparative characteristics of strains of *Bdellovibrio* isolated from river water and sewage. Mikrobiologiya 50:378–385

Afinogenova AV, Markelova NY, Lambina VA (1986) Detection of enzyme activities of some metabolic pathways in *Micavibrio admirandus* and *Bdellovibrio bacteriovorus*. Mikrobiologiya 141:471–475

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Andersson S, Karlberg O, Canback B, Kurland C (2003) On the origin of mitochondria: a genomics perspective. Philos Trans R Soc 358:165–179

Andersson S, Kurland C (1998) Reductive evolution of resident genomes. Trends Microbiol 6:263–278

Angert E, Clements K, Pace N (1993) The largest bacterium. Nature 362:239–241

Baer ML, Ravel J, Chun J, Hill RT, Williams HN (2000) A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax stolpii* comb nov and *Bacteriovorax starrii* comb nov, respectively. Int J Syst Environ Microbiol 50:219–224

Barel G, Jurkevitch E (2001) Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch Microbiol 176:211–216

Barel G, Sirota A, Volpin H, Jurkevitch E (2005) Fate of predator and prey proteins during the growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* and *Pseudomonas syringae* prey. J Bacteriol 187:329–335

Begunova EA, Stepnaya OA, Tsfasman IM, Kulaev IS (2004) The effect of the extracellular bacteriolytic enzymes of *Lysobacter* sp. on Gram-negative bacteria. Microbiol (Mikrobiologya) 73:267–270

Bengston S (2002) Origins and early evolution of predation. Paleontol Soc P 8:289–318

Beveridge TJ (1999) Structure of Gram-negative cell walls and their derived membrane vesicles. J Bacteriol 181:4725–4733

Bohannan BJM, Kerr B, Jessup CM, Hughes JB, Sandvik G (2002) Trade-offs and coexistence in microbial microcosms. Antonie van Leeuwenhoek 81:107–115

Bonner J (1993) Life Cycles: Reflections of an Evolutionary Biologist. Princeton University Press, Princeton

Boussau B, Karlberg EO, Frank AC, Legault BA, Andersson SG (2004) Computational inference of scenarios for alpha-proteobacterial genome evolution. Proc Natl Acad Sci USA 101:9722–9727

Brocks JJ, Logan GA, Buick R, Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. Science 285:1033–1036

Buckling A, Rainey PB (2002) The role of parasites in sympatric and allopatric host diversification. Nature 420:496–499

Burnham J, Collart S, Highison B (1981) Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of *Myxococcus xanthus*. PCO2 Arch Microbiol 129:285–294

Burnham J, Collart S, Daft ML (1984) Myxococcal predation of the cyanobacterium *Phormidium luridium* in aqueous environments. Arch Microbiol 137:220–225

Byrd J, Zeph L, Casida LE Jr (1985) Bacterial control of *Agromyces ramosus* in soil. Can J Microbiol 31:1157–1163

Cain CC et al. (2003) Synergistic antimicrobial activity of metabolites produced by a non-obligate bacterial predator. Antimicrob Agen Chemo 47:2113–2117

Casida LE Jr (1980) Bacterial predators of *Micrococcus luteus* in soil. Appl Environ Microbiol 39:1035–1041

Casida LE Jr (1982) *Ensifer adhaerens* gennov, sp nov: a bacerial predator bacteria in soil. Int J Syst Bacteriol 32:339–345

Casida LE Jr (1983) Interaction of *Agromyces ramosus* with other bacteria in soil. Appl Environ Microbiol 46:881–888

Casida LE Jr (1987) Relation to copper of N-1, a non-obligate bacterial predator. Appl Environ Microbiol 53:151–1518

Casida LE Jr (1988) Response in soil of *Cupriavidus necator* and other copper-resistant bacterial predators of bacteria to addition of water, soluble nutrients, various bacterial species, or *Bacillus thuringiensis* spores and crystals. Appl Environ Microbiol 54:2161–2166

Casida LE Jr (1992) Competitive ability and survival in soil of *Pseudomonas* strain 679-2, a dominant, non-obligate bacterial predator of bacteria. Appl Environ Microbiol 58:32–37

Chan A, Kacsmarska I, Suttle C (1997) Isolation and characterization of a species-specific bacterial pathogen which lyses the marine diatom *Navicula pulchripora*. Am Soc Limnol Ocean, Santa Fe NM, February 1997

Chang L, Pate J, Betzig RJ (1984) Isolation and characterization of nonspreading mutants of the gliding bacterium *Cytophaga johnsonae*. J Bacteriol 159:26–35

Christensen P, Cook F (1978) *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. Int J Syst Bacteriol 28:367–393

Clements K, Bullivant S (1991) An unusual symbiont from the gut of surgeonfishes may be the largest known prokaryote. J Bacteriol 173:5359–5362

Coder DM, Starr MP (1978) Antagonistic associations of the chlorellavorus bacterium (*Bdellovibrio chlorellavorus*) with *Chlorella vulgaris*. Curr Microbiol 1:9–64

Cotter T, Thomashow MF (1992) Identification of a *Bdellovibrio bacteriovorus* genetic locus, *hit*, associated with the host-independent phenotype. J Bacteriol 174:6018–6024

Daft M, McCord S, Stewart W (1973) The occurrence of lytic bacteria as microbiological agents against blue-green algae and lytic bacteria at a waterworks in Scotland. Water Treat Exam 22:114–124

Daft M, Stewart W (1971) Bacterial pathogens of freshwater blue-green algae. New Phytol 70:819–829

Daft MJ, Burnham J, Yamamoto Y (1985) Lysis of *Phormidium luridum* by *Myxococcus fulvus* in continuous flow cultures. J Appl Bacteriol 59:73–80

Daft MJ, Stewart W (1973) Light and electron microscope observations on algal lysis by bacterium CP-1. New Phytol 72:799–808

Davidov Y, Friedjung A, Jurkevitch E (2006a) High diversity of *Bdellovibrio*-and-like organisms (BALOs) in soil revealed by culture-dependent and culture-independent methods. Environ Microbiol 8:1667–1673

Davidov Y, Huchon D, Koval SF, Jurkevitch E (2006b) A new α-proteobacterial clade of *Bdellovibrio*-like predators: implications for the mitochondrial endosymbiotic theory. Environ Microbiol (published online: 4-Aug-2006, http://www.blackwell-synergy.com/doi/full/10.1111/j.1462-2920.2006.01101.x)

Davidov Y, Jurkevitch E (2004) Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax–Peredibacter* clade as *Bacteriovoracaceae* fam. nov. Int J Syst Evolution Microbiol 54:1439–1452

Day T, Abrams P, Chasec J (2002) The role of size-specific predation in the evolution and diversification of prey life histories. Evol 5:877–887
Dias FF, Baht JV (1965) Microbial ecology of activated sludge. II. Bacteriophages, *Bdellovibrio*, coliforms, and other organisms. Appl Microbiol 13:257–261
Donze D, Mayo J, Diedrich D (1991) Relationship among the bdellovibrios revealed by partial sequences of 16S ribosomal RNA. Curr Microbiol 23:115–119
Doolittle RF, York AL (2002) Bacterial actins? An evolutionary perspective. Bioessays 24:293–296
Dworkin M (1996) Recent advances in the social and developmental biology of the myxobacteria. Microbiol Rev 60:70–102
Dworkin M (1999) Fibrils as extracellular appendages of bacteria: Their role in contact-mediated cell-cell interactions in *Myxococcus xanthus*. Bioessays 21:590–595
Elsherif M, Grossman F (1996) Role of biotic factors in the control of soil-borne fungi by fluorescent pseudomonads. Microbiol Res 151:351–357
Embly TM, Hirt RP (1998) Early branching eukaryotes? Curr Opin Genet Dev 8:624–629
Emelanov VV (2003) Mitochondrial connection to the origin of the eukaryotic cell. Eur J Biochem 270:1599–1618
Ensign JC, Wolfe RS (1965) Lysis of bacterial cell walls by an enzyme isolated from a myxobacter. J Bacteriol 90:395–402
Epstein D, Wensink P (1988) The alpha-lytic protease gene of *Lysobacter enzymogenes* The nucleotide sequence predicts a large prepropeptide with homology to pro-peptides of other chymotrypsin-like enzymes. J Biol Chem 263:16586–16590
Esteve I, Guerrero R, Montesinos E, Abella C (1983) Electron microscopy study of the interaction of epibiontic bacteria with *Chromatium minus* in natural habitats. Microb Ecol 9:57–64
Farmer J (1992) Grazing and bioturbation in modern microbial mats. Cambridge University Press, Cambridge
Fitzpatrick DA, Creevey CJ, McInerney JO (2006) Genome phylogenies indicate a meaningful a-proteobacterial phylogeny and support a grouping of the mitochondria with the *Rickettsiales*. Mol Biol Evol 23:74–85
Folman L, Postma J, vanVeen J (2003) Characterization of *Lysobacter enzymogenes* (Christensen and Cook 1978) strain 31T8, a powerful antagonist of fungal diseases in cucumber. Microbiol Res 158:107–115
Forde ES, Thompson JN, Bohannan BJM (2004) Adaptation varies through space and time in a coevolving host-parasitoid interaction. Nature 431:841–843
Fraleigh P, Burnham J (1988) Myxococcal predation on cyanobacterial populations–nutrient effects. Limnol Ocean 33:476–483
Fry JC (2000) Bacterial diversity and "unculturables". Microbiol Today 27:186–188
Fry J, Staples DG (1974) The occurrence and role of *Bdellovibrio bacteriovorus* in a polluted river. Water Res 8:1029–1035
Fry J, Staples DG (1976) Distribution of Bdellovibrio bacteriovorus in sewage works, river water, and sediments. Appl Environ Microbiol 31:469–474
Fussmann G, SP S, Shertzer K, Hairston Jr N (2000) Crossing the Hopf bifurcation in a live predator–prey system. Science 290:1358–1360
Germida JJ, Casida LE Jr (1983) *Ensifer adhaerens* predatory activity against other bacteria in soil, as monitored by indirect phage analysist. Appl Environ Microbiol 45:1380–1388
Gillespie D, Cook F (1965) Extracellular enzymes from strains of *Sorangium*. Can J Microbiol 11:109–118

Gabaldón T, Huynen MA (2003) Reconstruction of the proto-mitochondrial metabolism. Science 301:609

Guelin A, Maillet P-L (1978) Observation sur l'ultrastructure de micropredateurs de bacilles gram-positif et la degenerescence de *C perfringens*. CR Acad Sc Paris Serie D:1975–1978

Guerrero R (1991) Predation as prerequisite to organelle origin: *Daptobacter* as example. In: Margulis L, Fester R (eds) Symbiosis as a source of evolutionary innovation. The MIT Press, Cambridge, pp 107–117

Guerrero R, Esteve I, Pedros-Alio C, Gaju N (1987) Predatory bacteria in prokaryotic communities: The earliest trophic relationships. In: Endocytobiology III. The New York Academy of Sciences, New York

Guerrero R, Pedros-Alio C, Esteve I, Mas J, Chase D, Margulis L (1986) Predatory prokaryotes: predation and primary consumption evolved in bacteria. Proc Natl Acad Sci USA 83:2138–2142

Gray MW, Burger G, Lang BF (1999) Mitochondrial evolution. Science 283:1476–1481

Gromov BV, Mamkayeva KA (1980) Proposal of a new genus Vampirococcus for chlorellavorus bacteria previously assigned to Bdellovibrio. Mikrobiologya 49:165–167

Haska G (1981) Activity of bacteriolytic enzymes adsorbed to clay. Microb Ecol 7:331–341

Herschkovitz Y, Lerner A, Davidov Y, Okon Y, Jurkevitch E (2005) *Azospirillum brasilense* does not affect population structure of specific rhizobacterial communities of inoculated maize (*Zea mays*). Environ Microbiol 7:1847–1852

Hespell RB, Thomashow MF, Rittenberg SC (1974) Changes in cell composition and viability of *Bdellovibrio bacteriovorus* during starvation. Arch Microbiol 97:313–327

Hespell RB (1978) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* on heat-treated *Escherichia coli*. J Bacteriol 133:1156–1162

Hespell RB, Paster BJ, Macke TJ, Woese CR (1984) The origin and phylogeny of the Bdellovibrios. Syst Appl Microbiol 5:196–203

Hoeniger J, Ladwig R, Moor H (1972) The fine structure of resting bodies of *Bdellovibrio* sp. strain W developed in *Rhodospirillum rubrum*. Can J Microbiol 18:87–92

Hoenigsberg H (2002) Non-Darwinian and Darwinian prokaryotic and eukaryotic evolution – an enigma in cell biology conservation. Gen Mol Res 2:279–287

Horn M et al. (2004) Illuminating the evolutionary history of *Chlamydiae*. Science 304:728–730

Huber H, Burggraf S, Mayer T, Wyschkony I, Rachel R, Stetter K (2000) *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic Archaea, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. Int J Syst Evol Microbiol 50:2093–2100

Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417:63–67

Hutchens E, Radajewski S, Dumont MG, McDonald IR, Murrell JC (2004) Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing. Environ Microbiol 6:111–120

Imai I, Ishida Y, Sakaguchi K, Hata Y (1993) Killing of marine phytoplankton by gliding bacterium *Cytophaga* sp, isolated from the coastal sea of Japan. Mar Biol 116:527–532

Jessup CM, Kassen R, Forde SE, Kerr B, Buckling A, Rainey PB, Bohannan BJM (2004) Big questions, small worlds: microbial model systems in ecology. Trends Ecol Evol 19:189–197

Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66:2365–2371

Kadouri D, O'Toole GA (2005) Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 71:4044–4051

Karlberg EO (2004) Microbial evolution. Turning bugs into features. PhD Thesis, University of Uppsala, Sweden

Kato A, Nakaya S, Ohashi Y, Hirata H (1997) WAP-8294A2, a novel anti-MRSA antibiotic produced by *Lysobacter* sp. J Am Chem Soc 119:6680–6681

Kaunzinger CMK, Morin PJ (1998) Productivity controls food-chain properties in microbial communities. Nature 395:495–497

Kelley J, Williams H (1992) Bdellovibrios in *Callinectus sapidus*, the blue crab. Appl Environ Microbiol 58:1408–1410

Kelley J, Turng B, Williams H, Baer M (1997) Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic bdellovibrios. Appl Environ Microbiol 63:84–90

Kirchman D (2002) The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol Ecol 39:91–100

Kitchell JA (1983) Biotic interactions and siliceous marine phytoplankton: an ecological and evolutionary perspective. In: McCall PL (ed) Biotic Interactions in Recent and Fossils Benthic Communities. Plenum, New York, pp 285–329

Kobayashi DY, Reedy RM, Palumbo JD, Zhou J-M, Yuen GY (2005) A clp gene homologue belonging to the Crp gene family globally regulates lytic enzyme production, antimicrobial activity, and biological control activity expressed by *Lysobacter enzymogenes* strain C3. Appl Environ Microbiol 71:261–269

Koch A (1996) What size should a bacterium be? a question of scale. Ann Rev Microbiol 50:317–348

Koval S, Bayer ME (1997) Bacterial capsules: no barrier against *Bdellovibrio*. Microbiology 143:749–753

Kramer T, Westergaard J (1977) Antigenicity of bdellovibrios. Appl Environ Microbiol 33:967–970

Lambina VA, Afinogenova AV, Romai Penabad S, Konovalona SM, Pushkareva AP (1982) *Micavibrio admirandus* gen. et sp. nov. Mikrobiologiya 51:114–117

Lambina VA, Afinogenova AV, Romay Penabad S, Konovalona SM, Andreev LV (1983) A new species of exoparasitic bacteria from the genus *Micavibrio* destroying gram-negative bacteria. Mikrobiologiya 53:777–780

Larkin JM, Henck MC, Burton SD (1990) Occurrence of a *Thiothrix* sp. attached to mayfly larvae and presence of parasitic bacteria in the *Thiothrix* sp. Appl Environ Microbiol 56:357–361

Law R (1991) The symbiotic phenotype: origins and evolution. In: Margulis L, Fester R (eds) Symbiosis as a source of evolutionary innovation. The MIT Press, Cambridge

Lenski R (1988) Experimental studies of pleiotropy and epistasis in *Escherichia coli*. I. Variation in competitive fitness among mutants resistant to virus T4. Evol 42:425–432

Li Z, Clarke J, Beveridge T (1996) A major autolysin of *Pseudomonas aeruginosa*, its subcellular distribution, its potential role in cell growth and division, and its secretion in surface membrane vesicles. J Bacteriol 178:2479–2488

Li ZA, Clarke J, Beveridge TJ (1998) Gram-negative bacteria produce membrane vesicles which are capable of killing other bacteria. J Bacteriol 180:5478–5483

Lin SB, Wang HH (1983) *Bdellovibrio* in methylotrophic bacteria. Biotechnol Lett 8:549–554

Ludwig W et al. (1998) Bacterial phylogeny based on comparative sequence analysis. Electrophoresis 19:554–568

Makkar N, Casida Jr L (1987) *Cupriavidus necator* gen. nov., sp. nov.: a non-obligatebacterial predator in soil. Int J Syst Microbiol 37:323–326
Marbach A, Shilo M (1978) Dependence of marine bdellovibrios on potassium, calcium, and magnesium ions. Appl Environ Microbiol 36:169–177
Margalith P (1962) Bacteriolytic principles of *Myxococcus fulvus*. Nature 196:1335–1336
Margulis L (1970) Origin of eukaryotic cells. Yale University Press, New Haven, CT
Margulis L (1993) Symbiosis in Cell Evolution. 2nd edn. Freeman, New York
Mashburn LM, Whiteley M (2005) Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature 437:422–425
Martin MO (2002) Predatory prokaryotes: an emerging research opportunity. J Microbiol Biotechnol 4:467–477
Martin W, Embley TM (2004) Evolutionary biology—Early evolution comes full circle. Nature 431:134–137
Martinez E, Palacios R, Sanchez F (1987) Nitrogen-fixing nodules induced by *Agrobacterium tumefaciens* harboring *Rhizobium phaseoli* plasmids. J Bacteriol 169:2828–2834
Maynard-Smith J, Szathamary E (1995) The major transitions in evolution. Freeman, New York
McBride MJ, Zusman DM (1996) Behavioral analysis of single cells of *Myxococcus xanthus* in response to prey cells of *Escherichia coli*. FEMS Microbiol Lett 137:227–231
McBride MJ (2004) *Cytophaga-Flavobacterium* gliding motility. J Mol Microbiol Biotechnol 7:63–71
McBride MJ, Braun TF, Brust JL (2003) *Flavobacterium johnsoniae* GldH is a lipoprotein that is required for gliding motility and chitin utilization. J Bacteriol 185:6648–6657
Mira A, Klasson L, Andersson SGE (2002) Microbial genome evolution: sources of variability. Curr Opin Microbiol 5:506–512
Morris SA, Radajewski S, Willison TW, Murrell JC (2002) Identification of the functionally active methanotroph population in a peat soil microcosm by stable-isotope probing. Appl Environ Microbiol 68:1446–1453
Moulder J (1985) Comparative biology of intracellular paratism. Microbiol Rev 49:298–337
Nealson K, Conrad P (1999) Life: past, present and future. Philos Trans R Soc Lond 354:1923–1939
Nogales B, Guerrero R, Esteve I (1997) A heterotrophic bacterium inhibits growth of several species of the genus *Chlorobium*. Arch Microbiol 167:396–399
Nunez ME, Martin MO, Duong LK, Ly E, Spain EM (EM) Investigations into the life cycle of the bacterial predator *Bdellovibrio bacteriovorus* 109J at an interface by atomic force microscopy. Biophys J 84:3379–3388
Ogata H et al. (2001) Mechanisms of evolution in *Rickettsia conorii* and *R. prowazekii*. Science 293:2093–2098
Palumbo JD, Sullivan RF, Kobayashi DY (2003) Molecular characterization and expression in *Escherichia coli* of three {beta}-1,3-glucanase genes from *Lysobacter enzymogenes* strain N4–7. J Bacteriol 185:4362–4370
Pan et al. (1997) Isolation and identification of *Bdellovibrio* from coastal areas of Taiwan. Fish Sci 63:52–59
Pineiro SA, Sahaniuk GE, Romberg E, Williams H (2004) Predation pattern and phylogenetic analysis of *Bdellovibrionaceae* from the Great Salt Lake. Utah Curr Microbiol 48:113–117
Quinn R, Skerman V (1980) *Herpetosiphon*-Nature's scavenger? Curr Microbiol 4:57–62
Rappé M, Giovannoni S (2003) The uncultured microbial majority. Ann Rev Microbiol 57:369–394

Rashidan KK, Bird DF (2001) Role of predatory bacteria in the termination of a cyanobacterial bloom. Microbiol Ecol 41:97–105

Reichenbach H (2001) The genus *Lysobacter*. In: Dworkin M et al (ed) The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. Springer, Berlin Heidelberg New York

Reichenbach H, Gerth K, Irschik H, Kunze B, Hofle G (1988) Myxobacteria: A source of new antibiotics. Trends Biotechnol 6:115–121

Reichenbach H (1999) The ecology of the myxobacteria. Environ Microbiol 1:15–21

Rendulic S et al. (2004) A predator unmasked: Life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692

Rice TD, Williams HN, Turng BF (1998) Susceptibility of bacteria in estuarine environments to autochthonous bdellovibrios. Microb Ecol 35:256–264

Rittenberg SC (1983) *Bdellovibrio*: Attack, penetration and growth on its prey. ASM News 49:435–440

Rittenberg SC, Shilo M (1970) Early host damage in the infection cycle of *Bdellovibrio bacteriovorus*. J Bacteriol 102:149–160

Rivera MC, Lake JA (2004) The ring of life provides evidence for a genome fusion origin of eukaryotes. Nature 431:152–155

Rogel AM, Hernandez-Lucas I, Kuykendall D, Balkwill DL, Martinez-Romero E (2001) Nitrogen-Fixing nodules with *Ensifer adhaerens* harboring *Rhizobium tropici* symbiotic plasmids. Appl Environ Microbiol 67:3264–3268

Rosenberg E, Keller K, Dworkin M (1977) Cell density-dependent growth of *Myxococcus xanthus* on casein. J Bacteriol 129:770–777

Ruby EG, McCabe J (1988) Metabolism of periplasmic membrane-derived oligosaccharides by the predatory bacterium *Bdellovibrio bacteriovorus* 109J. J Bacteriol 170:646–652

Ruby EG, Rittenberg SC (1984) Attachment of diaminopimelic acid to bdelloplast peptidoglycan during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. J Bacteriol 158:597–602

Ruby EG, McCabe JB, Barke JI (1985) Uptake of intact nucleoside monophosphate by *Bdellovibrio bacteriovorus* 109J. J Bacteriol 163:1087–1094

Sacchi L, Bigliardi E, Corona S, Beninati T, Lo N, Franceschi A (2004) A symbiont of the tick *Inoxes ricinus* invades and consumes mitochondria in a mode similar to that of the parasitic bacterium *Bdellovibrio bacteriovorus*. Tissue Cell 36:43–53

Sachs JL, Mueller UG, Wilcox T, Bull JJ (2004) The evolution of cooperation. Quart Rev Biol 79:135–160

Sait M, Hugenholtz P, Janssen P (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. Environ Microbiol 4:654–666

Sanchez-Amat A, Torrella F (1989) Isolation and characterization of marine and salt pond halophylic bdellovibrios. Can J Microbiol 35:771–778

Sanchez-Amat A, Torrella F (1990) Formation of stable bdelloplasts as a starvation-survival strategy of marine bdellovibrios. Appl Environ Microbiol 56:2127–2125

Schopf J (1993) Microfossils of the Early Archean Apex chert: new evidence of the antiquity of life. Science 260:640–646

Schrag SJ, Mittler JE (1996) Host–parasite coexistence: the role of spatial refuges in stabilizing bacteria–phage interactions. Am Nat 148:348–377

Schulz HN, Brinkhoff T, Ferdelman TG, Hernandez Marine M, Teske A, Jorgensen BB (1999) Dense populations of a giant sulfur bacterium in Namibian shelf sediments. Science 284:493–495

Schwudke D, Strauch E, Krueger M, Appel B (2001) Taxonomic studies of predatory bdellovibrios based on 16S rRNA analysis, ribotyping and the hit locus and characterization of isolates from the gut of animals. Syst Appl Microbiol 24:385–394

Seidler RJ, Starr MP (1969a) Factors affecting the intracellular parasitic growth of *Bdellovibrio bacteriovorus* developing within *Escherichia coli*. J Bacteriol 97:912–923

Seidler R, Starr MP (1969b) Isolation and characterization of host-independent bdellovibrios. J Bacteriol 100:769–785

Seidler R, Mendel M, Baptist J (1972) Molecular heterogeneity of the bdellovibrios: evidence for two new species. J Bacteriol 109:209–217

Shemesh Y, Davidov Y, Koval S, Jurkevitch E (2003) Small eats big: ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator–prey interactions. Agronomie 23:433–439

Shemesh Y, Jurkevitch E (2004) Plastic phenotypic resistance to predation by *Bdellovibrio* and like organisms in bacterial prey. Environ Microbiol 6:8–12

Sheridan P, Freeman K, Brenchley J (2003) Estimated minimal divergence times of the major bacterial and archaeal phyla. Geomicrobiol J 20:1–14

Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H (2000) Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. Nature 407:81–86

Shilo M, Bruff B (1965) Lysis of Gram negative bacteria by host-independent ectoparasitic *Bdellovibrio bacteriovorus* isolates. J Gen Microbiol 40:317–328

Shilo M (1970) Lysis of blue-green algae by myxobacter. J Bacteriol 104:453–461

Shimkets LJ (1990) Social and developmental biology of myxobacteria. Microbiol Rev 54:473–501

Simpson AGB, Roger AJ (2002) Eukaryotic evolution: Getting to the root of the problem. Curr Biol 12:R691–R693

Singh B (1947) Myxobacteria in soils and composts: Their distribution, number and lytic action on bacteria. J Gen Microbiol 1:1–10

Sitkin B, Tsfasman I, Stepnaya O (2003) May the bacterial autolysins be the precursors of extracellular bacteriolytic enzymes? Dokl Biochem Biophys 392:260–262

Snyder AR, Williams HN, Baer ML, Walker SOC (2002) 16S rDNA sequence analysis of environmental *Bdellovibrio*-and-like organisms (BALO) reveals extensive diversity. Int J Syst Evol Microbiol 52:2089–2094

Stackebrandt E, Goebel BM (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Stanley SM (1973) An ecological theory for the sudden origin of multicellular life in the late Precambrian. Proc Natl Acad Sci USA 70:1486–1489

Starr MP, Seidler R (1971) The Bdellovibrios. Ann Rev Microbiol 25:649–678

Stechmann A, Cavalier-Smith T (2002) Rooting the eukaryote tree by using a derived gene fusion. Science 297:89–91

Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, Mitchell W, Olinger L, Tatusov RL, Zhao Q, Koonin EV, Davis RW (2001) Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 282:754–759

Stepnaya OA et al. (2004) The mechanism of action of the extracellular bacteriolytic enzymes of *Lysobacter* sp on Gram-positive bacteria: The role of the cell wall anionic polymers of target bacteria. Microbiol (Mikrobiologya) 73:404–409

Stewart J, Brown R (1971) Algicidal nonfruiting myxobacteria with high G+C ratios. Arch Microbiol 80:176–190

Stolp H, Pertzold H (1962) Untersuchungen uber einen obligat parasitischen Mikroorganismus mit lytischer Aktivitat fur *Pseudomonas*-Bakterien. Phytopathol Z 45:364–390

Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* gen. et sp., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek 29:217–248
Stolp H (1967) Lysis von bacterien durch den parasiten *Bdellovibrio bacteriovorus*. Film C972. IWF, Gottingen
Sudo S, Dworkin M (1972) Bacteriolytic enzymes produced by *Myxococcus xanthus*. J Bacteriol 110:236–245
Thomashow LS, Rittenberg S (1985a) Waveform analysis and structure of flagella and basal complexes from *Bdellovibrio bacteriovorus* 109J. J Bacteriol 163:1038–1046
Thomashow LS, Rittenberg S (1985b) Isolation and composition of sheathed flagella from *Bdellovibrio bacteriovorus* 109J. J Bacteriol 163:1047–1054
Thomashow MF, Rittenberg S (1978a) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: solubilization of *Escherichia coli* peptidoglycan. J Bacteriol 135:998–1007
Thomashow MF, Rittenberg SC (1978b) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: attachment of long-chain fatty acids to *Escherichia coli* peptidoglycan. J Bacteriol 135:1015–1023
Thomashow MF, Rittenberg SC (1978c) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: N-Deacetylation of *Escherichia coli* peptidoglycan amino sugars. J Bacteriol 135:1008–1014
Torrella F, Guerrero R, Seidler R (1978) Further taxonomic characterization of the genus *Bdellovibrio*. Can J Microbiol 24:1387–1394
Tudor JJ, Conti SF (1977) Ultrastructural changes during encystment and germination of *Bdellovibrio* sp. J Bacteriol 131:323–330
Tudor JJ, Conti SF (1978) Characterization of germination and activation of *Bdellovibrio* bdellocysts. J Bacteriol 133:130–138
Tudor JJ, McCann M, Acrich IA (1990) A new model for the penetration of prey cells by bdellovibrios. J Bacteriol 172:2421–2426
van den Ent F, Amos LJL (2001) Prokaryotic origin of the actin cytoskeleton. Nature 413:39–44
Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60:407–438
Vandamme P, Coenye T (2004) Taxonomy of the genus *Cupriavidus*: a tale of lost and found. Int J Syst Environ Microbiol 54:2285–2289
van der Giezen M, Tovar J (2005) Degenerate mitochondria. EMBO Rep 6:525–530
Varon M, Dickbusch S, Shilo M (1974) Isolation of host-dependent and nonparasitic mutants of the facultative parasitic *Bdellovibrio* UKi2. J Bacteriol 119:635–637
Varon M (1979) Selection of predation-resistant bacteria in continuous culture. Nature 277:386–388
Varon M, Shilo M (1968) Interaction of *Bdellovibrio bacteriovorus* and host bacteria I Kinetic studies of attachment and invasion of *Escherichia coli* B by *Bdellovibrio bacteriovorus*. J Bacteriol 95:744–753
Varon M, Shilo M (1969) Attachment of *Bdellovibrio bacteriovorus* to cell wall mutants of *Salmonella* spp and *Escherichia coli*. J Bacteriol 97:977–979
Varon M, Zeigler B (1978) Bacterial predator–prey interaction at low prey density. Appl Environ Microbiol 36:11–17
von Dohlen C, Kohler S, Aslop S, McManus W (2001) Mealybug [beta]-proteobacterial endosymbionts contain [gamma]-proteobacterial symbionts. Nature 412:433–436
von Tigerstrom R (1980) Extracellular nuclease of *Lysobacter enzymogenes*: production of the enzymes and purification and characterization of an endonuclease. Can J Microbiol 26:1029–1037

Waters E et al. (2003) The genome of Nanoarchaeum equitans: Insights into early archaeal evolution and derived parasitism. Proc Natl Acad Sci USA 100:12984–12988

Wilkinson CR (1979) *Bdellovibrio*-like parasite of cyanobacteria symbiotic in marine sponges. Arch Microbiol 123:101–103

Wilkinson MHF (2001) Predation in the presence of decoys: an inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems. J Theor Biol 208:27–36

Willems AF-L et al. (2003) Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Casida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb nov Request for an opinion. Int J Syst Environ Microbiol 53:1207–1217

Williams H, Falkler W, Shay D (1982) Seasonal distribution of bdellovibrios at the mouth of the Patuxent river in the Chesapeake bay. Can J Microbiol 28:111–116

Williams H (1987) The recovery of high numbers of bdellovibrios from the surface water microlayer. Can J Microbiol 33:572–575

Williams HN (1988) A study of the distribution of bdellovibrios in estuarine sediment over an annual cycle. Microb Ecol 15:9–20

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Wood DW et al. (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science 294:2317–2323

Wright D, Graham L, Jennings P (1998) Cloning of a *Lysobacter enzymogenes* gene that encodes an arginyl endopeptidase (endoproteinase Arg-C). Biochim Biophys Acta 1443:369–374

Yoshida T, Jones L, Ellner S, Fussmann G, Hairston N Jr (2003) Rapid evolution drives ecological dynamics in a predator–prey system. Nature 424:303–306

Young JM (2003) The genus name *Ensifer* Casida 1982 takes priority over *Sinorhizobium* Chen et al. 1988, and "*Sinorhizobium morelense*" Wang et al. 2002 is a later synonym of *Ensifer adhaerens* Casida 1982. Is the combination Sinorhizobium adhaerens (Casida 1982) Willems et al. 2003 legitimate? Request for an Opinion. Int J Syst Environ Microbiol 53:2107–2110

Yu X-J, Walker DH (2000) In: Dworkin M et al (ed) The Order Rickettsiales. The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community. 3rd edn, release 3.7, November 2, 2001. Springer, Berlin Heidelberg New York, http://link.springer-ny.com/link/service/books/10125/

Zeph L, Casida LE Jr (1986) Gram-negative versus Gram-positive (Actinomycete) nonobligate bacterial predators of bacteria in soil. Appl Environ Microbiol 52:819–823

Zlatanova J (1997) Archaeal chromatin: Virtual or real? Proc Natl Acad Sci USA 94:12251–12254

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_053/Published online: 14 October 2006

Predation on Bacteria and Bacterial Resistance Mechanisms: Comparative Aspects Among Different Predator Groups in Aquatic Systems

Klaus Jürgens

Baltic Sea Research Institute Warnemünde, Seestrasse 15, 18119 Rostock, Germany
klaus.juergens@io-warnemuende.de

Abstract Predation on bacteria is now considered as an essential component of aquatic and terrestrial food webs, with important implications for many ecosystem processes. Studies in recent years have also focused on the mechanisms of predation and its implications for the structure of bacterial communities. This chapter gives an overview on predation on free-living bacteria by the major predator groups encountered in aquatic systems: bacteriophages, predatory prokaryotes, protists and metazoans. Quantitative as well as qualitative predation impacts on bacterial communities by the different predators, as derived from field studies and laboratory experiments, are summarized. The different predator types encompass several orders of magnitude in size and differ with respect to foraging strategy, consumption rates and selectivity. Bacteria have evolved various strategies to reduce predatory mortality. These have been studied most extensively with respect to protist predation and encompass behavioural, morphological and physiological adaptations, which act at different stages of the predator–prey interactions between bacteria and bacterivores. Field studies and food web manipulation experiments in pelagic systems have also demonstrated the relevance of predation for the taxonomic and morphological composition of natural bacterial assemblages. Compared to bacterivorous protists, much less is known about the predation impact of the smallest, parasitoid-like predators such as bacteriophages and prokaryotic predators (e.g. *Bdellovibrio*) and the resulting anti-predator strategies of bacteria.

1 Introduction

Predation on bacteria is an interesting phenomenon, both from an ecological and an evolutionary point of view. As prokaryotic microorganisms (*Bacteria*, *Archaea*) are the driving agents of all biogeochemical cycles and as they constitute a global biomass comparable to that of green plants (Whitman et al. 1998), it is crucial for ecosystem research and community ecology to understand predation as a regulating and structuring mechanisms for natural, complex microbial communities. From an evolutionary point of view, predation has been suggested to have promoted the development and evolution of new life forms within the microbial world, for example by the incorporation of prokaryotic cells as precursors of eukaryotic organelles (Guerrero et al. 1986; Margulis 1996). Predator–prey co-evolution is assumed to have shaped the phenotypic traits of both microbial predators and prey organisms, and the origin of phagotrophy has been suggested to have become a selective pressure for multicellularity (Stanley 1973). This view has been supported by culture experiments, which have demonstrated the development of predation-resistant, colonial forms from unicellular prey in bacteria (Hahn et al. 2000; Matz et al. 2002b) and algae (Boraas et al. 1998).

In this review I will deal mainly with predation impacts that have ecological implications on shorter time scales, i.e. affecting the structure and function of bacterial communities and promoting bacterial adaptations to reduce predatory mortality, but which also offer insights into predation as an evolutionary selective force. I use "predation" here in its widest sense, including phagotrophic organisms engulfing and digesting bacteria (protists, some metazoans) as well as predators that multiply inside bacteria (bacteriophages, predatory bacteria) and that could rather be termed parasitoid. For the latter, the bacterial prey cells are synonymously called host cells. There are other antagonistic interactions between microorganisms that are not considered here, for example antibiotic- or bacteriocin-mediated inhibition of competitors. The practical, generic term "bacteria", which is used throughout this chapter, includes the phylogenetic domains *Bacteria* and *Archaea* as there is no evidence that archaea microorganisms behave fundamentally different with respect to predatory interactions.

Traditionally, microbiologists have focused more on the abiotic physicochemical conditions and substrate supply, so called "bottom-up" control, as factors determining bacterial growth and survival in the environment (Roszak and Colwell 1987). However, predation ("top-down" control) has been identified as a major mortality and selection factor for bacteria in many aquatic and terrestrial systems, impacting their abundance, composition and activity. Further, many ecological functions of prokaryotes, such as detritus decomposition and nutrient remineralization, might not be possible without bacterial predators (Fenchel and Harrison 1976). Ecologically realistic models

now consider both driving forces, control by resources and predation, in order to understand the functioning and regulation of microbial communities in the environment (Thingstad 2000a). Therefore, in recent years predation on bacteria has gained more attention as a regulating and structuring force, for which it is important to achieve a mechanistic understanding (see reviews by Jürgens and Matz 2002; Pernthaler 2005).

Much of our current knowledge of predatory effects on bacteria and microbial predator–prey interactions stems from studies on aquatic ecosystems, particularly freshwater and marine plankton communities and their respective model systems. This field was particularly stimulated when the new conceptual role of microorganisms in aquatic systems was formulated, stating that bacteria are an essential component of aquatic food webs, with high biomass and productivity, and dominate energy and nutrient fluxes (Azam et al. 1983). Understanding the fate of bacteria was central for a comprehensive picture of the major carbon flow pathways in the pelagial of the ocean. Small protists (flagellates, ciliates) had been identified as the major bacterivores in pelagic systems, their grazing activity controlling bacterial numbers and consuming bacterial production (Fenchel 1982b, 1984). After the generally recognized importance of bacteria in aquatic food webs, quantitative studies on bacterial mortality due to protist grazing and, later, due to viral lysis were performed. These studies resulted in the general notion that predatory mortality of bacteria is more or less in balance with bacterial production and, therefore, the major loss factor for planktonic prokaryotes (Pace 1988; Sherr et al. 1989; Sanders et al. 1992).

In the last few years, qualitative aspects of predation came more into focus and aspects such as prey selectivity, grazing impact on bacterial community composition, and predation avoidance mechanisms were studied in field and laboratory experiments (see reviews by Jürgens and Matz 2002; Pernthaler 2005; Matz and Kjelleberg 2005). Initially, indirect evidence suggested strong qualitative impacts of bacterial predation (Jürgens and Güde 1994). This has been confirmed by many experimental studies that demonstrated significant predation effects on the phenotypic and genotypic composition of bacterial communities, as well as on their physiological functions. Most insights have been gained on mechanisms of bacteria–protist interactions in planktonic systems. Results from the whole spectrum of approaches, from field observations to controlled laboratory experiments with isolated strains, gave a rather comprehensive picture of the nature of this predator–prey relationship. Comparatively little is still known on the quantitative and qualitative implications of prokaryotic predation (e.g. by *Bdellovibrio*) and bacteria–virus interactions on natural bacterial communities, and on resulting bacterial resistance mechanisms.

Meanwhile, we do not only have a reasonable quantitative estimate of bacterial grazing losses in aquatic systems, we also have insights into the protist grazing impact on the bacterial community composition, functional proper-

ties, and the development of bacterial resistance mechanisms. This high level of comprehensive information might be due to the fact that predation is of particular importance for suspended bacteria in pelagic habitats where few spatial refuges exist. Further, such systems are also most amenable to the analysis of predatory interactions with appropriate methods both in situ as well as in model laboratory systems with cultured organisms. Although the importance of predation has also been demonstrated for other habitats such as soil (Ekelund and Ronn 1994), sediment (Dietrich and Arndt 2000) and biofilms (Matz et al. 2004a), much less is known about the regulating mechanisms.

In this review I will mainly refer to results obtained from predatory interactions in aquatic (mainly planktonic) ecosystems in order to illustrate general mechanisms of microbial predator–prey interactions and to summarize known impacts of bacterivory and grazing resistance mechanisms in bacteria. These insights might serve as a general example and as framework to be used in other systems and with other bacterial predators. When appropriate, I will also include examples from other aquatic and terrestrial habitats. For comparison, I will outline the basic principles of predatory strategies of the other major aquatic predator groups (bacteriophages, prokaryotic predators and filter-feeding metazoans) and briefly discuss evidence for their in situ predation impact and potential bacterial resistance mechanisms.

2
Predation as a Selective Force for Bacteria in Aquatic Systems

It is common knowledge that predation is a major and probably the most important mortality factor for bacteria in all kinds of aquatic systems, from freshwater to marine, from groundwater and rivers to the ocean (Pace 1988; Sherr and Sherr 2002). In order to elucidate whether predation by a particular predator group creates a significant selective pressure on bacterial communities, information on the in situ abundance and predation rates across temporal and spatial scales is needed and should be combined with detailed mechanistic studies of foraging strategies, particle capture and potential bacterial adaptations. Ideally, such information is gathered by a combination of well-controlled laboratory experiments (to elucidate specific mechanisms) and in situ observations and field experiments (to estimate the importance in the environment). Such a comprehensive level of information, which is outlined in more detail below, is at present only available for bacteria–protist interactions in planktonic systems.

Aquatic ecosystems encompass a large structural and functional diversity, implying a diversity of predators and different selection pressures on bacteria. Depending on the structure of the habitat and whether bacteria are mainly in suspension (e.g. plankton) or attached (biofilms), different functional predator groups have to be considered. Freshwater and marine systems

further differ with respect to the dominating groups of phagotrophic organisms that potentially consume bacteria. This is most evident in the case of filter-feeding zooplankton, which is much more dominant in lakes than in the ocean (Sommer and Stibor 2002). Further, the abiotic constraints of the specific habitat determine which predator group is of importance. Harsher environments (e.g. anoxia, very high or very low temperature, high pressure etc.) harbour a significantly lower diversity of predator groups and, in the most extreme ones, potentially none at all. For example, it is not known whether predation plays any role in microbial communities in the deep subsurface, the habitat which contains the majority of prokaryotes on earth (Whitman et al. 1998). It is beyond the scope of this review to discuss predation on bacteria in this whole spectrum of habitats, and most of the examples are from predation on suspended bacteria in the water column or on attached bacteria in biofilms surrounded by water. However, even here we have to deal with different predator groups, encompassing a size range of more than eight orders of magnitude, from viruses to large bivalves (Table 1), which all might potentially consume similar sized bacterial prey. Therefore, obviously, huge differences in the type of predatory interactions with bacteria, in regulating mechanisms and in the predation impact in different environments have to be expected. Only some very general features are sketched in Table 1 and then

Table 1 Basic characteristics of different groups of predators on bacteria in aquatic systems

Predator group	Size	Nutrition	Predatory strategy	Occurrence	Selectivity
Bacteriophages	nm	Bacteriovorous	Parasitoid	Ubiquitous	High
Prokaryotes					
Bdellovibrio	μm	Bacteriovorous	Parasitoid	Aquatic, soil	High
Protozoans					
Flagellates	2–20 m	Bacteriovorous, omnivorous	Direct interception, filtration	Aquatic, soil	Moderate
Ciliates	10–100 m	Bacteriovorous, omnivorous	Filter feeders	Aquatic, soil	Low (size)
Metazoans					
Cladocerans	0.5–5 mm	Herbivorous, omnivorous	Filter feeders	Freshwater plankton	Low (size)
Tunicates	mm–15 cm	Omnivorous	Filter feeders	Marine plankton	Low (size)
Bivalves	mm–1 m	Omnivorous	Filter feeders	Marine, freshwater benthos	Low (size)

outlined more in detail. Bivalves are only one among several groups of benthic filter feeders that can potentially retain bacteria and are mentioned here as a representative group. Bryozoans, corals, sponges and different marine invertebrate larvae must also be considered (Riisgard and Larsen 2001a). A major distinction can be made between the smallest predators, bacteriophages and predatory bacteria, which multiply inside the bacterial host, and protistan and metazoan predators, which ingest whole bacterial cells.

The grazing pressure in the environment depends on predator abundance and their feeding rates. This information is not trivial to obtain, particularly not for the smallest predator groups. Although many new techniques to quantify bulk grazing rates on bacteria have been developed, particularly for protist grazing (Landry 1994; Sleigh and Zubkov 1998), there is still a great deal of uncertainty in all bacterial grazing rate estimates (Vaqué et al. 1994). Precision and reliability in measurements seem to decrease with decreasing size of predators: whereas the impact of filter-feeding zooplankton can be quite accurately assessed, grazing rate measurements of protists are tedious to determine and all methods are subject to different kinds of biases. Estimates on virally induced mortality are only assessed indirectly and depend on several empirical conversion factors (Weinbauer 2004). In situ predation rates by predatory bacteria (e.g. *Bdellovibrio*) cannot be assessed at all with the methods currently in use.

In order to understand the particular selective force exerted by the different predator groups on bacteria we also have to consider foraging strategies and particle capture mechanisms. Such information is partly available from autecological studies with cultivated organisms, particularly for planktonic protists (Fenchel 1982a; Jürgens and De Mott 1995). However, in the case of microbial predators (protists, prokaryotes, phages) it is not clear whether the studied isolates are representative of the majority of the natural assemblages.

The degree of prey selection (or host specificity) is of overall importance for estimating the ecological impact of the different predator types. Natural, complex microbial assemblages are characterized by an enormous heterogeneity, which encompasses not only the phylogenetic affiliation but also physiological states and phenotypic properties of the cells. There is no totally unselective predator, in the sense that all different phenotypic and genotypic bacterial cells are consumed in the same proportions as they occur in the environment. The bacterial phenotypic properties that govern prey selection, such as size and morphology, can be related to the physiological state of the cells, to specific taxonomic characters or to a mixture of both. Bacterial cell size is of overall importance for all predatory interactions, particularly in planktonic systems. The predatory strategy determines the selectivity between different bacteria, which is naturally highest for the parasitoids and low for those organisms that retain small particles by specialized filtering structures (e.g. cilia). Here, particle size is the main decisive prey selection criteria. However, even within the different ciliary filter feeders different particle col-

lecting mechanisms can be found (Riisgard and Larsen 2001b). It also makes a difference for the realized selective pressure whether predators are specialized bacterivores that feed nearly exclusively on bacteria or whether bacteria are only one component of the diet (omnivores). In this latter case, the dynamics of the biotic interactions between bacteria and their consumers are influenced largely by other factors (e.g. alternative food resources).

As mentioned above, all these data are best documented for the interaction of bacteria and phagotrophic protists in planktonic systems. However, some major characteristic features for the different predators on bacteria, which are relevant for an understanding of the nature of their selective force, can be extracted from the current literature. In the following, I will therefore briefly summarize existing information on the four major predator groups in aquatic systems and will address the general occurrence, characteristics of the foraging strategy (which determines possible anti-predator defenses), evidence for environmental relevance and potential impact on bacterial communities. Except for metazoans, these predator groups are also relevant in soil ecosystems.

2.1 Predation by Bacteriophages

Viruses occur in all aquatic systems at high concentrations, typically being 10–100 times more abundant than prokaryotes (see reviews by Fuhrman 2000; Wommack and Colwell 2000; Weinbauer 2004). Probably most of the viruses are in fact bacteriophages, infecting heterotrophic bacteria and cyanobacteria. Electron microscopy studies revealed that a significant fraction, typically in the range of 5–20%, of planktonic bacteria are infected by phages. Estimates for the fraction of bacterial mortality in aquatic systems due to viral lysis are in the range of 5–40% (Fuhrman 1999) and largely differ between different systems and different times of the year. On certain occasions, viral mortality can be of similar or higher magnitude than protist grazing (Fuhrman and Noble 1995; Fischer and Velimirov 2002). The range of viral mortality estimates is, however, very large and there is considerable uncertainty with regard to the precision of the methods used. From a compilation of all current bacterial mortality assessments, it seems reasonable to conclude that viral lysis is the second most important bacterial mortality factor after protist grazing in non-extreme environments (such as the pelagial of oceans and lakes) and in sediments (Pedrós-Alió et al. 2000). However, the relative importance of viral mortality changes seasonally and with depth (Bettarel et al. 2004) and viral mortality probably becomes the major bacterial mortality factor in more "extreme" environments with respect to the abiotic conditions, such as anoxic (Weinbauer and Höfle 1998; Bettarel et al. 2004) and hypersaline (Guixa-Boixareu et al. 1996) waters. This seems simply due to the fact that bacteriophages tolerate a wider range of conditions than do eukaryotic predators. Altogether, bacteriophages are now considered to have

a strong and significant effect on the composition of bacterial communities, on the production of dissolved organic matter (DOM) and the cycling of bacterially bound nutrients in pelagic systems (Thingstad et al. 1993; Fuhrman 1999; Weinbauer and Rassoulzadegan 2004). Data from other environments are much scarcer but high viral abundances have also been reported from sediments, microbial mats and soil (Weinbauer 2004).

Different life cycles can be observed in bacteriophages, the major ones being lytic and lysogenic infections. In the lysogenic cycle, the viral genome remains in the bacterial host in a dormant state (prophage) and replicates together with the host until a lytic cycle is induced by certain environmental factors (e.g. UV radiation). A large fraction of cells (10–40%) within natural bacterial assemblages are lysogenic (Wommack and Colwell 2000; Weinbauer 2004) but it is under debate how much prophage induction contributes to phage production. Although it resembles a parasitic interaction, the life cycle of a lytic phage is of a real predatory nature. The adsorption of the phage by passive diffusion to specific cell surface structures (e.g. transporter proteins, flagella, pili) of a suitable host bacterium involves a reversible phase and an irreversible binding between the phage and a receptor. After enzymatic treatment of the bacterial cell wall the viral nucleic acids are injected and later integrated into the host genome. This causes the host cell to produce numerous progeny phages, leading to bursting of the cell, release of the new phages and the start of a new cycle.

The contact rate between bacteriophages and bacteria depend on their respective concentrations and can be modelled by including diffusive transport, temperature, bacterial swimming speed and cells size (Fischer and Velimirov 2002). Host specificity is of crucial importance for understanding the in situ selective predation pressure by bacteriophages. A common view is that the host range is rather narrow and phages do not "trespass generic boundaries" (Ackermann and DuBow 1987). This is supported by the fact that most isolated aquatic viruses examined to date show species or strain specificity (Wommack and Colwell 2000). However, for some phages, and on several occasions, broader host ranges have been demonstrated (Wichels et al. 1998). Broad host range (polyvalence) has been particularly demonstrated for cyanophages infecting marine *Synechococcus* (Suttle and Chan 1993; Waterbury and Valois 1993; Lu et al. 2001). Presently, there is too little data available to draw conclusions on the host range and specificity of natural bacteriophage communities.

From field and laboratory experiments, evidence shows that viral infection influences the diversity of the bacterial host communities (Wommack and Colwell 2000; Weinbauer 2004). It had been assumed intuitively, and later supported by models, that bacterial strains that are most successful in exploiting a substrate pulse and in developing higher concentrations are also most susceptible to viral infection due to the increased virus–host encounter. This "killing the winner" concept has been used in food web models

(Thingstad 2000b) but experimental evidence for this concept is still rather scarce. Heterotrophic bacteria and cyanobacteria can quickly acquire resistance to lytic phages when exposed to strong predation pressure, for example in long-term chemostat cultures with one bacterial prey and one phage (Bohannan and Lenski 1997), but also in more complex experimental systems (Middelboe et al. 2001). The rapid development of resistant mutant strains means that bacteriophages only have a temporal effect on specific host populations. Despite potentially resistant host cells in natural waters, the high virus abundance observed can be explained by the fact that resistance has physiological costs, thereby promoting the co-occurrence of less competitive but resistant, and of sensitive but faster growing host bacteria (Bohannan et al. 2002). Because of this, it has been argued that viral lysis is more significant as a factor for influencing clonal diversity than for controlling bacterial abundance (Waterbury and Valois 1993; Middelboe et al. 2001). There can also be an evolutionary response of virulent bacteriophages towards resistant host populations by phage mutants that can utilize alternate host receptors (host range mutants) (Buckling and Rainey 2002). This suggests the existence of co-evolving populations of bacteria and phages with endless cycles of resistance and counter-resistance mutations (Lenski and Levin 1985).

2.2 Predation by Prokaryotes

Prokaryotic predation on other prokaryotes seems to be a widespread survival mode among different taxonomic groups of bacteria (see chapter by Jurkevitch and Davidov in this volume) and probably affects to some extent a wide prey range among Gram positive and Gram negative bacteria. Predatory prokaryotes have only been known since the early 1960s when *Bdellovibrio* was discovered (Stolp and Starr 1963). *Bdellovibrio* and like organisms (BALO), which have been isolated from many aquatic and terrestrial environments, are now the best studied group of predatory prokaryotes. Much less information is available on predatory bacteria from other phylogenetic groups (e.g. *Ensifer*, *Micavibrio*), some of which are non-obligate predators on bacteria and some only partially described and not available in culture collections (see overview by Jurkevitch and Davidov).

In order to judge the ecological role and implications of prokaryotic predation on bacteria, field data on predator and prey abundance and rates of successful attacks and multiplication time within hosts are required. Some of this data is available for BALOs, which seem to occupy a wide spectrum of ecological niches (Williams et al. 1995; Markelova and Kerzhentsev 1998; Yair et al. 2003), including oxygen-deficient environments (Schoeffield et al. 1996). The in situ quantification of BALOs has up to now been performed by counting plaque-forming units on specific host bacteria. This technique yields numbers ranging usually from tens to tens of thousands of plaque-forming

units (pfu) per gram or milliliter of sample, with the lowest values of < 10 pfu from open water (Varon and Shilo 1978; Williams et al. 1982). Somewhat larger numbers can be found in sediments (Williams 1988), the air–surface interface (Williams 1987), biofilms (Williams et al. 1995; Markelova and Colwell 1999) and soils (Jurkevitch et al. 2000). Also, seasonal fluctuations, partly correlated with temperature, were shown to occur (Williams et al. 1982; Sutton and Besant 1994). There is evidence that BALO concentrations increase with bacterial concentrations and are thus found in more productive environments such as sewage effluents and polluted rivers (Fry and Staples 1974, 1976). Dependence on high prey concentrations fits with the observations that bdellovibrios prefer to associate with surfaces (Kelley et al. 1997) and can efficiently prey on and eliminate bacterial biofilms (Kadouri and O'Toole 2005; Nunez et al. 2005).

If we assume that every BALO has a certain host preference range, the plaque-counting technique faces the same limitation (and probably severe underestimation) as does the classical quantification of bacteriophages, for which direct microscopic counts yielded several orders of magnitude higher concentrations (Bergh et al. 1989). Within one habitat, different taxa of predatory bacteria co-exist, and even within the genus *Bdellovibrio* ecologically different strains occur that have different bacterial utilization patterns (Jurkevitch et al. 2000; Pineiro et al. 2004). Therefore, the real concentrations of predatory prokaryotes in the environment are probably much higher. However, we cannot make conclusions at present on the real numbers of BALOs or other predatory prokaryotes in the environment. This might change in the near future with the application of newly designed oligonucleotide probes (Davidov et al. 2006), which could be used for quantification by fluorescent in situ hybridization or quantitative PCR (see chapter by Koval in this volume).

Many laboratory studies demonstrated that isolated BALOs from soil and water can be efficiently grown on suspended and attached pure or mixed cultures of prey bacteria, which become efficiently reduced during the grazing experiments. From growth experiments with suspended bacteria it seems that BALOs need minimum prey concentrations in the range 10^5–10^6 mL^{-1} for survival (Varon and Shilo 1978). Then, it depends on the in situ host specificity whether a significant impact on the natural bacterial community can be expected. This also agrees with the fact that BALOs are more abundant in productive systems and are particularly associated with surfaces where bacterial biomass is found in a more concentrated form.

A significant predation impact by BALOs or other predatory prokaryotes has not been confirmed yet for natural systems. Reports on the ecological importance of predatory prokaryotes are, besides the quantification by the plaque assays, mostly descriptive without quantitative assessments of grazing impact on bacterial communities. For example, high abundance of predatory prokaryotes has been observed during blooms of purple sulfur bacteria, with

a high percentage of prey cells under attack by *Vampirococcus* (Guerrero et al. 1986). Though this indicated potential ecological relevance, it was not clear whether all attached cells contributed to host damage as host mortality rates were not determined. Experimental studies with river bacterioplankton assemblages did not reveal a significant impact of *Bdellovibrio* on the bacterial community (Fry and Staples 1974).

The different groups of prokaryotic predators have evolved different predatory strategies to attack bacteria (Martin 2002). One strategy does not even require cell-to-cell contact: group predation ("wolfpack strategy") by *Myxococcus* and *Lysobacteria* in which the production of hydrolytic extracellular enzymes results in localized prey damage, e.g. in dense cyanobacterial blooms. A probably more common foraging strategy involves attachment to the outer cell wall (epibiotic) and subsequent degradation and assimilation of the host cell through specialized structures (e.g. *Vampirococcus*) or the generation of a lytic factor (presumably in *Ensifer*). Epibiotic predation in field samples might be confused with epibiotic attachment because surfaces of larger prokaryotes can be colonized by other prokaryotes. Direct invasion of predators into the cytoplasm (e.g. by *Daptobacter*) has also been reported (Esteve et al. 1983; Guerrero et al. 1986) but so far not further investigated or observed.

Invasion into the periplasm of Gram negative cells by BALOs is the best studied process, both by field observation as well as by laboratory experiments with isolated cultures. It is outlined in detail in the chapters by Jurkevitch and Davidov and Tudor and McCann in this volume. Whereas foraging by excretion of lytic enzymes is probably only effective at dense, non-motile prey concentrations (e.g. microbial mats, biofilms), predation involving cell-to-cell contact might also be relevant for suspended bacteria in a dilute habitat such as plankton. BALOs in the attack phase have limited substrate uptake capacity and therefore may only have a relatively short time (in the range of hours) to search for appropriate host cells with high velocity until their energy reserves are exhausted (see chapter by Strauch et al. in this volume). Similarly to other heterotrophic bacteria and protists they are attracted by chemotactic behaviour towards labile substrates (e.g. amino acids), which enhances the probability of finding patches of higher bacterial concentrations. The dynamics of predator–prey interactions between BALOs and their substrate cells is described in the accompanying chapter by Wilkinson in this volume. A random collision model has been suggested similar to that for phage–bacteria interactions,(Martin 2002).

The crucial factor that determines the magnitude of prokaryotic predation as a selective factor and its ecological impact on the bacterial communities is the host specificity. Although a wide variety of Gram-negative bacteria can be infected by BALOs, the prey range susceptible to specific BALO strains varies strongly. Interestingly, colony-forming bacteria growing on agar plates seem to be particularly susceptible: around 80% of the bacteria isolated from wa-

ter, sediment and oyster-shell surfaces were susceptible to BALO predation from the same sample site, as revealed by the plaque-forming technique (Rice et al. 1998). From these numbers the authors extrapolated to the whole bacterial assemblage, concluding that sufficient prey bacteria should be present to support in situ growth of BALOs. This extrapolation is of course problematic as we now know that the bacteria growing on agar plates (in the study by Rice et al. 1998 mainly from the genera *Pseudomonas, Vibrio, Aeromonas*) represent only a small fraction of the in situ assemblage, which is dominated by hitherto uncultivated bacterial taxa (Rappé and Giovannoni 2003). A more realistic picture of the in situ dynamics of BALOs and their prey populations would require direct measurements of predator abundance and infection rates in the environment. One approach could be the monitoring of added prey bacteria (Lambert et al. 2003) in combination with BALO-specific probes.

Bacterial resistance to prokaryote predation has also to be considered in order to understand the in situ population dynamics. When exposed to strong predation by *Bdellovibrio*, for example in experimental monocultures of prey bacteria, a rapid development of predation-resistant prey bacteria can be observed (Shemesh and Jurkevitch 2004). The underlying reasons may be both phenotypic plasticity (Shemesh and Jurkevitch 2004) as well as the development of resistant mutants (Varon 1979). In summary, due to the lack of field studies on prokaryote predation impact on bacterial communities and populations, it remains to be proven that predatory prokaryotes might have an impact comparable to predation by bacteriophages and protists.

2.3 Predation by Protists

Phagotrophic protists are ubiquitous grazers of bacteria in marine, freshwater and terrestrial environments and have been the focus of numerous investigations in the past three decades. This line of research was promoted by the general notion that heterotrophic bacteria and picocyanobacteria account for a large fraction of primary and secondary production in marine pelagic food webs and that protist grazing is the major mortality factor for suspended prokaryotes (Azam et al. 1983; Fenchel 1984). A number of different techniques for obtaining grazing rate estimates have been developed, resulting in a large data base particularly for planktonic habitats (Landry 1994; Vaqué et al. 1994; Sleigh and Zubkov 1998). The data revealed that protist grazing often balances bacterial production, thereby keeping bacterial numbers rather constant within a given system (Sherr et al. 1989; Sanders et al. 1992). Protist grazing is considered an important term in all aquatic food web models, which include microorganisms (Gasol 1994; Thingstad 2000a). Less data are available for grazing on attached bacterial communities in biofilms, which dominate in benthic systems, small running waters and all below-

ground habitats. Here some of the regulating mechanisms might be different from planktonic systems, and the interactions between bacteria and bacterivores on biofilms have only recently been examined in more detail (Matz et al. 2004a; Weitere et al. 2005). Besides the quantitative aspects of protist bacterivory, grazing has also functional impacts, such as the stimulation of microbial nutrient regeneration and detritus decomposition (Fenchel and Harrison 1976; Caron et al. 1988; Jürgens and Sala 2000), and of bacterial production (Posch et al. 1999).

Small (2–10 μm), flagellated heterotrophic or mixotrophic protists have been identified as the major bacterivores in pelagic systems, able to thrive exclusively on suspended bacteria as food (Fenchel 1984, 1986a). This functional group, consisting of very different phylogenetic lineages (Patterson 1994; Arndt et al. 2000), is often collectively termed heterotrophic nanoflagellates (HNF). Their abundance (range roughly 10^2–10^4 cells mL^{-1}) increases, together with that of bacteria, with the trophic state of the system (Sanders et al. 1992) but shows strong temporal dynamics within a system due to food web interactions with higher trophic levels (e.g. Gasol and Vaqué 1993; Jürgens and Stolpe 1995). In most situations HNF grazing accounts for the major portion of protist bacterivory. Threshold bacterial concentrations which support HNF growth are in the range of 10^5–10^6 bacteria mL^{-1} (Eccleston-Parry and Leadbeater 1994). However, this has been mainly deduced from laboratory experiments in which neither the bacteria nor the HNF taxa might be representative for the in situ situation. Further, the incipient limiting in situ level of bacterial concentration is less clear as it depends on the size and food quality of the bacteria as well as on the HNF taxa. When HNF are found in habitats with extremely low bacterial concentrations (groundwater, oligotrophic ocean) some taxa might also depend on the occurrence of spots with higher concentrations of bacteria (e.g. on aggregates, biofilms) (Caron 1987).

For ecological studies, HNF are treated generally as one trophic level (bacterivores). Although this might be reasonable for estimates of major carbon flows it is a simplification as most flagellates are rather omnivorous, ingesting small algae, other flagellates and detritus (Boenigk and Arndt 2000) as well as bacteria. HNF are also considered a black box in terms of species composition, ignoring the huge phylogenetic diversity of this functional group. Dominant taxonomic groups in pelagic habitats are dinoflagellates, choanoflagellates, heterokont taxa (e.g. chrysomonads, bicosoecids) and katablepharids, euglenids in benthic communities, bodonids, thaumatomastigids, cermomonads and apusomonads (Arndt et al. 2000). The in situ species composition within these groups has only rarely been studied in more detail but probably differs between freshwater, brackish and marine systems. Further, there are many groups of as-yet uncertain phylogenetic position ("protista incertae sedis") (Patterson 1994). In recent years, novel groups among the phyla stramenopiles and alveolates have been discovered in the ocean by 18S clone libraries (Lopez-Garcia et al. 2001; Moon-van der Staay et al. 2001; Diez et al.

2001). Some of these still uncultivated groups seem to be abundant and globally distributed in marine systems (Massana et al. 2004). Although there is evidence that some of these picoeukaryotes are bacterivorous (Massana et al. 2002), their ecological role still has to be examined.

Nanoflagellates possess behavioural and morphological adaptations to concentrated food particles in suspension, being able to clear about 10^5 times their own cell volume of water per hour (Fenchel 1982a, 1986a). Even in pelagic habitats a large portion of HNF is loosely attached to seston particles as this is assumed to allow higher clearance rates (Fenchel 1986a). Different principal mechanisms of capturing and concentrating suspended food particles can be distinguished (Fenchel 1986a; Boenigk and Arndt 2002): interception feeding, which depends on the random encounter between individual prey cells and the predator; filter feeding, in which prey organisms are concentrated by sieving through a ciliary or pseudopodial filter; and diffusion feeding, in which motile prey collide with a sticky surface on the predator. Filtration and direct interception, the two most common modes in HNF, are achieved by undulating flagella. These produce a water current that transports particles towards the cell surface where phagocytosis and food vacuole formation is induced. In benthic habitats many HNF taxa grasp attached bacteria, often aided by specialized feeding organelles to detach bacteria from biofilms.

Beyond this distinction in basic feeding mechanisms, a large variability with regard to feeding strategy, food uptake and selection mechanisms exist, even among a single feeding type group (Boenigk and Arndt 2002). This results in different prey size preferences even within the micrometre range of prey particles (see Fig. 1), and probably also different prey selectivities based on other bacterial properties. A complex prey selection behaviour has been discovered in different protist taxa (Verity 1988; Jürgens and De Mott 1995). It suggests that there may exist many species-specific interactions between bacteria and bacterivorous protists. These, together with the development of bacterial defence mechanisms, would allow the coexistence of many bacterivorous protist taxa and maintain bacterial diversity despite a substantial grazing exerted by the functional group of HNF.

Many filter-feeding ciliate species are also able to consume bacteria. Strictly bacterivorous taxa seem to be adapted to environments with very high bacterial concentrations (e.g. in decomposing organic material) (Fenchel 1980). However, some planktonic ciliates (e.g. oligotrichs) can also exert significant feeding rates on bacteria although they are probably omnivorous, consuming pico- and nanosized plankton organisms (Sherr and Sherr 1987; Šimek et al. 2000).

Size-selective prey uptake occurs in nearly all bacterivorous protist groups. In filter-feeding taxa this is governed by the morphology of the filtering structure, the distance between ciliary membranelles in ciliates or between the tentacles of the collar in chaoanoflagellates and helioflagellates (Fenchel

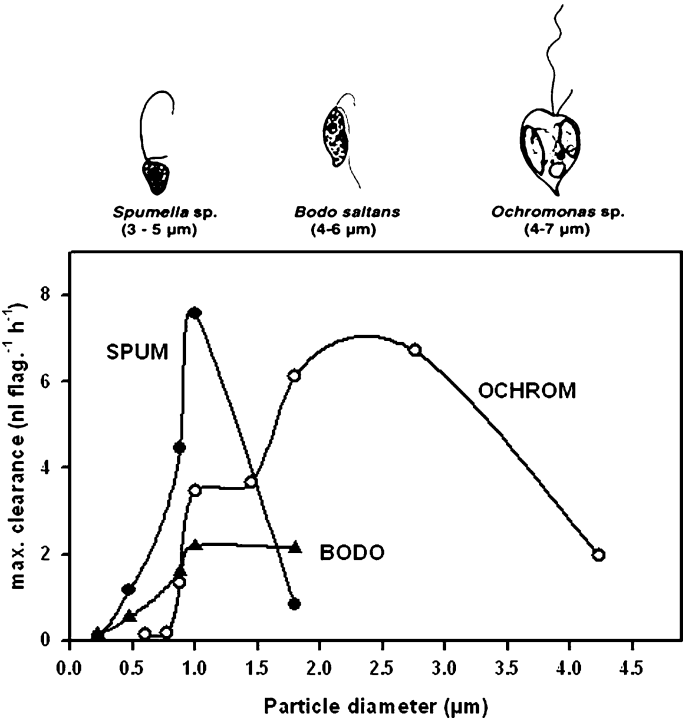


Fig. 1 Size-selective feeding efficiency and prey size range of three species of interception-feeding flagellates (*Spumella* sp., *Ochromonas* sp., *Bodo saltans*), determined by uptake experiments with fluorescent latex beads. For each particle size, ingestion rates were measured as a function of particle concentration (functional response) and fitted to a hyperbolic function. For each flagellate and particle size, maximum ingestion rate (I_m) and half-saturation constant (K_s) were determined and used to calculate maximum clearance rate by I_m/K_s. (from Jürgens & Matz 2002, with permission from the publishers)

1986b). The porosity of the filter structures determines the particle size that can be retained. Therefore, as a first-order approximation, filter feeders are characterized by the absence of food particle discrimination, except through mechanical properties (Fenchel 1986b). The bacterial cell size is the decisive character determining the efficiency with which a bacterial cell is retained. Whereas among larger bacterivores (ciliates, some metazoan filter feeders) only filtration is realized, many nanoflagellates are interception feeders. However, even this feeding type has shown to be strongly size selective, achieving much higher clearance rates with larger food particles (Chrzanowski and Šimek 1990; Gonzalez et al. 1990). Prey size-selectivity in interception-feeding flagellates requires neither particular filter structures nor active predator choice, but can be explained by simple geometric models in which the encounter rate between prey and predator increases proportional to the prey radius (Fenchel 1982a). Therefore, small differences in bacterial cell size can

result in large differences in feeding efficiency (roughly proportional to the square of the prey radius), which can be demonstrated in feeding experiments with artificial particles of defined size (Fig. 1). Besides this general pattern, differences in predator size, feeding currents and mode of prey uptake can nevertheless result in distinct prey size ranges, and therefore in niche differentiation, among bacterivorous nanoflagellates (Fig. 1).

Interception-feeding flagellates are the only phagotrophic organisms that handle and process individual bacterial cells, thereby potentially interacting with their prey at the biochemical, molecular level. This has considerable implications for recognition and prey selection as well as for bacterial defence mechanisms. Bacterial features such as size, motility and the physicochemical properties of surface structures strongly influence the HNF predation success and the escape probability of bacteria (see below). Additionally, the flexibility in the expression of certain bacterial characters that are relevant for the predatory interactions implies that phenotypic plasticity can be involved in the formation of resistant phenotypes.

2.4 Predation by Metazoans

There are no metazoans that feed exclusively on bacteria, but some groups can nevertheless not be ignored as bacterial consumers. These are, in planktonic systems, some mesozooplankton groups. In benthic habitats certain bivalve species and other benthic filtrating suspension feeders collect suspended particles from the water column. The possession of specialized filtration structures enables the retention of a range of particles sizes and it depends on the fine structure of the filtering organs whether particles in the bacterial size fraction can be captured. In freshwater planktonic systems, the most relevant group to be considered are cladocerans, particularly of the genus *Daphnia*. Their grazing impact affects all organisms of the microbial food web, from picoplankton to large ciliates (reviewed in Jürgens 1994). Many *Daphnia* species have fine filter mesh widths, which retain bacterial cells of around 0.5 μm (Brendelberger 1991). During *Daphnia* population maxima in lakes ("clear water phase") they can occasionally become the major bacterial consumer, partly due to the fact that the abundance of bacterivorous protists is strongly suppressed (Jürgens 1994). Additionally, some smaller cladoceran taxa and a number of rotifer species are able to filter suspended bacteria (Arndt 1993). Their overall impact on planktonic bacteria is, however, only occasionally comparable to *Daphnia*-dominated situations (Ooms-Wilms et al. 1995).

A functional counterpart of freshwater filter-feeding cladocerans in marine systems are pelagic tunicates, such as salps, doliolids and appendicularians (also referred to as gelatinous zooplankton) (Sommer and Stibor 2002). All pelagic tunicates are filter feeders and some of them possess fine-mesh filter

screens to retain even the smallest bacterial cells (Deibel and Lee 1992). Similarly to freshwater daphnids, they can temporarily have an important impact on lower trophic levels due to this particular filtering ability and due to their high population growth rates.

All planktonic metazoan filter feeders are characterized by the facts that:

1. Their predation pressure is generally spatially (due to patchy distribution) and temporally (seasonal succession) confined
2. Planktonic bacteria are at the lower boundary of ingestible particle sizes and the retention efficiency depends on the actual zooplankton species (and the mesh width of their filtration apparatus) and the size structure of the bacterial community
3. They do not solely depend on bacteria as food resource as they ingest a certain prey size spectrum

Other metazoans potentially able to feed on suspended bacteria are various organism groups of freshwater and marine benthic communities such as bivalves, ascidians and corals (Cotner et al. 1995; Bak et al. 1998). In particular, some bivalve species have sufficiently fine filter structures (laterofrontal cirri) to retain bacteria (Riisgard 1988; Silverman et al. 1997) and their impact on microbial communities in shallower aquatic systems has been demonstrated (Strayer et al. 1999).

Besides direct predation on bacteria, many invertebrate metazoans in aquatic systems can have stronger indirect effects on bacterial communities due to cascading predation effects, for example when bacterivores (e.g. protists) are efficiently controlled, thereby relieving predation pressure on bacteria (Zöllner et al. 2003).

3
Bacterial Resistance Mechanisms with Respect to Protist Predation

Heterotrophic or mixotrophic (photosynthesis and bacterivory) nanoflagellates can be easily maintained on a mixed or pure bacterial diet and have been extensively used for laboratory experiments in batch and continuous cultivation to study growth, feeding and interactions with bacterial populations. These studies, at well-defined conditions, provided insights into basic principles and mechanisms, for example of protist feeding and foraging behaviour (Jürgens and De Mott 1995; Boenigk et al. 2001) and of bacterial resistance mechanisms (Jürgens and Matz 2002; Matz et al. 2002a). These results can further be related to observations from field experiments and in situ studies of bacteria–protist interactions in order to deduce their relevance for the natural situation. For bacterial cell size, which is probably the most relevant phenotypic character of suspended bacteria for determining predation mortality, evidence is now available both from laboratory studies as well as from field

experiments and observations in different systems. Thereby, we have a comprehensive insight into the importance of bacterial size for the interactions with bacterial consumers, which will be summarized below.

Size-selective grazing by most protists and an increase in predation rates with increasing bacterial cell size has repeatedly been demonstrated and has already been mentioned (see Fig. 1). Small bacteria obviously suffer less predation mortality than large cells and this fact is one reason for the dominance of small bacterial cells in pelagic habitats. However, above a certain cell size bacteria clearly become too large to be ingested by nanoflagellates or other small protists and might become grazing-resistant. Resistant bacterial morphologies include different types of filament-forming bacteria and cells growing in aggregates or microcolonies (Fig. 2). The development of predation resistance in bacteria has frequently been studied in bacteria–protist continuous culture systems with mixed bacterial prey communities. In those systems, grazing becomes the major selective force and predation-mediated phenotypic and taxonomic changes and the selection of more or less resistant strains can be analysed. This was most obvious in the case of filamentous bacteria, which sometimes became the dominant morphotype in such bacteria–protist chemostats (Šimek et al. 1997; Hahn and Höfle 1999; Matz and Jürgens 2003). It confirmed the previous observation from lake plankton where the occurrence of filamentous bacteria was associated with increased grazing pressure by nanoprotists (Güde 1989; Jürgens et al. 1994; Jürgens and Stolpe 1995; Sommaruga and Psenner 1995). Besides filamentous bacteria, other bacteria without unusual morphologies also often survived protist grazing in mixed chemostat cultures (Pernthaler et al. 1997; Pernthaler et al. 2001; Matz and Jürgens 2003), indicating the existence of other resistance mechanisms.

The isolation of bacterial strains able to grow in the form of long filaments and the maintenance of axenic protist cultures allowed the performance of one prey/one predator experiments (Hahn and Höfle 1999). These gave some

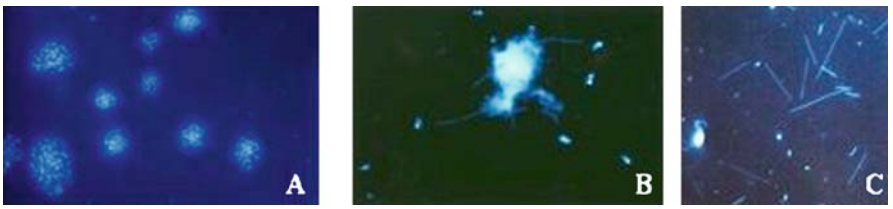


Fig. 2 Examples of grazing-resistant bacterial morphologies, observed in response to increased protist predation pressure. **a** Pure culture experiment with *Pseudomonas* sp., growing in resistant microcolonies in the presence of protists (Matz et al. 2002b); **b** Bacterial aggregates and filaments developing in response to protist grazing in microcosm experiments (Jürgens et al. 1997); **c** Filamentous bacteria observed in a mesocosm experiment during increased protist abundance (Jürgens et al. 1994)

insight into the underlying mechanisms of development of resistant bacterial morphotypes. When using monocultures of isolated bacterial strains as prey, a remarkable degree of phenotypic plasticity in some of the studied bacterial strains was observed. This plasticity resulted in reversible morphological changes towards grazing-resistant growth forms such as filamentous cells (Hahn et al. 1999; Corno and Jürgens 2006) or cells growing in the form of microcolonies (Hahn et al. 2000; Matz et al. 2002b) (see also Fig. 2). From chemostat experiments, as well as from field observations, three different principal mechanisms could be distinguished by which filamentous bacteria can become dominant in a complex bacterial assemblage under enhanced protist grazing pressure (Jürgens and Matz 2002):

1. *Permanently resistant morphotypes*: Certain bacterial taxa are permanently resistant to ingestion by nanoprotists, for example due to complex, large morphologies. Although generally not abundant, they increase in number when edible (and presumably more competitive) bacteria are eliminated during increased grazing pressure. Many bacterial taxa with a permanent complex morphology (e.g. stalked and filamentous cells), have been observed and isolated from freshwater and marine plankton (e.g. Hirsch 1974; Schmaljohann et al. 1987). Occasionally an increased in situ abundance of such taxa with peculiar morphology was observed in response to increased protist predation (e.g. *Ancalomicrobium*; Bianchi 1989).
2. *Growth rate-related morphological changes*: Enhanced substrate supply and high bacterial growth rates often result, within a mixed bacterial assemblage, in an increased proportion of larger bacteria, bacterial clumps and aggregates, which are all less available for nanoprotists (e.g. Jürgens and Sala 2000). At the level of single bacterial strains, the shift towards resistant morphotypes can be mediated by phenotypic plasticity and directly related to increased protist grazing pressure. For example, the bacterial strains *Comamonas acidovorans* and *Flectobacillus* sp. both showed a shift towards inedible filaments when grown in chemostat cultures in the presence of bacterivorous flagellates (Hahn et al. 1999). Because this increase in cell length is similar to the increase that can be observed at higher growth rates, it was concluded that the grazers enhanced the specific bacterial growth rate due to recycling of nutrients and elimination of competitors, thereby indirectly triggering the shift towards resistant cell sizes (Hahn et al. 1999).
3. *Chemically induced morphological changes*: The induction of phenotypic changes due to specific signals released by the predators (kairomones) has been demonstrated for many other plankton organisms (Tollrian and Harvell 1999) and also assumed to exist for bacteria. However, evidence for chemically induced plasticity in bacteria was obtained only recently from chemostat studies with a *Flectobacillus* strain in which resistant bac-

terial filaments developed despite bacteria and protists being separated by dialysis membranes (Corno and Jürgens 2006). However, it remains to be demonstrated whether a predator-released "infochemical" is involved or whether protist excretions change the bacterial growth medium in a way that favours filamentous morphotypes. Until the active chemical compound that triggers morphological changes in bacteria is determined, it will not be possible to answer this question.

Predation experiments with single bacterial strains also revealed the importance of morphological polymorphism within a clonal bacterial population, including vulnerable and resistant (e.g. elongated cells and filaments) morphotypes. Phenotypic heterogeneity is a recognized property of bacterial cultures, including morphological and metabolic properties (Booth 2002). In the case of strains with high phenotypic plasticity, such as the filament-forming genus *Flectobacillus* (Hahn et al. 1999; Corno and Jürgens 2006), this polymorphism should enhance the adaptability and the potential to survive environmental changes caused by abiotic or biotic factors. Phenotypic heterogeneity of bacterial strains with respect to vulnerability towards predators is certainly an important issue needing further research.

A more systematic investigation of potentially important bacterial phenotypic traits, other than bacterial size, that affect vulnerability towards bacterivorous protists has been performed using high-resolution video microscopy. Using this technique, the fate of single bacterial cells from well-characterized cultures could be followed during the protist feeding process (reviewed in Jürgens and Matz 2002). Such an analysis provided evidence for a complex selection behaviour and for the influence of bacterial properties on different stages of the process of food acquisition (Boenigk and Arndt 2000; Jürgens and Matz 2002; Matz et al. 2002a). Predator–prey interactions between flagellates and bacteria can be subdivided into several stages, starting with the encounter or contact of flagellate and bacterium, followed by capture and particle handling, ingestion of the particle, and finally digestion and assimilation of the prey (Boenigk and Arndt 2000a). A number of bacterial properties have been found to cause feeding failure, thus acting as successful defence mechanisms at different stages of this predator–prey interaction (Jürgens and Matz 2002). A schematic overview of the most important recent findings is shown in Fig. 3 and summarized in Table 2, outlining the crucial steps observed in the feeding behaviour of an interception-feeding bacterivorous flagellate and the effective bacterial resistance mechanisms.

Figure 3 only shows the mechanisms acting after the encounter of predator and prey. However, the encounter rate itself is already affected by bacterial size and motility and by bacterial exudates operating as attractant or repellent in chemoperceptive prey location of protozoans (Fenchel and Blackburn 1999). Once the bacterial cell encounters a flagellate predator, bacterial motility and surface charge seem to be effective as initial mechanisms of capture

Table 2 Properties of bacteria that influence predation success rates of bacterivorous nanoflagellates. See text for details

Properties	Remarks on mechanisms
Size	Decreased encounter and ingestion efficiency with decreasing size; resistance to ingestion by elongated, complex morphologies; phenotypic plasticity
Microcolonies	Resistance against ingestion; phenotypic plasticity
Hydrophobicity	Decreasing hydrophobicity reduces feeding rates
Charge	Reduced capture efficiency of particles with highly negative surface charges
Biochemical surface structures	Influences prey selection behaviour
Motility	High swimming speeds prevent capture
Capsules, exopolymers	Influences prey selection; potentially reduces digestion
Toxicity	Inhibitory or lethal effects on predators

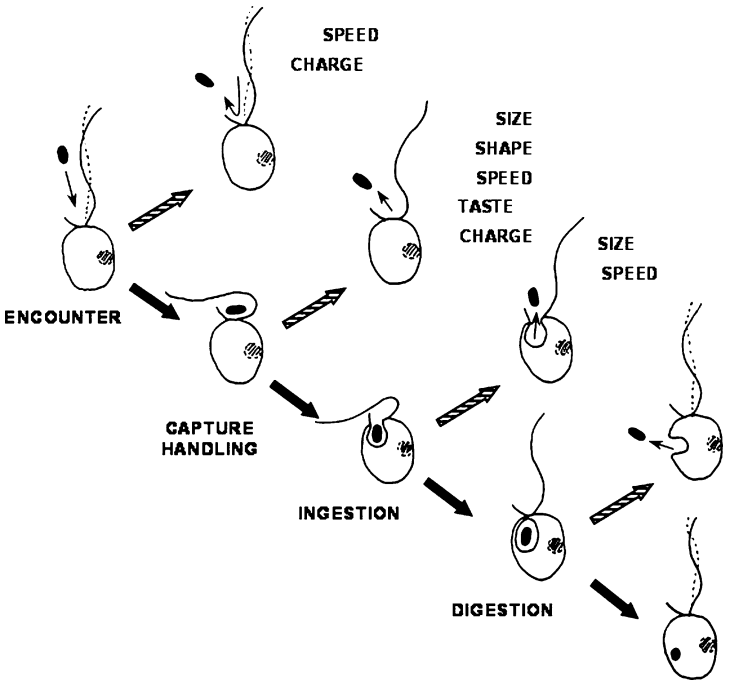


Fig. 3 Resistance mechanisms of suspended bacteria, acting at different stages in the predator–prey interactions with interception-feeding nanoflagellates. Events of potential feeding failure at each stage are indicated by *hatched arrows* (from Jürgens and Matz (2002) with permission from the publishers). See text for further explanations

avoidance (Fig. 3 Encounter): High swimming speed provides a mechanism to escape prior to the predator's reaction. Ingestion rates are already negatively affected at bacterial swimming speeds $> 25\ \mu m\ s^{-1}$ (Matz and Jürgens 2005) and significantly reduced at speeds of $40–60\ m\ s^{-1}$, a motility commonly found in planktonic bacteria (Fenchel 2001; Grossart et al. 2001; Johansen et al. 2002). Prey particles with extremely negative surface charges reduce capture probabilities due to repulsive surface forces (Matz and Jürgens 2001), whereas contradictory results were obtained for the impact of cell hydrophobicity (Monger et al. 1999; Matz and Jürgens 2001). Bacterial cells captured by a flagellate can still potentially escape, favoured by several bacterial features (Fig. 3 Capture handling): Very large and very small bacterial size and complex cell shape and morphology can cause handling problems. Further, extreme surface charge or "distasteful" biochemical surface compounds of the bacterial prey may result in active rejection of the captured bacteria. Even during the process of food vacuole formation (Fig. 3 Ingestion), oversized bacteria might be rejected and highly motile cells may escape from the phagocytosis process. Finally, resistance can also occur in the last stage (Fig. 3 Digestion) when unpalatable prey bacteria (shown for example for some cyanobacterial strains) can be prematurely egested (Boenigk et al. 2001) or some bacteria might be resistant to enzymatic digestion. Some bacterial strains produce highly toxic compounds (e.g. the pigment violacein), which result in immediate death of the predators after these compounds are released into the food vacuoles (Matz et al. 2004b).

Although this compilation already comprises a range of resistance mechanisms comparable to those known for higher aquatic organisms (see Tollrian and Harvell 1999), it is by no means exhaustive and presumably other mechanisms await discovery. It is not yet known which of these mechanisms is most relevant in natural systems. Although most evidence is available for bacterial size as the relevant character, this is biased by the fact that other bacterial phenotypic features that potentially confer resistance or reduced vulnerability are more difficult to assess in field samples. This is particularly the case for the physico-chemical surface structures of bacteria.

4
Bacterial Resistance against Other Predators

Experimental studies with bacterial isolates showed that bacterial strains can quickly acquire resistance against bacteriophages (Bohannan and Lenski 1997) and also against prokaryotic predators such as BALOs (Shemesh and Jurkevitch 2004). The dynamics of predator–prey interactions in a batch culture experiment for BALO or bacteriophage predation looks in some aspects quite similar to that for bacteria–protist interactions: After a phase of rapid predator growth and decline in prey bacteria, a relatively stable level

is achieved below which prey abundance does not decrease further. Eventually, re-growth of bacteria occurs despite continuing predation pressure. Such a pattern is evidence for the development of resistant bacterial prey and has been observed with protists (Jürgens and Güde 1994), BALOs (Shemesh and Jurkevitch 2004) and bacteriophages (Middelboe et al. 2001) as predators. In a continuous culture system, in which bacterial growth and predation are permanently present, the development of resistance is even more pronounced. This has been repeatedly documented with bacterivorous protists, as outlined above, but has also been observed for *Bdellovibrio* (Varon 1979) and bacteriophages (Lenski and Levin 1985; Bohannan and Lenski 1997). For the latter, coevolutionary arms races between phages and bacteria has been observed and modelled (Bohannan and Lenski 1997; Weitz et al. 2005). It should be kept in mind, however, that many predation studies were performed with monocultures of bacteria and bacterial predators. A lower degree of bacterial resistance might develop when multiple bacterial preys (Harcombe and Bull 2005) or when different bacterial predators are present (Massana and Jürgens 2003).

The underlying bacterial resistance mechanisms and their regulation towards predation by BALOs and bacteriophages have generally not been studied in as detailed a manner as for bacterivorous protists. Some studies on resistance to phage predation have been performed with economically or medically important bacterial strains (e.g. Allison and Klaenhammer 1998). During the first stage of phage infection (i.e. adhesion) the distinct tail fibres of the bacteriophage are presumed to exclusively absorb to specific conformations of bacterial surface receptors. In principle, resistance to phage absorption can most efficiently be attained by a lack of receptor expression or by a change in protein conformation (Lenski 1988). Relevant receptors include cell wall and membrane proteins, lipopolysaccharides, flagella and pili. It is not known what the major responsible receptors are in natural communities or what implications the lack of them has for the host cells. However, loss of receptors is not the only possible resistance mechanism, as seen, for example, from the detailed studies on phage resistance in lactic acid bacteria (Allison and Klaenhammer 1998). In this model, bacteria evolved a variety of mechanisms to interfere with phage development at different stages of the infection cycle. These included prevention of phage adsorption; inhibition of DNA injection, replication and transcription; restriction of phage DNA; and interference with the synthesis of phage proteins, phage assembly and release of phage progeny. Abortive infection is called a resistance mechanism and acts after phage adsorption and DNA injection and results in cell suicide. This is interpreted as an altruistic strategy to prevent the formation of phage progeny and ensuing infection of other cells within the population.

Biochemical and physico-chemical structures of the outer cell wall (e.g. lipopolysaccharides) might also constitute decisive factors characterising the

appropriate host cells of BALOs and of other prokaryotic predators. Changes in the biochemical surface structures of host bacteria would then result in predation resistance by inhibiting BALO attachment. However, the nature of the specific receptor sites of host cells is not clear yet and the problem of receptor sites and prey recognition seems to be quite complex (Gray and Ruby 1991) (see also chapter by Strauch and Appel in this volume). Bacterial capsules, which have been assumed to be involved in protection against bacteriophages and may also prevent digestion by larger bacterivores (Plante 2000), cannot prevent the penetration of *Bdellovibrio* (Koval and Bayer 1997) and may even increase the encounter rate with BALOs. On the hand, paracrystalline protein surface layers (S-layers) have been shown to protect Gram-negative cells against predation by *B. bacteriovorus* (Koval and Hynes 1991).

5 Impact of Predation on Natural Bacterial Communities

The rather extreme effects of predation on the morphology and composition of bacteria, as observed in experimental systems in the laboratory, can only seldom be found as clearly in natural systems. This is mainly due to the fact that bacterial predators in natural systems are also regulated by abiotic and biotic forces, and bacteria are subject to both bottom-up and top-down control. In the case of bacterivorous protists, food web interactions with higher trophic levels (Jürgens and Güde 1994) are most important, whereas for BALOs and bacteriophages the host dependency is probably the major governing force. Nevertheless, at least for protozoan and metazoan bacterivory, predation effects on natural bacterioplankton communities are well documented. For example, in situations when filter-feeding crustacean zoooplankton (*Daphnia*) dominate, i.e. during the seasonal succession in lakes, strong declines in bacterial abundance (reviewed by Jürgens 1994) and even bacterial diversity (Höfle et al. 1999) have been observed. In contrast, strong predation pressure by small protists (nanoflagellates) is only seldom associated with declines in bacteria but occasionally with the appearance of resistant filamentous bacterial cells (Güde 1989; Jürgens and Stolpe 1995; Pernthaler et al. 1996), indicating grazing impacts on bacterial community composition similar to those observed in chemostat studies. The contrasting effects of metazoan and protozoan predation on the bacterial size structure, which can be observed during the seasonal succession within one lake, have been described by Güde (1989) (Fig. 4). Recently, the population structure of the filamentous bacteria was elucidated with molecular techniques (Pernthaler et al. 2004; Schauer and Hahn 2005). It revealed that such grazing-resistant taxa can temporarily dominate bacterioplankton biomass (Pernthaler et al. 2004), thus demonstrating similar protist predation effects as previously shown in chemostat studies.

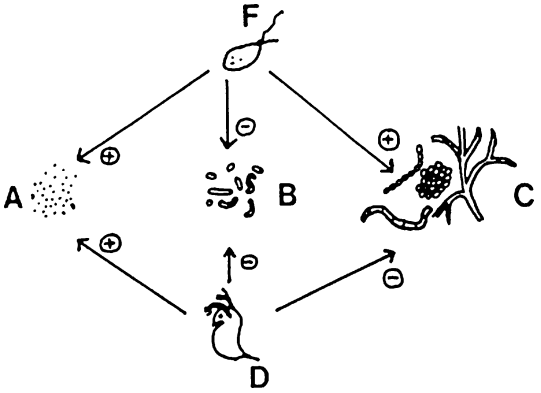


Fig. 4 Conceptual scheme showing the different impacts of metazoan filter feeders (*Daphnia* spp.) and protozoan (flagellates) predation on the bacterial size, structure and morphology as observed in Lake Constance (from: Güde 1989 with permission from the author and the publishers). Both predator groups preferentially remove medium-sized bacteria (*B*) whereas smallest cells (*A*) may accumulate. Complex bacterial morphologies such as filaments and aggregates (*C*) are resistant to protist predation but not to metazoans

There are certainly many other grazing-mediated shifts in bacterial community composition besides the shift towards filaments, which seems to be restricted to limnic and brackish habitats. For example, it has been shown that certain bacterial groups (e.g. *Alteromonas* and other strains within the γ-Proteobacteria in marine systems) generally occur in low numbers and only sporadically increase (e.g. due to substrate pulses). Such *r*-strategists and easily culturable bacteria are highly vulnerable to protist predation and become rapidly eliminated when grazers follow a bacterial peak (Beardsley et al. 2003). There are also indications that the dominance of small-sized *Actinobacteria* in lakes (Sekar et al. 2003) might be related to a reduced vulnerability towards protist grazers (Pernthaler et al. 2001). It remains to be shown whether this also plays a role in the dominance of ultra-microbacterial strains in the ocean (Morris et al. 2002).

To gain an overall understanding of predation on bacteria, food web control of the bacteria–protist relationship has to be considered. This was revealed in size-fractionation and enclosure experiments in which top-down control on HNF was relieved by removing larger predators (Jürgens et al. 1999; Šimek et al. 1999; Langenheder and Jürgens 2001; Šimek et al. 2002). In those studies, HNF abundance and the grazing pressure on bacteria were enhanced and resulted in stronger or weaker morphological and compositional changes of the bacterial assemblage, depending on the presence or absence of larger predators. Grazing-resistant bacterial morphotypes (filaments, aggregates) appeared within the major phylogenetic groups α-Proteobacteria, β-Proteobacteria and *Cytophaga-Flavobacteria*. Within each of these groups

there are examples of relative increases in abundance due to increased grazing pressure.

The abundance and production of bacterial communities, as well as their species composition and phenotypic structure, are regulated by the interplay of bottom-up and top-down forces. In a conceptual model by Šimek et al. (2002) the bacterial community composition is relatively stable at a given level of substrate supply and grazing pressure (steady-state-like situation) but shifts to a new composition (with better adapted taxa) when the ratio of bacterial growth and grazing is changed (Fig. 5). In planktonic systems, a tight predator–prey relationship between populations of bacterivorous protists and planktonic bacteria might rapidly mediate changes in the external forcing to the level of bacterial species composition.

Most of the evidence for the importance of trophic couplings and planktonic food web structure comes from studies in freshwater plankton. We are starting to gain a consistent picture on the possible cascading effects from zooplankton to bacteria. Considering all the available evidence from food web manipulation studies in enclosure experiments, as well as from field observations of seasonal succession, it now appears that there are strong linkages between the higher trophic levels and the microbial communities. Different metazooplankton communities exert different grazing pressure on the protozooplankton assemblage and the population density of bacterivorous protists, especially HNF, regulates the selective grazing pressure and feed-back mechanisms within the bacterioplankton.

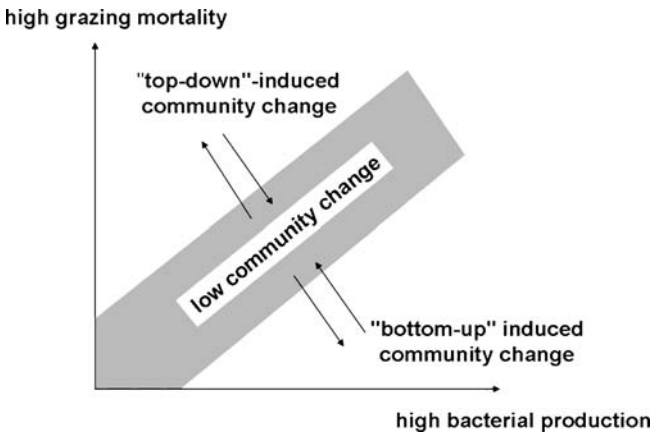


Fig. 5 Conceptual model of the regulation of bacterial community structure by a balance of production and bacterivory. Under steady state conditions of growth and grazing, the bacterial community composition is assumed to be near steady state (*shadowed area*), while a change in the ratio between growth and grazing will result in shifts in bacterial community composition towards a new state (after: Šimek et al. 2002, with permission from the authors and publishers)

6 Conclusions

Predation has long since been considered an important force, along with competition, in community and population ecology. It has major effects on organismal traits, population dynamics and community structure (see overviews in Kerfoot and Sih1987; Tollrian and Harvell 1999). Within the last two decades the relevance of this force has also been recognized in microbial ecology, particularly thanks to the study of aquatic microbial communities. It has become obvious that predatory mortality and selective predation are important factors to be considered for understanding a wide variety of issues, such as the flow of energy and material through ecosystems and the evolution of bacterial lifestyles and adaptations. It now seems possible that the distribution and diversity of free-living bacteria in the ocean is to some extent governed by the ability of microbes to survive in this hostile environment, in which predation quickly eliminates non-adapted strains (Pernthaler and Amann 2005). The development of new molecular tools in microbial ecology, which enable the monitoring of both bacterial population dynamics and of their physiological and phenotypic states, allows a more detailed comprehension of the interplay of resource supply and predation as shaping forces of bacterial assemblages. The current view has largely emerged from studies in pelagic environments, in which predation is naturally extremely important. It remains to be seen to what extent microbial communities in ecosystems less accessible to similar detailed studies, such as biofilms, below-ground food webs and the deep biosphere, show comparable patterns.

The numerous experimental studies with bacteria and bacterivorous protists have revealed several hitherto unknown mechanisms of predation resistance and predator foraging strategies, yet they probably still only represent the tip of the iceberg (Jürgens and Matz 2002). Moreover, our knowledge of the qualitative and quantitative impacts of other major predator groups, such as bacteriophages and predatory prokaryotes, is even smaller and lacks basic information such as in situ predation rates and bacterial defence mechanisms. More research efforts in this direction, as well as the development of new approaches, will be required to achieve a more comprehensive understanding, similar to the one we now have of bacteria–protist interactions.

Finally, we have to be aware that microorganisms are continuously faced with trade-offs, e.g. between resistance to predation by differently acting predator types or between resistance and competitive ability (Bohannan et al. 2002). Studies that have considered the interaction of substrate limitation and predation demonstrated that these factors should always be considered together (e.g. Matz and Jürgens 2003). It will be a challenge for experimental microbial ecologists, as well as for theoretical studies, to simulate trade-offs in a complex and permanently changing environment at the microscale level

in order to obtain a more realistic view of the consequences of predation for bacterial communities in the natural world.

References

Ackermann H, DuBow M (1987) Viruses of prokaryotes: general properties of bacteriophages. CRC, Boca Raton, FL

Allison GE, Klaenhammer TR (1998) Phage resistance mechanisms in lactic acid bacteria. Int Dairy J 8:207–226

Arndt H (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. Hydrobiologia 255/256:231–246

Arndt H, Dietrich D, Auer B, Cleven E-J, Grafenhan T, Weitere M, Mylinkov A (2000) Functional diversity of heterotrophic flagellates in aquatic ecosystems. In: Leadbeater B, Green J (eds) The flagellates. Taylor and Francis, London, pp 240–243

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Bak R, Joenje M, de Jong I, Lambrechts D, Nieuwland G (1998) Bacterial suspension feeding by coral reef benthic organisms. Mar Ecol Prog Ser 175:285–288

Beardsley C, Pernthaler J, Wosniok W, Amann R (2003) Are readily culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? Appl Environ Microbiol 69:2624–2630

Bergh Ø, Børsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature 340:467–468

Bettarel Y, Sime-Ngando T, Amblard C, Dolan J (2004) Viral activity in two contrasting lake ecosystems. Appl Environ Microbiol 70:2941–2951

Bianchi M (1989) Unusual bloom of a star-like prosthecate bacteria and filaments as a consequence of grazing pressure. Microb Ecol 17:137–142

Boenigk J, Arndt H (2000) Comparative studies on the feeding behavior of two heterotrophic nanoflagellates: The filter-feeding choanoflagellate Monosiga ovata and the raptorial-feeding kinetoplastid Rhynchomonas nasuta. Aquat Microb Ecol 22:243–249

Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie van Leeuwenhoek 81:465–480

Boenigk J, Matz C, Jürgens K, Arndt H (2001) The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. Microb Ecol 42:168–176

Bohannan BJM, Kerr B, Jessup CM, Hughes JB, Sandvik G (2002) Trade-offs and coexistence in microbial microcosms. Antonie van Leeuwenhoek 81:107–115

Bohannan BJM, Lenski RE (1997) Effect of resource enrichment on a chemostat community of bacteria and bacteriophage. Ecology 78:2303–2315

Booth IR (2002) Stress and the single cell: intrapopulation diversity is a mechanism to ensure survival upon exposure to stress. Int J Food Microbiol 78:19–30

Boraas M, Seale D, Boxhorn J (1998) Phagotrophy by a flagellate selects for colonial prey: A possible origin of multicellularity. Evol Ecol 12:153–164

Brendelberger H (1991) Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol Oceanogr 36:884–894

Buckling A, Rainey PB (2002) Antagonistic coevolution between a bacterium and a bacteriophage. Proceed R Soc Lond B Biol Sci 269:931–936

Caron DA (1987) Grazing of attached bacteria by heterotrophic microflagellates. Microb Ecol 13:203
Caron DA, Goldman JC, Dennett MR (1988) Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. Hydrobiologia 159:27–40
Chrzanowski TH, Šimek K (1990) Prey-size selection by freshwater flagellated protozoa. Limnol Oceanogr 35:1429–1436
Corno G, Jürgens K (2006) Direct and indirect effects of protist predation on the population size structure of a bacterial strain with high phenotypic plasticity. Appl Environ Microbiol 72:78–86
Cotner JB, Gardner WS, Johnson JR, Sada RH, Cavaletto JF, Heath RT (1995) Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton – evidence for both size-selective consumption and growth stimulation. J Great Lakes Res 21:517–528
Davidov Y, Friedjung A, Jurkevitch E (2006) High diversity of *Bdellovibrio*-and-like organisms (BALOs) in soil revealed by culture-dependent and culture-independent methods. Environ Microbiol 8:1667–1673
Deibel D, Lee SH (1992) Retention efficiency of submicrometer particles by the pharyngeal filter of the pelagic tunicate *Oikopleura vanhoeffeni*. Mar Ecol Prog Ser 81:25–30
Dietrich D, Arndt H (2000) Biomass partitioning of benthic microbes in a Baltic inlet: relationships between bacteria, algae, heterotrophic flagellates and ciliates. Mar Biol 136:309–322
Díez B, Pedrós-Alió C, Massana R (2001) Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl Environ Microbiol 67:2932–2941
Eccleston-Parry JD, Leadbeater BSC (1994) A comparison of the growth kinetics of six marine heterotrophic nanoflagellates fed with one bacterial species. Mar Ecol Prog Ser 105:167–177
Ekelund F, Ronn R (1994) Notes on protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amoebae and their ecology. FEMS Microbiol Rev 15:321–353
Esteve I, Guerrero R, Montesinos E, Abella C (1983) Electron microscopy study of the interaction of epibiontic bacteria with *Chromatium minus* in natural habitats. Microb Ecol 9:57–64
Fenchel T (1980) Suspension feeding in ciliated protozoa: Feeding rates and their ecological significance. Microb Ecol 6:13–25
Fenchel T (1982a) Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar Ecol Prog Ser 8:211–223
Fenchel T (1982b) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9:35–41
Fenchel T (1984) Suspended marine bacteria as a food source. In: Fasham MJ (ed) Flows of energy and materials in marine ecosystems. Plenum, New York, London, pp 301–315
Fenchel T (1986a) The ecology of heterotrophic microflagellates. Adv Microb Ecol 9:57–97
Fenchel T (1986b) Protozoan filter feeding. Progr Protistol 1:65–113
Fenchel T (2001) Eppur si muove: many water column bacteria are motile. Aquat Microb Ecol 24:197–201
Fenchel T, Blackburn N (1999) Motile chemosensory behaviour of phagotrophic protists: Mechanisms for and efficiency in congregating at food patches. Protist 150:325–336
Fenchel T, Harrison P (1976) The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In: Anderson JM, Macfadyen A (eds)

The role of terrestrial and aquatic organisms in decomposition processes. Blackwell, pp 285–299
Fischer UR, Velimirov B (2002) High control of bacterial production by viruses in a eutrophic oxbow lake. Aquat Microb Ecol 27:1–12
Fry JC, Staples DG (1974) Occurrence and role of *Bdellovibrio bacteriovorus* in a polluted river. Water Res 8:1029–1035
Fry JC, Staples DG (1976) Distribution of *Bdellovibrio bacteriovorus* in sewage works, river water, and sediments. Appl Environ Microbiol 31:469–474
Fuhrman J (2000) Impact of viruses on bacterial processes. In: Kirchman D (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 327–350
Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548
Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol Oceanogr 40:1236–1242
Gasol JM (1994) A framework for the assessment of top-down vs bottom-up control of heterotrophic nanoflagellate abundance. Mar Ecol Prog Ser 113:291–300
Gasol JM, Vaqué D (1993) Lack of coupling between heterotrophic nanoflagellates and bacteria: A general phenomenon across aquatic systems? Limnol Oceanogr 38:657–665
González JM, Sherr EB, Sherr BF (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl Environ Microbiol 56:583–589
Gray KM, Ruby EG (1991) Intercellular signalling in the *Bdellovibrio* developmental cycle. In: Dworkin M (ed) Microbial cell–cell interactions. American Society for Microbiology, Washington DC, pp 333–366
Grossart HP, Riemann L, Azam F (2001) Bacterial motility in the sea and its ecological implications. Aquat Microb Ecol 25:247–258
Güde H (1989) The role of grazing on bacteria in plankton succession. In: Sommer U (ed) Plankton ecology. Succession in plankton communities. Springer, Berlin Heidelberg New York, pp 337–364
Guerrero R, Pedrós-Alió C, Esteve I, Mas J, Chase D, Margulis L (1986) Predatory prokaryotes predation and primary consumption evolved in bacteria. Proc Natl Acad Sci USA 83:2138–2142
Guixa-Boixareu N, Calderón-Paz JI, Heldal M, Bratbak G, Pedrós-Alió C (1996) Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat Microb Ecol 11:215–227
Hahn MW, Höfle MG (1999) Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. Appl Environ Microbiol 65:4863–4872
Hahn MW, Moore ERB, Höfle MG (1999) Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. Appl Environ Microbiol 65:25–35
Hahn MW, Moore ERB, Höfle MG (2000) Role of microcolony formation in the protistan grazing defense of the aquatic bacterium *Pseudomonas* sp. MWH1. Microb Ecol 39:175–185
Harcombe WR, Bull JJ (2005) Impact of phages on two-species bacterial communities. Appl Environ Microbiol 71:5254–5259
Hirsch P (1974) Budding bacteria. Annu Rev Microbiol 28:391–444
Höfle MG, Haas H, Dominik K (1999) Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. Appl Environ Microbiol 65:3164–3174

Johansen JE, Pinhassi J, Blackburn N, Zweifel UL, Hagström Å (2002) Variability in motility characteristics among marine bacteria. Aquat Microb Ecol 28:229–237
Jürgens K (1994) Impact of *Daphnia* on planktonic microbial food webs – A review. Mar Microb Food Webs 8:295–324
Jürgens K, Arndt H, Rothhaupt KO (1994) Zooplankton-mediated changes of bacterial community structure. Microb Ecol 27:27–42
Jürgens K, Arndt H, Zimmermann H (1997) Impact of metazoan and protozoan grazers on bacterial biomass distribution in microcosm experiments. Aquat Microb Ecol 12:131–138
Jürgens K, De Mott WR (1995) Behavioral flexibility in prey selection by bacterivorous nanoflagellates. Limnol Oceanogr 40:1503–1507
Jürgens K, Güde H (1994) The potential importance of grazing-resistant bacteria in planktonic systems. Mar Ecol Prog Ser 112:169–188
Jürgens K, Matz C (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Antonie van Leeuwenhoek 81:413–434
Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl Environ Microbiol 65:1241–1250
Jürgens K, Sala MM (2000) Predation-mediated shifts in size distribution of microbial biomass and activity during detritus decomposition. Oikos 91:29–40
Jürgens K, Stolpe G (1995) Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. Freshwater Biol 33:27–38
Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66:2365–2371
Kadouri D, O'Toole GA (2005) Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 71:4044–4051
Kelley JI, Turng BF, Williams HN, Baer ML (1997) Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic bdellovibrios. Appl Environ Microbiol 63:84–90
Kerfoot WC, Sih A (1987) Predation. Direct and indirect impacts on aquatic communities. University Press of New England, Hanover, New Hampshire
Koval SF, Bayer ME (1997) Bacterial capsules: No barrier against *Bdellovibrio*. Microbiology-Uk 143:749–753
Koval SF, Hynes SH (1991) The effect of paracrystalline protein surface layers on predation by *Bdellovibrio bacterivorus*. J Bacteriol 173:2244–2249
Lambert C, Smith MC, Sockett RE (2003) A novel assay to monitor predator–prey interactions for *Bdellovibrio bacteriovorus* 109 J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol 5:127–132
Landry MR (1994) Methods and controls for measuring the grazing impact of planktonic protists. Mar Microb Food Webs 8:37–57
Langenheder S, Jürgens K (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr 46:121–134
Lenski R (1988) Dynamics of interactions between bacteria and virulent bacteriophage. Adv Microb Ecol 10:1–44
Lenski RE, Levin BR (1985) Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. Am Nat 125:585–602
López-García P, Rodriguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603–607

Lu J, Chen F, Hodson RE (2001) Distribution, isolation, host specificity, and diversity of cyanophages infecting marine *Synechococcus* spp. in river estuaries. Appl Environ Microbiol 67:3285–3290

Margulis L (1996) Archaeal-eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. Proc Natl Acad Sci USA 93:1071–1076

Markelova NY, Colwell RR (1999) Two-component bacterial predator–prey system immobilized on the surface of a transparent plastic substrate is a promising model for investigation of the role of bacterial predators in ecosystems. Microbiol 68:328–331

Markelova NY, Kerzhentsev AS (1998) Isolation of a new strain of the genus *Bdellovibrio* from plant rhizosphere and its lytic spectrum. Microbiol 67:696–699

Martin MO (2002) Predatory prokaryotes: An emerging research opportunity. J Mol Microbiol Biotechnol 4:467–477

Massana R et al. (2004) Phylogenetic and ecological analysis of novel marine stramenopiles. Appl Environ Microbiol 70:3528–3534

Massana R, Guillou L, Díez B, Pedrós-Alió C (2002) Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl Environ Microbiol 68:4554–4558

Massana R, Jürgens K (2003) Composition and population dynamics of planktonic bacteria and bacterivorous flagellates in seawater chemostat cultures. Aquat Microb Ecol 32:11–22

Matz C, Bergfeld T, Rice SA, Kjelleberg S (2004a) Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. Environ Microbiol 6:218–226

Matz C, Boenigk J, Arndt H, Jürgens K (2002a) Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp. Aquat Microb Ecol 27:137–148

Matz C et al. (2004b) Impact of violacein-producing bacteria on survival and feeding of bacterivorous nanoflagellates. Appl Environ Microbiol 70:1593—1599

Matz C, Deines P, Jürgens K (2002b) Phenotypic variation in *Pseudomonas* sp. CM10 determines microcolony formation and survival under protozoan grazing. FEMS Microbiol Ecol 39:57–65

Matz C, Jürgens K (2001) Effects of hydrophobic and electrostatic cell surface properties of bacteria on feeding rates of heterotrophic nanoflagellates. Appl Environ Microbiol 67:814–820

Matz C, Jürgens K (2003) Interaction of nutrient limitation and protozoan grazing determines the phenotypic structure of a bacterial community. Microb Ecol 45:384–398

Matz C, Jürgens K (2005) High motility reduces grazing mortality of planktonic bacteria. Appl Environ Microbiol 71:921–929

Matz C, Kjelleberg S (2005) Off the hook – how bacteria survive protozoan grazing. Trends Microbiol 13:302–307

Middelboe M et al. (2001) Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. Microb Ecol 42:395–406

Monger BC, Landry MR, Brown SL (1999) Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnol Oceanogr 44:1917–1927

Moon-van der Staay SY, De Wachter R, Vaulot D (2001) Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature 409:607–610

Morris RM et al. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420:806–810

Nunez ME, Martin MO, Chan PH, Spain EM (2005) Predation, death, and survival in a biofilm: *Bdellovibrio* investigated by atomic force microscopy. Colloids Surf B-Biointerfaces 42:263–271

Ooms-Wilms AL, Postema G, Gulati RD (1995) Evaluation of bacterivory of Rotifera based on measurements of in situ ingestion of fluorescent particles, including some comparisons with Cladocera. J Plankton Res 17:1057–1077

Pace ML (1988) Bacterial mortality and the fate of bacterial production. Hydrobiologia 159:41–49

Patterson D (1994) Protozoa: evolution and systematics. In: Hausmann K, Hülsmann N (eds) Progress in protozoology. Gustav Fischer, Stuttgart

Pedrós-Alió C, Calderón-Paz JI, Gasol JM (2000) Comparative analysis shows that bacterivory, not viral lysis, controls the abundance of heterotrophic prokaryotic plankton. FEMS Microbiol Ecol 32:157–165

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. Nature Rev Microbiol 3:537–546

Pernthaler J, Amann R (2005) Fate of heterotrophic microbes in pelagic habitats: focus on populations. Microbiol Mol Biol Rev 69:440–461

Pernthaler J, Posch T, Šimek K, Vrba J, Amann R, Psenner R (1997) Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. Appl Environ Microbiol 63:596–601

Pernthaler J et al. (2001) Predator-specific enrichment of Actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. Appl Environ Microbiol 67:2145–2155

Pernthaler J, Sattler B, Šimek K, Schwarzenbacher A, Psenner R (1996) Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community. Aquat Microb Ecol 10:255–263

Pernthaler J, Zöllner E, Warnecke F, Jürgens K (2004) Bloom of filamentous bacteria in a mesotrophic lake: Identity and potential controlling mechanism. Appl Environ Microbiol 70:6272–6281

Pineiro SA, Sahaniuk GE, Romberg E, Williams HN (2004) Predation pattern and phylogenetic analysis of Bdellovibrionaceae from the Great Salt Lake, Utah. Curr Microbiol 48:113–117

Plante CJ (2000) Role of bacterial exopolymeric capsules in protection from deposit-feeder digestion. Aquat Microb Ecol 21:211–219

Posch T et al. (1999) Predator-induced changes of bacterial size, structure and productivity studied on an experimental microbial community. Aquat Microb Ecol 18:235–246

Rappé M, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394

Rice TD, Williams HN, Turng BF (1998) Susceptibility of bacteria in estuarine environments to autochthonous bdellovibrios. Microb Ecol 35:256–264

Riisgard H (1988) Efficiency of particle retention and filtration rate in six species of Northeast American bivalves. Mar Ecol Prog Ser:217–223

Riisgard H, Larsen P (2001a) Comparative ecophysiology of active zoobenthic filter-feeding, essence of current knowledge. J Sea Res 44:169–193

Riisgard H, Larsen P (2001b) Minireview: Ciliary filter feeding and bio-fluid mechanics – present understanding and unsolved problems. Limnol Oceanogr 46:882–891

Roszak DB, Colwell RR (1987) Survival strategies of bacteria in the natural environment. Microbiol Rev 51:365–379

Sanders RW, Caron DA, Berninger UG (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters – An inter-ecosystem comparison. Mar Ecol Prog Ser 86:1–14

Schauer M, Hahn MW (2005) Diversity and phylogenetic affiliations of morphologically conspicuous large filamentous bacteria occurring in the pelagic zones of a broad spectrum of freshwater habitats. Appl Environ Microbiol 71:1931–1940

Schmaljohann R, Pollingher U, Berman T (1987) Natural populations of bacteria in Lake Kinneret: Observations with scanning electron and epifluorescence microscopy. Microb Ecol 13:1–12

Schoeffield AJ, Williams HN, Turng BF, Falkler WA (1996) A comparison of the survival of intraperiplasmic and attack phase bdellovibrios with reduced oxygen. Microb Ecol 32:35–46

Sekar R, Pernthaler A, Pernthaler J, Warnecke F, Posch T, Amann R (2003) An improved protocol for quantification of freshwater Actinobacteria by fluorescence in situ hybridization. Appl Environ Microbiol 69:2928–2935

Shemesh Y, Jurkevitch E (2004) Plastic phenotypic resistance to predation by Bdellovibrio and like organisms in bacterial prey. Environ Microbiol 6:12–18

Sherr BF, Sherr EB, Pedros-Alio C (1989) Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine water. Mar Ecol Prog Ser 54:209–219

Sherr EB, Sherr BF (1987) High rates of consumption of bacteria by pelagic ciliates. Nature 325:710–711

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek 81:293–308

Silverman H, Nichols SJ, Cherry JS, Achberger E, Lynn JW, Dietz TH (1997) Clearance of laboratory-cultured bacteria by fresh-water bivalves: differences between lentic and lotic unionids. Can J Zool 75:1857–1866

Šimek K, Jürgens K, Nedoma J, Comerma M, Armengol J (2000) Ecological role and bacterial grazing of *Halteria* spp.: small freshwater oligotrichs as dominant pelagic ciliate bacterivores. Aquat Microb Ecol 22:43–56

Šimek K, Kojecka P, Nedoma J, Hartman P, Vrba J, Dolan JR (1999) Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. Limnol Oceanogr 44:1634–1644

Šimek K, Nedoma J, Pernthaler J, Posch T, Dolan JR (2002) Altering the balance between bacterial production and protistan bacterivory triggers shifts in freshwater bacterial community composition. Antonie van Leeuwenhoek 81:453–463

Šimek K et al. (1997) Morphological and compositional shifts in an experimental bacterial community influenced by protists with contrasting feeding modes. Appl Environ Microbiol 63:587–595

Sleigh MA, Zubkov MV (1998) Methods of estimating bacterivory by protozoa. Eur J Protistol 34:273–280

Sommaruga R, Psenner R (1995) Permanent presence of grazing-resistant bacteria in a hypertrophic Lake. Appl Environ Microbiol 61:3457–3459

Sommer U, Stibor H (2002) Copepoda-Cladocera-Tunicata: the role of three major mesozooplankton groups in pelagic food webs. Ecol Res 17:161–174

Stanley S (1973) An ecological theory for the sudden origin of multicellular life in the late Precambrian. Proc Nat Acad Sci 70:1486–1489

Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie Van Leeuwenhoek 29:217

Strayer DL, Caraco NF, Cole JJ, Findlay S, Pace ML (1999) Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. BioScience 49:19–27

Suttle CA, Chan AM (1993) Marine cyanophages infecting oceanic and coastal strains of *Synechococcus* – abundance, morphology, cross-infectivity and growth characteristics. Mar Ecol Prog Ser 92:99–109

Sutton DC, Besant PJ (1994) Ecology and characteristics of Bdellovibrios from three tropical marine habitats. Mar Biol 119:313–320

Thingstad T (2000a) Control of bacterial growth in idealized food webs. In: Kirchman D (ed) Microbial ecology of the oceans. Wiley-Liss, New York

Thingstad TF (2000b) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45:1320–1328

Thingstad TF, Heldal M, Bratbak G, Dundas I (1993) Are viruses important partners in pelagic food webs? Trends Ecol Evol 8:209–212

Tollrian R, Harvell C (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey

Vaqué D, Gasol JM, Marrase C (1994) Grazing rates on bacteria: The significance of methodology and ecological factors. Mar Ecol Prog Ser 109:263–274

Varon M (1979) Selection of predation-resistant bacteria in continuous culture. Nature 277:386–388

Varon M, Shilo M (1978) Ecology of aquatic bdellovibrios. In: Droop M, Jannash H (eds) Advances in microbial ecology. Academic, London, pp 1–48

Verity PG (1988) Chemosensory behavior in marine planktonic ciliates. Bull Mar Sci 43:772–782

Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl Environ Microbiol 59:3393–3399

Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28:127–181

Weinbauer MG, Höfle MG (1998) Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Appl Environ Microbiol 64:431–438

Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? Environ Microbiol 6:1–11

Weitere M, Bergfeld T, Rice SA, Matz C, Kjelleberg S (2005) Grazing resistance of *Pseudomonas aeruginosa* biofilms depends on type of protective mechanism, developmental stage and protozoan feeding mode. Environ Microbiol 7:1593–1601

Weitz JS, Hartman H, Levin SA (2005) Coevolutionary arms races between bacteria and bacteriophage. Proc Natl Acad Sci USA 102:9535–9540

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. Proc Natl Acad Sci USA 95:6578–6583

Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, Schütt C (1998) Bacteriophage diversity in the North Sea. Appl Environ Microbiol 64:4128–4133

Williams H (1987) The recovery of high numbers of bdellovibrios from the surface water microlayer. Can J Microbiol 33:572–575

Williams H (1988) A study of the distribution of bdellovibrios in estuarine sediment over an annual cycle. Microb Ecol 15:9–20

Williams H, Falkler W, Shay D (1982) Seasonal distribution of bdellovibrios at the mouth of the Patuxent river in the Chesapeake bay. Can J Microbiol 28:111–116

Williams HN, Kelley JI, Baer ML, Turng BF (1995) The association of bdellovibrios with surfaces in the aquatic environment. Can J Microbiol 41:1142–1147

Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64:69–114

Yair S, Yaacov D, Susan K, Jurkevitch E (2003) Small eats big: ecology and diversity of Bdellovibrio and like organisms, and their dynamics in predator–prey interactions. Agronomie 23:433–439

Zöllner E, Santer B, Boersma M, Hoppe HG, Jürgens K (2003) Cascading predation effects of *Daphnia* and copepods on microbial food web components. Freshwater Biol 48:2174–2193

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_054/Published online: 27 October 2006

Mathematical Modelling of Predatory Prokaryotes

Michael H. F. Wilkinson

Institute for Mathematics and Computing Science, University of Groningen, PO Box 800, 9700 AV Groningen, The Netherlands
m.h.f.wilkinson@rug.nl

Abstract Predator–prey models have a long history in mathematical modelling of ecosystem dynamics and evolution. In this chapter an introduction to the methodology of mathematical modelling is given, with emphasis on microbial predator–prey systems, followed by a description of variants of the basic two-species system. Then the two-species system is extended to incorporate effects such as predator satiation and prey escape strategies, after which multi-species effects, including alternative prey, protector species and decoy effects, are discussed. Simulations are used to discuss the effect of several model parameters.

1 Introduction

Mathematical models of predator–prey systems are amongst the oldest in biology (Lotka 1925; Volterra 1926). Though usually referred to as predator–prey systems, host–parasite and plant–herbivore systems are in many ways fundamentally the same: one species grows at the expense of another (e.g. Bulmer 1994; DeAngelis 1992). The mathematical treatment is therefore often similar, so predator–prey models are really a cornerstone of ecological modelling. Many, and certainly all the earliest, predator–prey models were concerned with macroscopic organisms. Though it has been shown that microbial ecosystems require a slightly different approach in some aspects, many of the same effects apply to all scales (e.g. Jost et al. 1973; Marchand and Gabignon 1981; Kooi and Kooijman 1994a).

This chapter has three main objectives: (1) to give a review of modelling predator–prey systems in general, (2) to review such work that has focussed on *Bdellovibrio bacteriovorus* and related species, and (3) to discuss a number of hypotheses proposed in other predator–prey systems in the context of predatory prokaryotes.

The chapter is organized as follows. First, modelling methodology is discussed, dealing with some basic concepts from dynamical systems theory. After this, various predator–prey models will be discussed, starting with the classical Lotka–Volterra model (Lotka 1925; Volterra 1926), followed by the introduction of variants to account for predator satiation and limitations on prey growth. Different models specific to the predatory prokaryotes are dealt with after this, including a comparison to bacteriophage models. Various strategies exist for bacterial predators, and the differences in modelling these mathematically are also dealt with. Furthermore, some models for evasion strategies for the prey are discussed.

In most cases I will assume the ecosystem is perfectly mixed (such as in chemostats), which means the spatial dimensions can be ignored. Certain ecosystems are not modelled well using this assumption, so techniques for dealing with spatial distributions and transport processes are dealt with briefly in Sect. 2.2.

Of course, no real ecosystem consists of just one predator and one prey. Therefore, multiple-species effects are dealt with after that. The most important of these are the alternative prey (Mallory et al. 1983), protector species (Pius and Leberg 1998) and decoy effects (Christensen et al. 1976; Wilkinson 2001). Though some of these have been suggested or even observed in a microbial setting, the protector species effect has not. A model for this effect in the context of predatory prokaryotes is presented here. The chapter ends with a discussion of the state of the art in modelling predatory prokaryotes and future directions for research.

2
Methodologies in Ecological Modelling

Mathematical modelling of ecosystems has two major aims, which are closely related: (1) understanding the dynamics of the system, given the behaviour of the organisms within the system, and (2) understanding the evolutionary processes by which different behaviours occur. Two approaches to modelling have been used traditionally: (1) dynamical systems, in particular through the use of differential equations, and (2) game theory, which focuses on best choices of behaviour given some model for the "payoff" of each possible strategy. These two approaches are no longer considered to be completely separate: replicator equations yield game-theory-based dynamical systems (e.g. Hofbauer and Sigmund 1998). Other taxonomies of modelling approaches split models into *tactical* and *strategic* models (Levins 1968). The former aim at accurate predictions for a specific system, but low general applicability, whereas the latter aim at wide applicability, but without accurate predictive capability. Strategic models are mainly interested in what kind of dynamics may occur in a given class of systems. We may also distinguish between individual-based modelling, in which the population is represented as a system of N interacting individuals, versus population-density-based modelling, in which the system is represented by M densities, each representing a particular species (generally $M \ll N$). It is assumed that each of the densities is a continuous variable, which is plausible if the populations are large enough. Density-based models are far easier to treat analytically, whereas individual-based models can handle inter-individual difference within a population more easily, potentially showing a richer diversity in behaviour. This is why individual-based modelling has become popular only after the availability of (lots of) cheap computing power. Fortunately, the population numbers in microbial predator–prey systems easily run into billions of individuals, so modelling on a population density basis is feasible, which is why the main focus is on this type of modelling.

Many textbooks on theoretical (evolutionary) ecology exist (e.g. Bulmer 1994; DeAngelis 1992; Hofbauer and Sigmund 1998; McGlade 1999), each of which provides a solid background in the topic of predator–prey modelling. A specific textbook on mathematical modelling in microbial ecology is by Koch et al. (1998).

2.1
Dynamical Systems

The cornerstone of population modelling is through dynamical systems. A dynamical system is represented mathematically by its state variables. In typical predator–prey systems, the two most obvious state variables are predator density and prey density. In general we will have an N-dimensional

state vector $\boldsymbol{x} = (x_1, x_2, ..., x_N)^{\mathrm{T}}$, in which each x_i represents, for example, a species or resource density. Apart from the state vector, a dynamical system is defined by its differential equation, which has the general form

$$\frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}t} = \boldsymbol{f}(\boldsymbol{x}, t)\,, \tag{1}$$

in which $\boldsymbol{x}$ is some (vector-valued) function, which takes the state vector $\boldsymbol{x}$ and time t as its inputs, and returns the rate of change of the state vector. In other words, the rate of change in time of each of the state variables is determined by the current state of the system, and the time. The latter may be used to introduce circadian or seasonal effects into biological systems, or any other time-dependent external influence. In many cases, and indeed most of the cases reviewed in the rest of the chapter, we are interested in *time-independent* ordinary differential equations (ODEs) which ignore the influence of time, i.e.

$$\frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}t} = \boldsymbol{f}(\boldsymbol{x})\,. \tag{2}$$

In this case, we can study the equilibrium condition

$$\frac{\mathrm{d}\vec{x}}{\mathrm{d}t} = \boldsymbol{f}(\boldsymbol{x}) = 0\,,$$

or

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = f_i(\boldsymbol{x}) = 0\,, \quad \text{for all} \quad i \in \{1, 2, ..., N\}\,, \tag{3}$$

in which f_i denotes the ith element of $\boldsymbol{f}$. Each of these N equations yields a *zero-isocline* for state variable x_i, which is a manifold in N-dimensional space along which the rate of change of x_i is precisely zero. In 2-D these zero-isoclines are just curves, in 3-D they are curved surfaces. The equilibrium points are found where all N isoclines intersect. An example is shown in Fig. 1c,d, in which the zero-isoclines for prey and predator are drawn for a predator–prey system. Thus, solving for equilibrium requires solving N (non-linear) equations. Given the non-linear nature of the equations, there may be any number of equilibria. Each of these equilibria can be (locally) stable, neutrally stable, or unstable. If the system is in a stable equilibrium it will return to the same state after any small disturbance. Neutral stability means that the system will not return to equilibrium, but neither will it move further away if disturbed. Instability means that a small disturbance will cause the system to move ever further from the equilibrium. In the case of time-independent ODEs, we can perform *local stability analysis* of the result very easily (e.g. DeAngelis 1992).

Rather than simply focussing on equilibria, we often want to use ODEs to determine the evolution of the state of the system in time. In the simplest cases, an exact, analytical solution can be computed, but often we have to

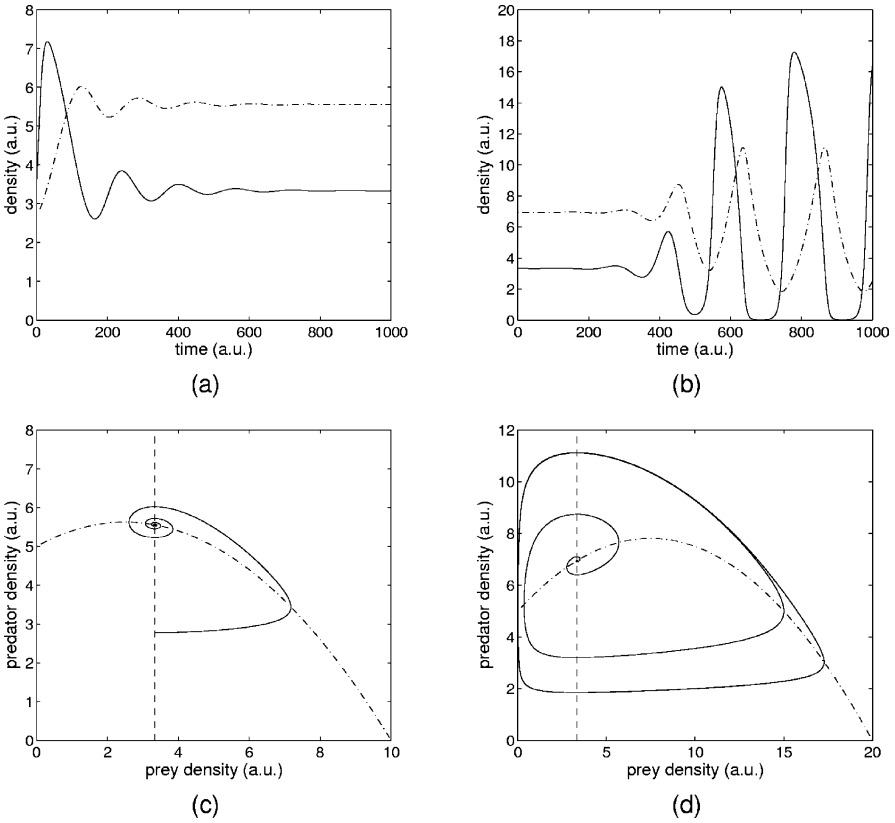


Fig. 1 Predator–prey dynamics using logistic growth for prey and Holling type II for the predator. **a** Predator (*dash-dot line*) and prey (*solid line*) densities vs. time, for carrying capacity $K = 10$; **b** same as **a** but with $K = 20$; **c** and **d** predator vs. prey density (*solid line*) for the same settings as **a** and **b**, respectively. The predator (*dashed line*) and prey (*dash-dot line*) zero-isoclines are shown as well. In the case of $K = 10$ the system stabilizes, even when released far from equilibrium, whereas for $K = 20$ the system spirals away from equilibrium for even the smallest perturbation. a.u.: arbitrary units

resort to numerical treatment. The most common type of problem concerning ODEs is the so-called initial value problem. In this case the state of the system is known at some time t_0, and we wish to compute the state of the system at a series of points in time t_1, t_2,...,t_m. This can be done using one of many ODE solvers, the best-known of which are probably the Euler method and the Runge–Kutta method (Press et al. 1986; Van Loan 1997). Various scientific packages such as MATLAB (The Mathworks, Inc.) contain a variety of methods to solve ODEs, both analytically and numerically (Palm 2005; Van Loan 1997).

A variant of ODEs are *delay differential equations* (DDEs), in which the rate of change does not only depend on the current state of the system $\boldsymbol{x}(t)$,

but also on the state at various points in the past $\boldsymbol{x}(t-\tau_1), \boldsymbol{x}(t-\tau_2), \ldots, \boldsymbol{x}(t-\tau_K)$. Their general form is

$$\frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}t} = \boldsymbol{f}\left(\boldsymbol{x}(t), \boldsymbol{x}(t-\tau_1), \boldsymbol{x}(t-\tau_2), \ldots, \boldsymbol{x}(t-\tau_K)\right) , \tag{4}$$

and the equilibrium condition becomes

$$f_i\left(\boldsymbol{x}(t), \boldsymbol{x}(t), \ldots, \boldsymbol{x}(t)\right) = 0 , \quad \text{for all} \quad i \in \{1, 2, \ldots, N\} , \tag{5}$$

because $\boldsymbol{x}(t) = \boldsymbol{x}(t-\tau_j)$ for all j at equilibrium. Though finding equilibria is often straightforward, and similar to the ODE case, stability analysis and numerical treatment are in general more difficult in the case of DDEs. However, packages such as MATLAB also support DDE solvers. DDEs have been used to model bacterium–phage systems (Campbell 1961; Levin et al. 1977; Bohannan and Lenski 1997) and *B. bacteriovorus–Escherichia coli* systems (Marchand and Gabignon 1981; Dulos and Marchand 1984; see also Sect. 3.3).

2.2 Spatial Models

In the population-density models presented previously the spatial extent of the ecosystem was ignored. This may be done for two reasons. First, the analysis of the system becomes much easier. Second, spatial extent is irrelevant if the ecosystem is well mixed, as in a chemostat (excluding surfaces supporting biofilm growth). Including spatial extent can have a profound effect on the dynamical behaviour of an ecosystem. For example, the chaotic oscillations predicted by a non-spatial model for a gypsy moth population were changed into regular wave trains by diffusion in a spatial model of the same population (Wilder et al. 1995). Generally, when introducing a spatial dimension to the system we must change the ODEs to partial differential equations (PDEs), due to the transport processes. The simplest and most commonly used transport process is diffusion, which for any substance X is modelled using the following PDE:

$$\frac{\partial X}{\partial t} = d\nabla^2 X = d\left(\frac{\partial^2 X}{\partial x^2} + \frac{\partial^2 X}{\partial y^2} + \frac{\partial^2 X}{\partial z^2}\right) ,$$

in which ∇^2 is the Laplacian (a second-order spatial derivative) and d is a diffusion constant. If only diffusion is used, the system becomes a reaction–diffusion system, in which the growth model determines the type of reaction. PDEs are generally more difficult to handle, both analytically and numerically. To allow computer simulation, the spatial extent is discretized in some way, after which the PDE can be turned into a (complex) ODE. An introduction to solving PDEs numerically is found in the book by Press et al. (1986).

Spatial extent can be added in various stages, each adding complexity to the model. In microbial ecology, much work is done in chemostats (Monod

1950; Levin et al. 1977; Chao et al. 1977; Gerritse et al. 1992; Kooi and Kooijmans 1994a,b), eliminating the need for spatial extent in the mathematical model. A slightly more complex approach is to use N cascaded chemostats, the effluent of number i being the inflowing material for $i + 1$, thus effectively discretizing the spatial extent into N compartments (Itoh and Freter 1989; Gibson and Wang 1994; Alander et al. 1999; Forde et al. 2004). This can readily be modelled using mN coupled ODEs, with m the number of coupled ODEs needed to model a single chemostat. For the intestinal microbial ecosystem, one spatial (axial) dimension can be added in models of plug-flow reactors (Ballyk and Smith 1999; Ballyk et al. 2001; Jones and Smith 2000), in which PDEs are used to model transport, and ODEs growth and wall attachment. A 2-D approach (one axial, one radial dimension) has also been used for the same ecosystem, in the MIMICS cellular automaton (Kamerman and Wilkinson 2002; Wilkinson 2002).

Full-blown PDE-based analysis in microbial ecosystems is essential in, e.g., microbial mats (de Wit et al. 1995) or sediments (Jahnke et al. 1982), which may show a distinct layered structure. They have also been used to explain the diversity of bacteriocins in microbial populations (Frank 1994). Frank's analysis is a two-stage approach: first several coupled ODEs are used to analyse the dynamics of a system consisting of a bacteriocin-producing species and a susceptible species in a chemostat-like environment. This system is bistable: either the susceptible species survives, or the producer survives, but coexistence is impossible. He then extends the model to include a spatial dimension in which several nutrient-rich patches separated by low-nutrient regions exist. In this system coexistence of susceptible and producer species is possible. A slightly different lattice-based spatial model used by Isawa et al. (1998) yields a similar result. Other spatial models include those of bacterial chemotaxis, reviewed by Ford and Cummings (1998), and pattern formation in growing colonies (Ben-Jacob et al. 1995; Tyson et al. 1999; Kawasaki et al. 1997) and various biofilm models (Dockery and Klapper 2001; Hermanowicz 1998).

As a final note it should be said that other forms of structure within a population, such as size structure, age structure, or resource–reserve structure, can equally be modelled through PDEs, as in the dynamic energy budget model of Kooijmans (1993) which has been applied to microbial predator–prey systems (Kooi and Kooijmans 1994a,b).

3
Two-Species Systems

In the following discussion X will denote the number, biomass or density of the prey species, and Y will denote the number, biomass or density of the predator species. The classical model of predator–prey systems is the Lotka–

Volterra system, which is set of ODEs of the form

$$\frac{\mathrm{d}X}{\mathrm{d}t} = F(X) - G(X,Y) \tag{6a}$$

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \eta G(X,Y) - H(Y)\,, \tag{6b}$$

in which F is a function denoting the growth of prey, G is a function denoting the reduction of prey due to predation by Y, η is a yield factor coupling prey losses to predator gains, and H is a function determining the predator starvation rate in the absence of prey. The latter term is often called the maintenance energy term (Nisbet et al. 1983). The very simplest form the Lotka–Volterra system can take is

$$\frac{\mathrm{d}X}{\mathrm{d}t} = fX - gXY \tag{7a}$$

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \eta gXY - hY\,, \tag{7b}$$

with f, g and h constants. The interpretation of these equations is the following. Prey has a constant relative growth rate, and therefore grows exponentially in the absence of predators. Conversely, predators starve at a constant relative rate, leading to exponential decay of predator numbers in the absence of prey. The predation rate is modelled as proportional to the number of predator–prey encounters, and is thus proportional to the product of predator and prey numbers. Setting the right-hand sides of Eqs. 7a,b to zero yields a non-trivial equilibrium point of $X = h/(\eta g)$ and $Y = f/g$. This equilibrium is neutrally stable: any deviation from this point does not result in the system returning to the equilibrium, but in predator–prey oscillations of an amplitude depending on the initial deviation from equilibrium.

Despite the simplicity of the model, it already explains the existence of oscillations in the populations of predators and prey. Having said that, Eqs. 7a,b suffer from many shortcomings. The most glaring is the fact that the prey species will grow to infinity if predators are absent. This can be corrected by using logistic growth to model the prey, i.e.

$$F(X) = rX\left(1 - \frac{X}{K}\right)\,, \tag{8}$$

in which r is the maximum relative growth rate and K the carrying capacity of the ecosystem. The equilibrium position for X remains the same, but for Y we have

$$Y = \frac{r}{g}\left(1 - \frac{X}{K}\right)\,, \tag{9}$$

which is now a function of X. The equilibrium point is now found by intersecting the two zero-isoclines, in this case inserting the equilibrium

position of X in Eq. 9. This means that at equilibrium, we have $Y = r(1 - h/(\eta gK))/g$. In this case the equilibrium is stable: any deviation from equilibrium results in damped oscillations, and the system slowly returns to equilibrium.

Curiously, the above improvement does not explain the persistent predator–prey oscillations observed in nature. This is due to the other main shortcoming of Eqs. 7a,b, which is that the relative growth rate of the predators will go to infinity as the number of prey increases. In reality, predator growth rate is limited by various other factors, the most obvious of which are the maximal fecundity of the predator and the "handling time", which is the time needed to process the prey, during which the predator generally cannot attack another prey item. Improvements to Eqs. 7a,b are given in the following subsections, focussing on microbial predator–prey systems. Note that although the Lotka–Volterra equation was intended to model predator–prey systems, it has also been used to model mutualistic interactions between species (Neuhauser and Fargione 2004).

3.1 Improvements to the Predator Model

The improvements to the predator model focus on G, rather than on H, which is usually modelled as a constant starvation rate. As in the case of simple exponential (or Malthusian) growth for the prey, some saturation of the predator growth rate, and therefore of predation, is required. The Holling type II (Holling 1959) model is given by

$$G(X,Y) = \frac{gXY}{k_1 + X}\,, \tag{10}$$

in which k_1 is a saturation constant. It is based on the notion that any predator will spend some time processing the prey after having encountered it. The Holling type II model is essentially the same as the Monod model for bacterial growth (Monod 1950). If prey densities are high, the predator grows at a maximum relative growth rate g, whereas at low prey densities G approximates the Lotka–Volterra model, asymptotically approaching gXY/k as X approaches zero.

The Holling type II model shows several changes in the dynamical behaviour of the predator–prey system as compared to the Lotka–Volterra system with logistic growth of the prey. In this case the zero-isocline for the prey is a parabola, and depending on where the zero-isocline for the predator intersects it, the result may be locally stable, neutrally stable, or unstable (e.g. DeAngelis 1992). Thus, this system can explain many of the features in real predator–prey systems. This is shown in a hypothetical predator–prey system in Fig. 1. In this system, prey dynamics are modelled by logistic growth with $r = 0.2$ and a variable value of K. The Holling type II model

is used for the predator–prey interaction, with $g = 0.2$ and $k_1 = 5$; predator parameters are $\eta = 0.25$, and starvation rate $h = 0.02$. Figure 1a,c plots the predator–prey dynamics for $K = 10$. In this case the system is stable, because the (linear) predator isocline intersects the prey isocline after the maximum of the parabola, as shown in Fig. 1c. Even if the system is released from a point quite far from equilibrium, the system converges to the point of intersection of the isoclines. In Fig. 1b and d where $K = 20$, we see strong oscillations. In this case, the intersection of the isoclines lies before the maximum in the prey isocline, and even if the system is released very close to equilibrium the system veers away from it, finally approaching a stable limit cycle.

Numerous variants have been proposed (for a discussion see, e.g., DeAngelis 1992, pp 81–87). The Holling type III (Holling 1959) model is given by

$$G(X,Y) = \frac{gX^2Y}{k_1 + X^2}\,. \tag{11}$$

In this case, the saturation behaviour is as in Eq. 10, but the behaviour at low prey densities becomes quadratic, rather than linear, in the number of prey: as X approaches zero, G approaches gX^2Y/k. This models the difficulty predators may have in finding prey at lower densities, or the fact that any remaining prey may be harder to detect. Jost et al. (1973) proposed a variant of this

$$G(X,Y) = \frac{gX^2Y}{\left(k_1 + X\right)\left(k_2 + X\right)}\,, \tag{12}$$

with k_1 and k_2 saturation constants, which has similar behaviour. The Holling type III form appears to model vertebrate predators better than insects (DeAngelis 1992, pp 81–87) or microbes (Canale 1969; Kooi and Kooijmans 1994a), which are often modelled by the Holling type II model. The model of Jost et al. (1973) was also proposed in the context of a microbial ecosystem.

Another effect that may occur is that of interference (Arditi et al. 2004; Beddington 1975; DeAngelis et al. 1975; Hassel and Varley 1969), i.e. at high predator densities the efficiency of the predator declines, not because of plummeting prey numbers, but through predator–predator interactions. In all the above models G is a linear function of Y. This means that the *relative* predation rate is independent of Y. To include interference we should introduce a non-linear term to replace the linear one. Based on observations, Hassel and Varley (1969) propose the following modification of the standard Lotka–Volterra form

$$G(X,Y) = gXY^{1-m}\,, \tag{13}$$

which becomes

$$G(X,Y) = \frac{gXY^{1-m}}{k_1 + Y^{-m}X}, \tag{14}$$

in the Holling type II case (Arditi and Akçakaya 1990). Strictly speaking we should replace Y^{-m} by $(Y/Y_0)^{-m}$, with Y_0 the predator density corresponding to a single predator in the entire ecosystem (Arditi et al. 2004). If m is zero, we have no interference, whereas if m is negative we have co-operation. The interference parameter m can readily be determined empirically. Both equations are essentially empirical, so interpretation of the meaning of m is difficult. Beddington (1975) uses a behavioural argument to introduce a different form of interference

$$G(X,Y) = \frac{gXY}{k_1 + X + k_2(Y - Y_0)}, \tag{15}$$

by arguing that predators will lose some time in predator–predator encounters. In essence, this is a form of competitive inhibition, with k_1 the saturation constant as before, and k_2 the inhibition constant. DeAngelis et al. (1975) derive a very similar equation, in which Y_0 is omitted. In practice there is no difference between the two. It is often assumed that both forms of interference tend to damp out oscillations and increase the stability of the ecosystem, but recent analysis by Arditi et al. (2004) shows that this may not be the case for high interference levels if exponential growth of the prey is assumed (it remains stable if logistic growth is used). This effect can be seen in Fig. 2.

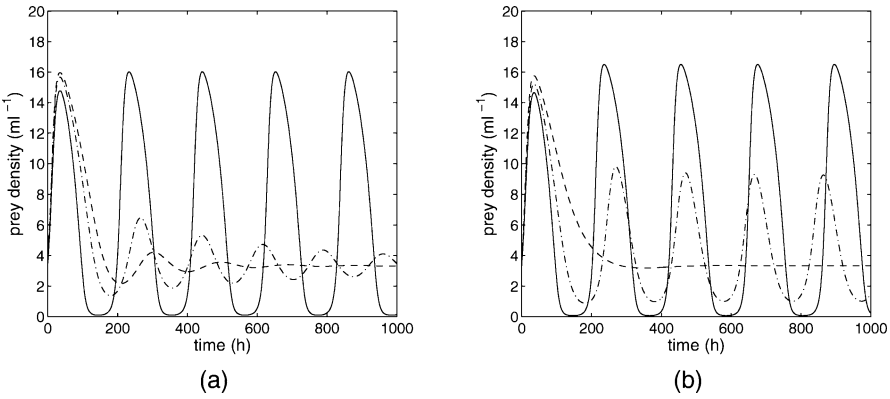


Fig. 2 Stabilizing effect on prey oscillations in the same system as in Fig. 1b but with mutual interference among predators: **a** according to model of Hassel and Varley (1969) with $m = 0.01$ (*solid line*), $m = 0.04$ (*dash-dot line*) and $m = 0.05$ (*dashed line*); **b** according to model of Beddington (1975) and DeAngelis et al. (1975) with $k_2 = 1$ (*solid line*), $k_2 = 5$ (*dash dot line*), and $k_2 = 10$ (*dashed line*). Whatever the model, the system is stabilized by mutual interference

Here the unstable system of Fig. 1b,d is used to show the stabilizing effect. In Fig. 2a the Hassel and Varley model is shown (only prey oscillations) for $m = 0.01$, 0.04 and 0.05. In Fig. 2b the model of Beddington (1975) and DeAngelis et al. (1975) is used for $k_2 = 1$, 5 and 10. In either case increasing the interference parameter increases stability.

3.2
Improvements to the Prey Model in the Microbial Case

As mentioned before, logistic growth is often used to model the prey dynamics. Though generally thought to be suitable for macroscopic prey, for microbes the Monod model is more suitable (Monod 1950; Koch 1998). In the following we assume the predator–prey system is contained within a chemostat with dilution rate constant D. Assuming that X_0 denotes the limiting substrate concentration, X_1 the prey concentration and S the concentration of substrate in the inflowing fluid, the set of differential equations becomes

$$\frac{\mathrm{d}X_0}{\mathrm{d}t} = D(S - X_0) - V_1 \frac{X_0}{K_1 + X_0} X_1 \tag{16a}$$

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \mu_1 \frac{X_0}{K_1 + X_0} X_1 - DX_1 - G(X_1, Y) \tag{16b}$$

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \eta G(X_1, Y) - H(Y) - DY\,, \tag{16c}$$

with μ_1 the maximum specific relative growth rate, K_1 the saturation constant and V_1 the maximum specific uptake rate of X_0 by X_1. In the absence of predators, growth of the prey must precisely balance the dilution term DX_1, and the equilibrium concentrations of X_0 and X_1 become

$$X_0 = \frac{DK_1}{\mu_1 - D} \tag{17a}$$

$$X_1 = \frac{\mu_1}{V_1}\left(S - \frac{DK_1}{\mu_1 - D}\right)\,. \tag{17b}$$

Curiously, the equilibrium concentration of food is not a function of S, whereas the equilibrium concentration of the prey is a linear function of S. Note that this is only meaningful if

1. $\mu_1 > D$, otherwise X_0 is negative at equilibrium.
2. $DK_1/(\mu_1 - D) < S$, or else X_1 is zero or negative at equilibrium.

The first condition means that the bacterium must be able to grow at more than the dilution rate, the second that sufficient food must be available for it to grow at precisely the dilution rate.

Since Monod, many people have put forward improvements to Eqs. 16a,b. One objection that has been raised against this is that no maintenance en-

ergy term similar to $H(Y)$ in Eq. 6b is used (Nisbet et al. 1983), but this can either be assimilated into D, or added explicitly as an extra term. In some cases, multiple pathways for uptake of the same substrate are present, e.g. for low and high substrate availability, and this can be accommodated by multiple Monod terms, each with its own μ_i and K_i (Gerritse et al. 1992). Gerritse et al. (1992) also provide a model for aerobic and anaerobic behaviour, which was extended and used by Kamerman and Wilkinson (2002) and by Wilkinson (2002). A further refinement is that of a cascade of enzymes, or transporter protein mediated reactions which limit growth (Button 1991; Koch 1982). Many models also focus on the physiology of slow growth, which can be of particular importance in low-nutrient environments such as lakes (Button 1991, 1993; Koch 1997). On the other side of the spectrum we have substrate inhibition models (e.g. Tan et al. 1996), which deal with situations in which there is a sudden glut of food. A number of alternatives to the Monod equation are reviewed by Koch (1998), in which not only enzyme-mediated steps are considered, but also diffusion processes. Koch (1998) concludes that, while there are many shortcomings to the Monod model, it does describe the overall behaviour of bacteria growing in chemostats quite well, and (with caveats) can serve as a basis for qualitative and even quantitative modelling of bacterial growth. Especially when designing strategic models of microbial dynamics, its use seems justified (Gottschal 1993; Kooi and Kooijmans 1994a; Wilkinson 2001, 2002). This is why I will use the simple Monod model for prey throughout the rest of this chapter.

3.3 Modelling a Microbial Predator–Prey System

An early model for a microbial predator–prey system was put forward by Canale (1969). He used the Monod/Holling type II model for growth of both predator and prey. When modelling predatory bacteria or protozoa, maintenance energy must be taken into account (Nisbet et al. 1983), but not in the case of bacteriophages. Therefore, the growth of microbial predators Y on prey species X_1 is modelled as

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \frac{\mu_y X_1}{K_X + X_1} Y - (D + d_y) Y \,, \tag{18}$$

in which μ_y is the maximum specific growth rate, K_X is the saturation constant, D is the dilution rate of the chemostat, and d_y is the starvation rate. The differential equation for species X_1 is

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - \frac{V_y X_1}{K_X + X_1} Y - D X_1 \,. \tag{19}$$

in which V_y is the maximum specific uptake rate of prey by predator. The differential equation for the limiting substrate becomes

$$\frac{\mathrm{d}X_0}{\mathrm{d}t} = D\left(S - X_0\right) - V_1 \frac{X_0}{K_1 + X_0} X_1 \,, \tag{20}$$

as before.

The behaviour of the set of ODEs defined by Eqs. 18–20 is similar to that of the logistic growth/Holling type II model shown in Fig. 1. This can be shown by stability analysis (Wilkinson 2001). Four different "phases" of the system can be identified (Kooi and Kooijman 1994a): (0) total washout of both species; (I) stable prey population with washout of predator; (II) stable coexistence of predator and prey, and (III) unstable coexistence (limit cycle behaviour). Levin et al. (1977) split phase III into two subphases: (IIIa) in which the limit cycle is itself stable (neither species is driven to extinction), and (IIIb) in which either the predator or both species are driven to extinction by increasing oscillations. The boundaries between these two subphases were determined by numerical analysis (Levin et al. 1977). In phases II and III, where predator and prey coexist, we can assume that all concentrations are non-zero, and we find the equilibrium point by equating the right-hand sides of Eqs. 18 and 20 to zero. A little algebra yields:

$$X_0 = \frac{1}{2}\left(S - K_1 - \frac{V_1 K_X}{\mu_y - D - d_y} \pm \sqrt{\left(S - K_1 - \frac{V_1 K_X}{\mu_y - D - d_y}\right)^2 + 4K_1 S}\right) \tag{21a}$$

$$X_1 = \frac{\left(D + d_y\right) K_X}{\mu_y - D - d_y} \tag{21b}$$

$$Y = \frac{D}{D + d_y} \frac{\mu_y}{V_y} \left(\frac{\mu_1}{V_1}\left(S - X_0\right) - X_1\right) \,. \tag{21c}$$

Thus it can be seen that the equilibrium concentration of prey is directly proportional to saturation constant K_X. The boundaries between the phases 0, I and II as a function of the chemostat's control parameters (dilution rate D and input concentration of the limiting substrate S) can be obtained analytically (Wilkinson 2001), whereas the boundary between phases II and III was obtained by local stability analysis of the steady-state solution. All boundaries are shown in Fig. 3b. Figure 3a shows the transient behaviour of the system using parameter values from Nisbet et al. (1983) and Kooi and Kooijman (1994a) (i.e. $K_1 = 8\,\mathrm{mg\,l^{-1}}$, $K_X = 9\,\mathrm{mg\,l^{-1}}$, $\mu_1 = 0.5\,\mathrm{h^{-1}}$, $\mu_y = 0.2\,\mathrm{h^{-1}}$, $V_1 = 1.25\,\mathrm{h^{-1}}$ and $V_y = 0.3333\,\mathrm{h^{-1}}$). Note that in all these studies the inflowing substrate levels are held constant. If they fluctuate, the dynamics become more complicated, allowing multiple prey species to coexist on a single limiting substrate (Grover 1988, 1990).

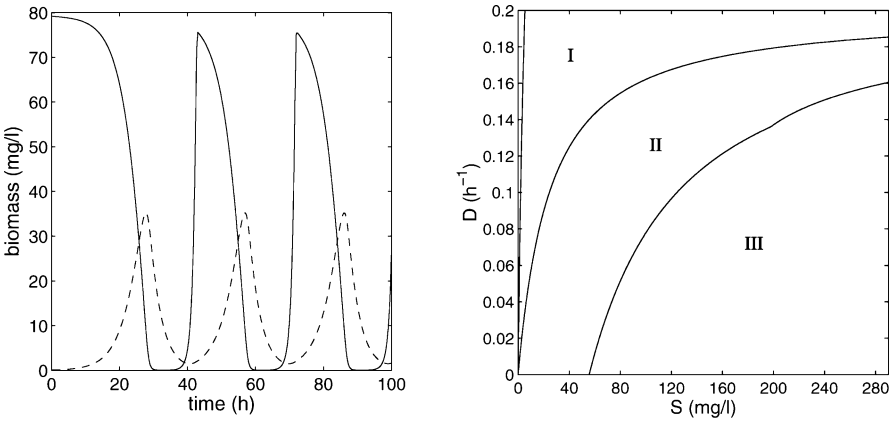


Fig. 3 Phase boundaries and transient behaviour of microbial predator–prey system described by Eqs. 18–20, with parameter settings according to Nisbet et al. (1983), i.e. $K_1 = 8\,\mathrm{mg\,l^{-1}}$, $K_X = 9\,\mathrm{mg\,l^{-1}}$, $\mu_1 = 0.5\,\mathrm{h^{-1}}$, $\mu_y = 0.2\,\mathrm{h^{-1}}$, $V_1 = 1.25\,\mathrm{h^{-1}}$ and $V_y = 0.3333\,\mathrm{h^{-1}}$. **a** Transient behaviour showing strong predator (*dashed line*)–prey (*solid line*) oscillations; **b** boundaries between phases as a function of dilution rate D and inflowing substrate concentration S. Note how the probability for oscillations increases with enrichment of the ecosystem. See text for details

3.4 Modelling Bacterium–Phage Systems

Bacterium–phage systems in chemostats have long been studied as "ideal" predator–prey systems, due to their small scale and short generation time (Campbell 1961; Chao et al. 1977; Levin et al. 1977; Bohannan and Lenski 1997; Weld et al 2004). Because the phage life cycle is similar to the life cycle of *Bdellovibrio* and similar organisms, I will present the methods used to model phages before describing predatory prokaryotes proper. All the work cited above uses DDEs to model a phage's life cycle using a single delay τ, which is the time between invasion and phage release. Following Levin et al. (1977), rather than Campbell (1961) who uses logistic prey growth, we use Monod growth as before. Note that the predator can be in two phases: the free and the reproductive phase within the infected host. Let X_1 be the prey as before; Y_{free} denotes the free predators and $[X_1\,Y]$ denotes the complex formed when prey is bound to predator (the infected bacteria). Furthermore, assume the rate of *productive* collisions (i.e. which result in prey capture or penetration) between predator and prey is r per unit of prey species, per unit of predator. Note that the actual collision rate may be larger by an order of magnitude or more. The prey/predator complex dissociates after a time delay τ, yielding $y_x + 1$ new predators. Because one predator is lost in the infection, we have

a net yield of y_x. We now obtain

$$\frac{dX_0}{dt} = D(S - X_0) - V_1 \frac{X_0}{K_1 + X_0}\left(X_1 + [X_1 Y]\right) \tag{22a}$$

$$\frac{dX_1}{dt} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - rY_{\text{free}} X_1 - DX_1 \tag{22b}$$

$$\frac{dY_{\text{free}}}{dt} = (y_x + 1)e^{-D\tau} X_1' Y' - rX_1 Y_{\text{free}} - DY_{\text{free}} \tag{22c}$$

$$\frac{d[X_1 Y]}{dt} = -e^{-D\tau} X_1' Y' - D[X_1 Y] + rX_1 Y_{\text{free}}\,, \tag{22d}$$

in which X_1' and Y' denote the density of X_1 and Y at time $t - \tau$, respectively. Note that it is assumed that infected prey also use the substrate. The term $e^{-D\tau} X_1' Y'$ denotes the amount of $[X_1 Y]$ which formed at $t - \tau$, and has not yet been washed out of the chemostat. In the last term in Eq. 22a, the factor $(X_1 + [X_1 Y])$ means that the complex $[X_1 Y]$ consumes substrate, which may be partly right for phage-infected prey, but much less for *Bdellovibrio*-infected prey.

Approximations using (slightly more tractable) ODEs have also been proposed. Payne and Jansen (2001) transform the time delay τ into a lysis rate $k_1 = 1/\tau$. In this case we obtain the set of ODEs

$$\frac{dX_0}{dt} = D\big(S - X_0\big) - V_1 \frac{X_0}{K_1 + X_0}\big(X_1 + [X_1 Y]\big) \tag{23a}$$

$$\frac{dX_1}{dt} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - rY_{\text{free}} X_1 - DX_1 \tag{23b}$$

$$\frac{dY_{\text{free}}}{dt} = \big(y_x + 1\big)\, k_1\, [X_1 Y] - rX_1 Y_{\text{free}} - DY_{\text{free}} \tag{23c}$$

$$\frac{d[X_1 Y]}{dt} = -k_1 [X_1 Y] - D![X_1 Y] + rX_1 Y_{\text{free}}\,. \tag{23d}$$

Figure 4 shows the result of a simulation using both models for an *E. coli*–T2 phage system, using the parameters from Levin et al. (1977). The overall behaviour of both models is similar, though the ODE method tends to overestimate predator growth (Weld et al. 2004). Another small difference is the slightly later onset of the oscillations in the case of the DDE approach. Many variants of the DDE method have been proposed, and the interested reader is referred to Weld et al. (2004).

3.5 Modelling Predatory Prokaryotes

To model prokaryote predators we must of course start with an understanding of how they attack and consume their prey. Martin (2002) distinguishes four types of predatory prokaryotes (in reverse order compared to Martin):

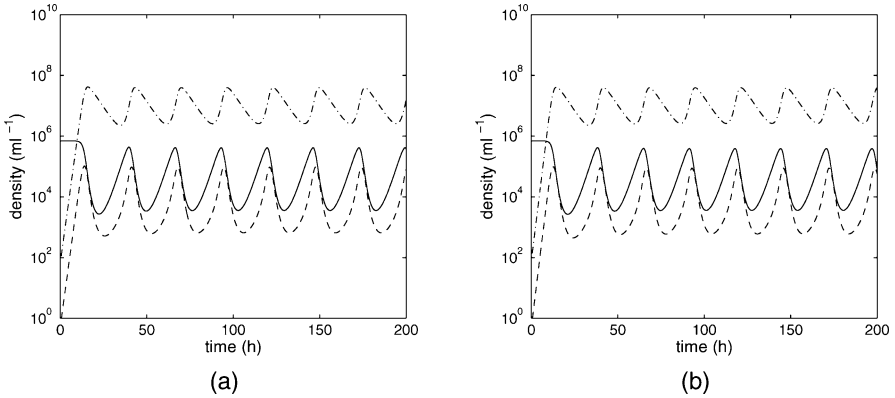


Fig. 4 DDE vs. ODE models for *E. coli*–T2 phage systems, showing prey density (*solid line*), infected prey density (*dotted line*) and phage density (*dash-dot line*). **a** DDE model according to Levin et al. (1977); **b** ODE equivalent according to Payne and Jansen (2001). The chief difference is a slight delay in the onset of oscillations in the DDE case

(1) periplasmic, in which the predator invades the periplasmic space of Gram-negative cells as in the case of *Bdellovibrio* and *Bacteriovorax* species; (2) direct invasion into the cytoplasm as in *Daptobacter* (Guerrero et al. 1986); (3) epibiotic, i.e. attached to the surface such as done by *Vampirococcus*; and (4) the "wolf-pack" approach, in which no physical contact is needed, but the predatory bacteria release lytic substances which break down the prey, as seen in *Myxococcus* (Burnham et al 1981), *Lysobacter* (Lin and McBride 1996) and *Pseudomonas* strain 679-2 (Casida and Lukezic 1992; Cain et al. 2003). The last case may be considered a simple extension of the production of lytic bacteriocins, which is very common amongst bacteria (Chao and Levin 1981; Frank 1994; Riley and Gordon 1996; Iwasa et al. 1998). By simply absorbing the nutrients released by the destruction of competitors, all these bacteria could be considered non-obligately predatory prokaryotes (see the chapter by Jurkevitch and Davidov, this volume).

From the point of view of modelling, the first two types of predator are identical, because the model simply does not take the location of the predator within the cell into account. The third and fourth are slightly different, because multiple organisms may attack a single host (Esteve and Gaju 1999; Guerrero et al. 1986; Martin 2002). The mechanisms are slightly different and I will propose two different models for types (3) and (4) in the following subsection.

We must also make the distinction between obligate and non-obligate predators. The former can be modelled with a single substrate uptake process, whereas the latter requires two: one for the predatory mode and one for the non-predatory mode. Furthermore, we need to model a switch between these modes. This is discussed in Sect. 3.5.2.

3.5.1 Obligate Predators

We will start by modelling the best-known obligately predatory prokaryote: *B. bacteriovorus*. Given that the lifestyle of *B. bacteriovorus* is similar to that of phages, the approach to phage modelling can be applied to *B. bacteriovorus*, as was done by Marchand and Gabignon (1981) and Dulos and Marchand (1984). They also used a DDE, similar to that of Levin et al. (1977), but with some simplifications. First of all they used exponential growth for the prey, without taking its substrate into account, which is an oversimplification. Secondly, the flush-out term $e^{-D\tau}$ was not included. This can be defended for low dilution rate D combined with a fairly small delay time τ. Dulos and Marchand (1984) used $D = 0.03\,\mathrm{h}^{-1}$ and $\tau = 3\,\mathrm{h}$, which means that $e^{-D\tau} = 0.9131$. Given the many inaccuracies in the measurements, this may be close enough to unity. Their set of coupled DDEs is

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \mu X_1 - rY_{\mathrm{free}}X_1 - DX_1 \tag{24a}$$

$$\frac{\mathrm{d}Y_{\mathrm{free}}}{\mathrm{d}t} = \left(y_x + 1\right) X_1' Y' - r\left(X_1 + [X_1 Y]\right) Y_{\mathrm{free}} - DY_{\mathrm{free}} \tag{24b}$$

$$\frac{\mathrm{d}\,[X_1 Y]}{\mathrm{d}t} = -X_1' Y' - D\,[X_1 Y] + rX_1 Y_{\mathrm{free}}\,, \tag{24c}$$

with μ the relative growth rate of prey. Note that multiple invasion is modelled in Eq. 24b by the additional $r[X_1 Y]Y_{\mathrm{free}}$ term. Though it is not apparent in this set of equations, Dulos and Marchand do model the starvation of free predators in their simulation program. They note the difficulty in modelling the starvation of those predators *who have not found a prey within the starvation time* $\tau_1 = 10\,\mathrm{h}$ within the framework of DDEs or ODEs. The reason for this is that the effect depends on the age structure of the free predator population. Ideally, this should be modelled through partial differential equations similar to the dynamic energy budget model (Kooijman 1993; Kooi and Kooijman 1994a,b). The approach in Dulos and Marchand (1984) is similar in that it effectively discretizes the age distribution of free prey, and transforms the problem back into a more complicated ODE, which they solve with a Euler approach with a fixed (20 min) time step. The predator can be modelled by an array of variables, each representing an age class. At each time step, we can first compute how many of each class find prey, and put any remaining predators in a higher age class. All new predators are put in the lowest class.

Following Dulos and Marchand's parameter settings we have $\tau = 1/k_1 \cong 3.0\,\mathrm{h}$. This system is shown in Fig. 5a, in which $\mu = 0.06\,\mathrm{h}^{-1}$, $y_x = 8$, $D = 0.03\,\mathrm{h}^{-1}$, $r = 3 \times 10^{-9}\,\mathrm{ml}^{-1}\,\mathrm{h}^{-1}$ and $k_1 = 1/3.0\,\mathrm{h}^{-1}$. The initial prey and predator densities are $3 \times 10^6\,\mathrm{ml}^{-1}$ and $10^7\,\mathrm{ml}^{-1}$, respectively. As can be seen the

oscillations increase in amplitude until one or both species go extinct. Figure 5b shows the effect of ignoring starvation, leading to more regular oscillations. We compare this to the DDE approach of Levin et al. (1977) with added starvation and similar parameter settings, but additionally $\mu_1 = V_1 = 1\,\mathrm{h}^{-1}$, $S = 3 \times 10^6\,\mathrm{ml}^{-1}$ and $K_1 = 10^6\,\mathrm{ml}^{-1}$ (note that the latter two are expressed in equivalent number of prey bacteria per millilitre), shown in Fig. 5c. Somewhat surprisingly, the change in prey model to a Monod-type substrate limited growth stabilizes the system dramatically. Only by raising r to $3 \times 10^{-8}\,\mathrm{ml}^{-1}\,\mathrm{h}^{-1}$ do we get any oscillations, as seen in Fig. 5d. If we follow the ODE approach and assume that the dilution rate constant D is small compared to the reaction constants, we can approximate this set as follows by so-called quasi-steady-state analysis. We assume that the prey capture and predator division reactions are fast enough to settle into equilibrium. At

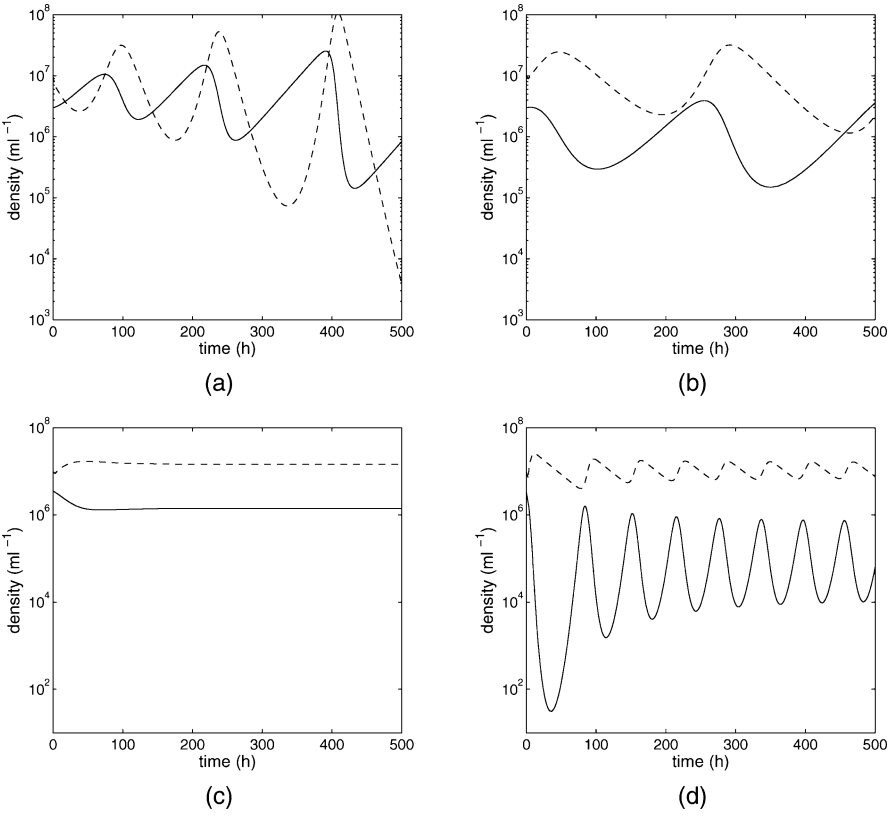


Fig. 5 Different models for the *B. bacteriovorus* (*dashed line*)–*E. coli* (*solid line*) system. **a** DDE according to Dulos and Marchand (1984); **b** same as **a** but ignoring starvation; **c** DDE according to Levin et al. (1977) with the same parameter settings as **a**; **d** only by increasing collision rate r by a factor of 10 do oscillations occur

(quasi-)steady state we have

$$[X_1 Y] = \frac{r}{k_1} X_1 Y_{\text{free}} ;$$

inserting this in Eqs. 23c,d and summing we arrive at

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = y_x k_1 \frac{X_1}{k_1/r + X_1} Y , \tag{25}$$

which is just the Holling type II model (Holling 1959). Whether quasi-steady-state analysis is justified depends very much on the situation. The same simulation as in Fig. 6a using the explicit form of Eqs. 22a–d was performed using the Holling type II approximation. The results are shown in Fig. 6b. Clearly, at a dilution rate of $D = 0.03\,\mathrm{h}^{-1}$, the Holling approximation is quite reasonable. This is not unexpected because the time constant k_1 is an order of magnitude larger than the dilution rate.

For epibiotic predatory bacteria, such as *Vampirococcus* spp., which attach to the outside and feed there, the Holling type II model is probably justified. In this case, multiple predator cells might attach to a single prey item (although this is not necessarily the case for all epibiotic interactions). This situation is more or less similar to "normal" feeding by bacteria, which is generally modelled through the use of Monod models, which are functionally identical to the Holling type II model. We can revert to the model given by Eqs. 18–20. Given the difficulties in culturing *Vampirococcus* in the laboratory (Martin 2002), no models have been put forward to date, so parameter estimation, let alone model validation, is difficult.

Finally, we have the wolf-pack type, which we can model using a combination of the model for bacteriocin production and susceptibility (Frank

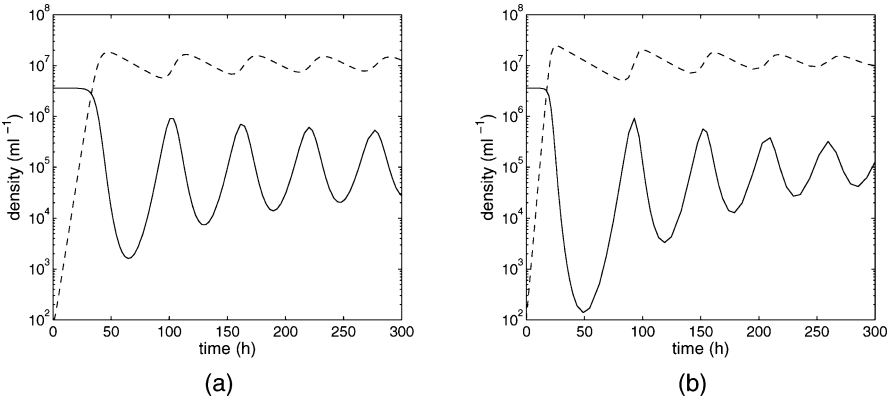


Fig. 6 Predator–prey oscillations in *Bdellovibrio*-type predator model. **a** Two curves are shown: prey density X_1 (*solid line*) and free predator density Y_{free} (*dashed line*). **b** The same model in a Holling type II approximation

1994; Wilkinson 2002), and allowing the predator to feed upon the materials released by lysis of the prey. The set of equations becomes somewhat more complex. Let T be a lytic toxin released by the predator Y. As before, we have the prey X_1 growing on X_0 through Monod kinetics. We assume the prey is destroyed at a rate proportional to the concentration of T. This reaction consumes some fraction of T. The destruction of X_1 by T leads to the formation of a substrate S_y on which Y grows directly, using Monod kinetics again. The set of differential equations becomes

$$\frac{\mathrm{d}X_0}{\mathrm{d}t} = D\left(S - X_0\right) - V_1 \frac{X_0}{K_1 + X_0} X_1 \tag{26a}$$

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - \kappa T X_1 - D X_1 \tag{26b}$$

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \frac{\mu_y S_y}{K_S + S_y} Y - \left(D + d_y\right) Y \tag{26c}$$

$$\frac{\mathrm{d}T}{\mathrm{d}t} = \alpha Y - \beta T X - D T \tag{26d}$$

$$\frac{\mathrm{d}S_y}{\mathrm{d}t} = \frac{V_y S_y}{K_S + S_y} Y - \eta \kappa T X - D S_y\,, \tag{26e}$$

with α, β and κ rate constants and η a conversion efficiency factor. For the sake of simplicity, we assume that the two substrates X_0 and S_y are different, which need not be the case. Rigorous analytical treatment of this set of ODEs is beyond the scope of this chapter. It may well be possible to simplify this set to a model similar to that of Eqs. 18–20, but this is not obvious. Preliminary numerical analysis suggests that Eqs. 26a–e resemble bacteriocin-mediated interactions in an important way. Bacteriocin-mediated interactions are bistable: either the susceptible species survives, or the producer species survives, but stable coexistence is impossible (Frank 1994; Wilkinson 2002). This is in part due to the positive feedback loop in this set of equations. This occurs because more predators means more toxin, means more substrate for the predators, means faster predator growth, etc. The reverse is also true: if predator numbers drop, so does the toxin level, and therefore substrate levels, meaning slower predator growth, etc. This means that once the predators, and therefore the toxin levels, have crossed a certain threshold, a runaway reaction takes place, killing all the prey. After this the predator population also collapses. In the alternative scenario, toxin levels are not high enough to kill enough prey, and the predator dies out. This means that if the predator is to be able to invade a system of only prey, it must produce a toxin potent enough to kill sufficient prey quickly, so that it can then grow at more than the dilution rate. Such potent toxins means that predators always wipe out the prey once their numbers start growing. This suggests that

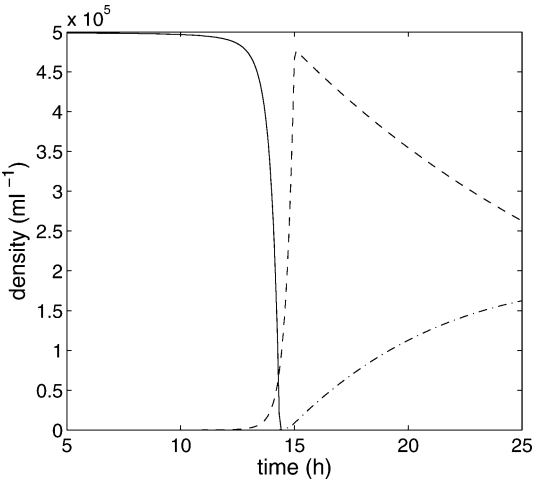


Fig. 7 A simulation for wolf-pack predators: prey (*solid line*), predator (*dashed line*) and toxin level (*dash-dot line*) are shown as a function of time. As predator numbers increase slowly, toxin levels rise gently so long as there are many prey to absorb the toxin. Once a certain threshold is reached, the prey kill rate outstrips the prey growth rate, leading to a collapse of the prey population, sudden release of substrate and an explosive growth of the predator, which then starves in the absence of prey

only predators that cannot invade pure prey systems might be able to coexist with prey.

We can conclude that, unless some damping mechanism is available, wolf-pack feeding does not appear to be stable. This suggests it will only occur in non-obligate predators, which is to some degree supported by observations (Martin 2002). A typical simulation run is shown in Fig. 7.

3.5.2 Non-Obligate Predators

Non-obligate predators may survive without prey, and in the prokaryote case often only switch to predatory behaviour under conditions of low substrate availability (Esteve and Gaju 1999; Guerrero et al. 1986; Martin 2002). Though *B. bacteriovorus* is probably the best-known predatory prokaryote, non-obligate predatory behaviour may actually be more common than obligate predatory behaviour. Modelling a non-obligate predator can be done by combining the standard Monod model for growth on regular substrate with, e.g., the Holling type II model for the predator phase. If the predatory behaviour only switches on below some minimum substrate level $S_{\min}$, we also need to model a switch function T. This function is zero at low substrate level, and switches rapidly, but preferably continuously, to 1 above $S_{\min}$. One

plausible model would be

$$T(S) = \frac{S^n}{S_{\min}^n + S^n} .$$

If n is larger than 1, this is a sigmoid function which switches rapidly from zero to 1 around $S_{\min}$, as is shown in Fig. 8 for various values of n. The equation for growth of a non-obligate predator then becomes

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = T(S)\mu_{\max}\frac{SY}{K_S + S} + \big(1 - T(S)\big)\,\mu_y\frac{X_1 Y}{K_X + Y} , \tag{27}$$

and for the prey (X_1 growing on X_0) we obtain

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \mu_1\frac{X_0 X_1}{K_1 + X_0} - \big(1 - T(S)\big)\,V_y\frac{X_1 Y}{K_X + Y} , \tag{28}$$

disregarding the dilution of the chemostat for the moment.

An alternative to this approach would be to model the predator not by one, but by two variables, Y_1 denoting the non-predatory mode and Y_2 the predatory mode. In this case, we must model the transfer rate from predatory to non-predatory mode in some way. Let $\tau_{\max}$ be the maximum switch rate. The forward switching function T_{12} could then be modelled as

$$T_{12}(S) = \tau_{\max}\frac{S_{12}^n}{S_{12}^n + S^n} , \tag{29}$$

in which S_{12} is the substrate concentration at which half the maximum forward switch rate is achieved. Obviously, if $S \gg S_{12}$ the forward switch rate is

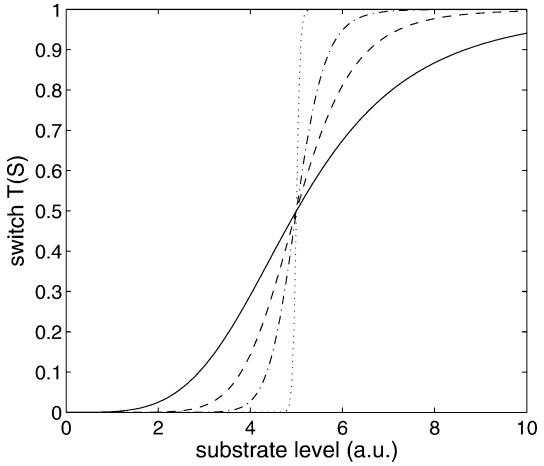


Fig. 8 The switch function $T(S)$ for $S_{\min} = 5$, and $n = 4$ (*solid line*), $n = 8$ (*dashed line*), $n = 16$ (*dash-dot line*) and $n = 128$ (*dotted line*). As n increases the sigmoidal shape progresses towards a more threshold-like behaviour. a.u.: arbitrary units

near zero. The reverse switch function T_{21} is modelled as

$$T_{21}(S) = \tau_{\max} \frac{S^n}{S_{21}^n + S^n}\,, \tag{30}$$

in which S_{21} is the concentration of S at which the reverse switch rate is half the maximum rate. It is possible to let the switch points be equal, i.e. $S_{12} = S_{21} = S_{\min}$. However, the above method is slightly more general. Assuming the offspring of Y_1 are also non-predatory and the offspring of Y_2 are all in predatory mode, the set of differential equations now becomes

$$\frac{\mathrm{d}Y_1}{\mathrm{d}t} = \mu_{\max} \frac{SY_1}{K_S + S} - T_{12}(S)Y_1 + T_{21}(S)Y_2 \tag{31a}$$

$$\frac{\mathrm{d}Y_2}{\mathrm{d}t} = \mu_y \frac{X_1 Y_2}{K_X + Y_2} + T_{12}(S)Y_1 - T_{21}(S)\,Y_2 \tag{31b}$$

$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = \mu_1 \frac{X_0 X_1}{K_1 + X_0} - V_y \frac{X_1 Y_2}{K_X + Y_2}\,. \tag{31c}$$

Note that many other switch functions could be used instead. These equations just serve to show how such predators could be modelled. I am not aware that similar types of ODEs have ever been used to mathematically model any of the known non-obligate prokaryote predators. Though easy to draw up, and fairly straightforward to simulate by computer, these equations are not easy to analyse, and the large number of parameters makes it hard to estimate them.

3.6 Prey Countermeasures

Another feature that could be modelled mathematically is that of prey countermeasures. As observed by Shemesh and Jurkevitch (2004), some prey species apparently respond to predation by switching to a resistant phenotype, in a similar way as bacteria may switch to an antibiotic-resistant phenotype when challenged by antibiotics (Balaban et al. 2004). Leaving aside the case of mutants, we assume that this resistant phenotype incurs some growth penalty, as observed in the case of antibiotic resistance (Balaban et al. 2004). If this were not the case, mutants only ever expressing that phenotype would dominate the population once they appeared. A simple way to model this is to model the prey species by two variables: X_1 which is susceptible and X_1^* which is not. The latter grows at a slightly lower growth rate determined by parameters μ_1^* and K_1^*. We will first treat the case of "type I persisters", as defined by Balaban et al. (2004). A predator–prey collision can now result in two outcomes: (1) either the prey is penetrated by the predator as before, with probability $1 - p$, or (2) the predator swims away and the prey is triggered to switch to resistant mode, with some probability p. In type II persisters, the switch to the resistant phenotype occurs at a constant rate in-

dependently of any trigger (Balaban et al. 2004). In either case, the resistant phenotype switches back to the sensitive strain at some rate s_r. Of course, the reverse switch rate could also be made dependent on some trigger (e.g. absence of collisions during some time interval). We arrive at the following set of differential equations

$$\frac{dX_0}{dt} = D\left(S - X_0\right) - V_1 \frac{X_0}{K_1 + X_0} X_1 - V_1^* \frac{X_0}{K_1^* + X_0} X_1^* \tag{32a}$$

$$\frac{dX_1}{dt} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - \left(r Y_{\text{free}} + D\right) X_1 \tag{32b}$$

$$\frac{dX_1^*}{dt} = \frac{\mu_1^* X_0}{K_1^* + X_0} X_1^* + p r Y_{\text{free}} X_1 - \left(s_r + D\right) X_1^* \tag{32c}$$

$$\frac{dY_{\text{free}}}{dt} = \left(y_x + 1\right) k_1 \left[X_1 Y\right] - \left(\left(1 - p\right) r X_1 + D\right) Y_{\text{free}} \tag{32d}$$

$$\frac{d\left[X_1 Y\right]}{dt} = -\left(k_1 + D\right)\left[X_1 Y\right] + r X_1 Y_{\text{free}} \,. \tag{32e}$$

The complexity of this system makes analysis and estimation of parameters quite hard. Nonetheless, we can use this set of ODEs to obtain some feeling for the importance of the parameters. The results of a number of simulations are shown in Fig. 9. In Fig. 9a the same system as in Fig. 5d is shown, which is the starting point of our modifications. In Fig. 9b–d we have set $\mu_1^* = 0.99\mu_1$, $K_1^* = K_1$ and $p = 0.5$, whilst doubling the collision rate r to ensure the same number of *productive* collisions with Y_{free} takes place. In Fig. 9b we set the reverse switch rate $s_r = 0.06\mu_1$, corresponding to a switch rate about 1/16 times the fastest doubling time (Shemesh and Jurkevitch 2004); in Fig. 9c we have $s_r = 0.01\mu_1$. Clearly having a $p > 0$ increases the survival rate, as suggested by Shemesh and Jurkevitch (2004). Increasing p to increase the forward switching rate does not change the dynamics of the system dramatically, it just leads to a further reduction of predator numbers. Reducing the reverse switch rate delays the return of the original phenotype to dominance as expected. Note that setting the reverse switch rate s_r to (nearly) zero, and making the forward switch probability small, and possibly independent of collision with Y_{free}, allows modelling of a genotypical switch (mutation) such as that observed in an *E. coli* bacteriophage PP01 system (Mizoguchi et al. 2003), rather than a phenotypic response using essentially the same set of equations. This is shown in Fig. 9d where $p = 0.09$ and $s_r = 0$. It is difficult to see in the plot, but the sensitive strain does recover very slowly after elimination of the predator, due to its slight growth advantage, as suggested by (Shemesh and Jurkevitch 2004). In the case described by Mizoguchi et al. (2003), the phage apparently responded to the prey response by mutating itself, potentially starting (or simply continuing) an arms race.

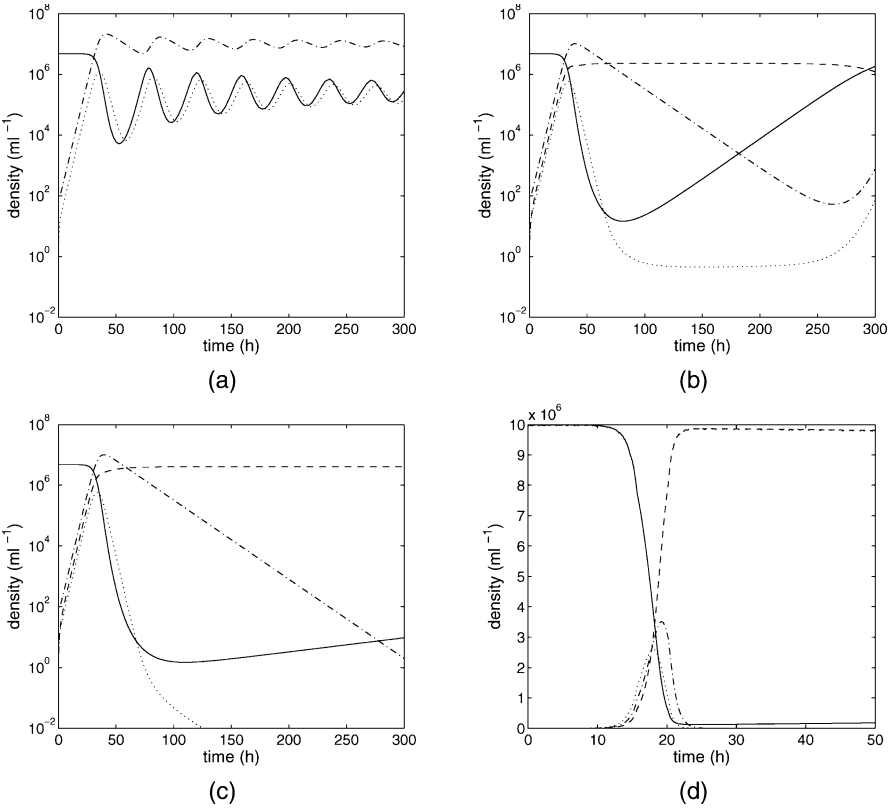


Fig. 9 Prey countermeasures by switching to a resistant state, showing susceptible state X_1 density (*solid line*), resistant state density X_1^* (*dashed line*), free predator density Y_{free} (*dash-dot line*), and bdelloplast density $[X_1 Y]$ (*dotted line*). **a** No countermeasure ($p = 0$); **b** prey switches to resistant mode after collision with Y_{free} with probability $p = 0.5$, collision rate r doubled with respect to **a**, and reverse switch rate $s_r = 0.06\mu_1$; **c** same as **b**, but with $s_r = 0.01\mu_1$; **d** $p = 0.09$ and $s_r = 0$, to mimic mutations rather than phenotypic switches

Equations 32a–e are only one way to model this type of countermeasure. If the switch is based on some active response by the prey, we could also model the signal transduction in the prey using density-dependent switches such as those in the model for non-obligate predators in Eqs. 31a–c. The effect described by Shemesh and Jurkevitch (2004) is by no means the only possible countermeasure. The prey species could also respond to predation by producing cidal or inhibitory toxins, such as bacteriocins. These could be modelled using the differential equations from Frank (1994) and Wilkinson (2002), similar to the lytic toxins used in Eqs. 26a–e. A further possibility is the production of decoys: objects which in some way distract the predator long enough to let the prey escape. This is similar to tail autotomy in certain

lizards (Dial and Fitzpatrick 1983), leg autotomy in arachnids (Punzo 1997) and the immune evasion strategies used by certain parasites (Donelson 1998; Ramasamy 1998). By casting off some part of the outer envelope, e.g. membrane vesicles (Beveridge 1999; Mashburn and Whiteley 2005), bacteria might be capable of something similar. The decoy effect is explained in detail in Sect. 4.2.

4 Third-Species Effects

Adding a third species to a system can have a profound effect, and just a few of the potential 704 different effects (Harmon and Andow 2002) will be discussed, in particular those which have been noted in a microbial context.

4.1 Alternative Prey

In the fields of control of potential pathogens in waste water (Mallory et al. 1983) and the control of insect pests (Harmon and Andrews 2002), the possibility of the so-called alternative prey model has been put forward. Before that it was also studied by Levin et al. (1977) in the setting of bacterium–phage systems. Suppose we wish to eliminate some pathogen X_1 in waste water by using predator Y. However, the levels of X_1 in the water may be too low to support the predator Y, let alone yield the predator–prey oscillations which would lead to a catastrophic collapse of the numbers of X_1. In this case the third, harmless species, X_2, is added to the water simultaneously with Y. This can enrich the ecosystem to the level that the "paradox of enrichment" effect takes place, i.e. the diversity reduces and ideally the pathogen disappears. The key issue is that the alternative prey effect occurs mainly in generalist predators, simply because true specialists do not have alternatives. Modelling this situation is straightforward. Simply add a third species X_2, which either grows on the same substrate as X_1 or on a different one, and add a second predation term to the model of the predator

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \frac{\mu_{y1} X_1}{K_{X1} + X_1} Y + \frac{\mu_{y2} X_2}{K_{X2} + X_2} Y - DY - d_y Y \,. \tag{33}$$

The results of the alternative prey effect depend on whether X_2 competes for the same substrate with X_1. If this is the case, X_1 is threatened both by increased predation and by competition. Nonetheless, X_1 could still eliminate X_2 provided it can grow faster than X_2. A few simulation runs using the same system as in Fig. 3 are shown in Fig. 10. Input substrate level S was lowered to $100\,\mathrm{mg\,l^{-1}}$ to obtain a stable predator–prey equilibrium. In all cases the

growth rate of X_2 is $0.99\mu_1$, to give it a slight disadvantage relative to X_1. Figure 10a,b concern the situation where X_2 is sustained by a separate substrate, i.e. it does not compete for X_0. In Fig. 10a the input level of the substrate for X_2 is equal to S, the input substrate level for X_1. Note the strong predator–prey oscillations, in which the levels of X_1 and X_2 are almost identical. The system behaves very much like the two-species system with double the amount of nutrients. In Fig. 10b the input substrate level for X_2 is $2S$, resulting in different predator–prey oscillations in which X_1 is suppressed more. In the case that X_1 and X_2 are in direct competition for the same resources the situation is very different, as shown in Fig. 10c,d. If X_2 is added at the equilibrium level of X_1, it fails to have any real impact, and the system will ultimately settle back

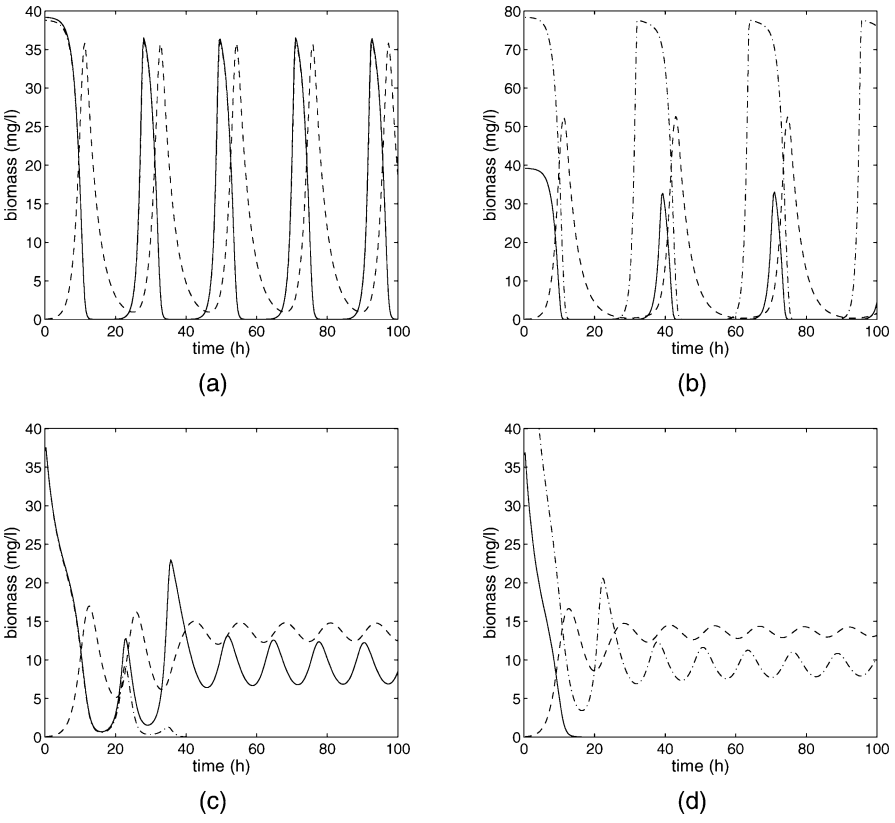


Fig. 10 Alternative prey effects in the same system as shown in Fig. 3. *Top row*: alternative prey X_2 (*dash-dot line*) that does not compete for the same substrate as regular prey X_1 (*solid line*): **a** equilibrium level (without predation) of X_2 equals that of X_1; **b** equilibrium level (without predation) of X_2 twice that of X_1. *Bottom row*: X_2 that does compete with X_1: **c** X_2 added at the same level as equilibrium of X_1; **d** X_2 added at twice the equilibrium level of X_1. Predator Y shown as *dashed line*

into equilibrium (Fig. 10c). However, if the initial density of X_2 is doubled, it leads to an eradication of X_1, despite the fact that the latter has a higher growth rate at identical substrate levels. The system now gradually converges to a two-species equilibrium of X_2 and Y (Fig. 10d). The mathematically interested reader is referred to Levin et al. (1977) and Deng et al. (2003) for a more thorough analysis.

4.2 Decoy Species

The decoy effect occurs whenever a third species interferes with the ability of a predator to detect or track its prey. It was described by Christensen et al. (1976) in a host–parasite system consisting of *Fasciola hepatica* (sheep liver fluke) miracidia, which infects the snail *Lymnaea trunculata*. The presence of non-host snails inhibits the ability of the parasite to find its host, depending in part on the non-host species (related/non-related). Similarly, Yousif et al. (1998) found that *Schistosoma mansoni* (schistosomiasis parasite) miracidia, which have the snail *Biomphalaria alexandrina* as host, were inhibited by the presence of several other snail species. More relevant to the study of predatory prokaryotes is the model for the decoy effect as described by Wilkinson (2001). This model is based on some attempts to use *B. bacteriovorus* and bacteriophages for pathogen control (Westergaard and Kramer 1977; Smith and Huggins 1983; Jackson and Whiting 1992; Fratamico and Whiting 1995; Sarkar et al. 1996). In particular, Drutz (1976) observed that *B. bacteriovorus* can waste time when encountering non-prey bacteria, in this case *Neisseria gonorrhoeae*. We essentially start at the model of Eqs. 23a–d and consider the addition of a non-prey species X_2, which is present at a constant level and has no direct effect on either X_0 or X_1. We make these assumptions to study the effect of the simple presence of a decoy species independently of any other competition effect. Rather than two states, the predator can now be in three states: free, bound to X_1, and bound to X_2. These complexes are denoted as $[X_1Y]$ and $[X_2Y]$. Again, assume the rate of collisions is r per unit of prey or non-prey species per unit of predator. Furthermore, the non-prey/predator complex dissociates at a rate of k_2. However, only the dissociation of the first complex yields new predators, again with a yield of $y_x + 1$. This leads to the following set of differential equations:

$$\frac{\mathrm{d}Y_{\mathrm{free}}}{\mathrm{d}t} = \left(y_x + 1\right) k_1[X_1Y] + k_2[X_2Y] - r\left(X_1 + X_2\right) Y_{\mathrm{free}} \tag{34a}$$

$$\frac{\mathrm{d}[X_1Y]}{\mathrm{d}t} = -k_1[X_1Y] + rX_1Y_{\mathrm{free}} \tag{34b}$$

$$\frac{\mathrm{d}[X_2Y]}{\mathrm{d}t} = -k_2[X_2Y] + rX_2Y_{\mathrm{free}}\,. \tag{34c}$$

At (quasi-)steady state we have

$$[X_1 Y] = \frac{r}{k_1} X_1 Y_{\text{free}} \quad \text{and} \quad [X_2 Y] = \frac{r}{k_2} X_2 Y_{\text{free}} .$$

Summing Eqs. 34a–c we find a growth rate of

$$\frac{\mathrm{d}Y}{\mathrm{d}t} = \frac{y_x k_1 X_1 Y}{k_1/r + X_1 + k_1 X_2/k_2} = \frac{\mu_y X_1 Y}{K_X + X_1 + K_{\text{inh}} X_2} , \tag{35}$$

with $K_X = k_1/r$ and $K_{\text{inh}} = k_1/k_2$. In this form we can recognize that the decoy effect is essentially a form of competitive inhibition (e.g. Vos et al. 2001; Wilkinson 2001). In effect the interference models of Beddington (1975) and DeAngelis et al. (1975) can be considered an *auto-decoy* effect. The above system could also be modelled using DDEs as in Eqs. 22a–d.

4.2.1 The Consequences for the Ecosystem

We can understand the consequences for the ecosystem by the usual analysis of the equilibria. The easiest way to do this is by absorbing the inhibition by the decoy into the saturation constant K_X. Substituting $K_X^* = K_X + K_{\text{inh}} X_2$ into Eqs. 21a–c we obtain equilibrium (in phase II or III) when

$$X_0 = \frac{1}{2}\left(S - K_1 - \frac{V_1 K_X^*}{\mu_y - D - d_y} \pm \sqrt{\left(S - K_1 - \frac{V_1 K_X^*}{\mu_y - D - d_y} \right)^2 + 4 K_1 S} \right) \tag{36a}$$

$$X_1 = \frac{\left(D + d_y\right) K_X^*}{\mu_y - D - d_y} \tag{36b}$$

$$Y = \frac{D}{D + d_y} \frac{\mu_y}{V_y} \left(\frac{\mu_1}{V_1} \left(S - X_0 \right) - X_1 \right) . \tag{36c}$$

Because the equilibrium density of X_1 is directly proportional to K_X^*, it should be a linear function of the density of decoys. Therefore, the decoy effect should be easy to quantify in an experimental setting, as suggested by Wilkinson (2001). To date, this has not been done. Figure 11 shows the stabilizing effect of decoys, with $K_X^* = 2K_X$. As can be seen, increasing K_X^* means that, for a given D, the predator can only be present in the ecosystem *at all* at a higher input substrate concentration than in the absence of decoys.

The decoy effect has been observed and modelled in the context of arthropod predator–prey systems (Vos et al. 2001). This model was slightly different in that multiple predator–prey couples were used, and some interference factor coupling these oscillators was postulated. For a more detailed review of the decoy effect in microbial and other ecosystems, see Wilkinson (2003).

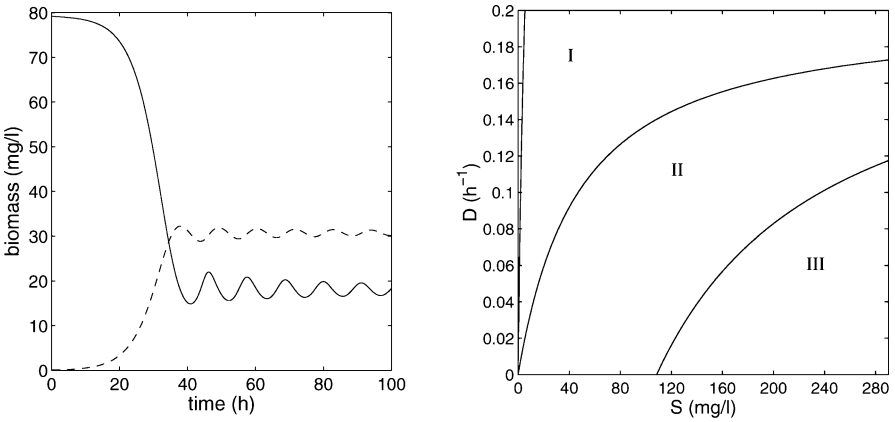


Fig. 11 The stabilizing effect of the presence of decoys, in the same system as shown in Fig. 3, shown for a decoy concentration such that $K_X^* = 2K_X$. **a** Transient behaviour shows damped oscillations between prey (*solid line*) and predator (*dashed line*); **b** phase boundaries show that the region of phase III (unstable oscillations) is greatly reduced with respect to Fig. 3b. See text for details

At this juncture it should be noted that the prey countermeasures suggested in Sect. 3.6, Eqs. 32a–e, do not include a decoy effect. Collisions between Y_{free} and X_1^* are not taken into account. It is expected that these would also lead to a decoy effect, further stabilizing the ecosystem and damping out oscillations.

4.3 Protector Species

The protector species effect is mainly known from nesting colonies of birds (Pius and Leberg 1998), in which a smaller, less aggressive species benefits from the presence of larger, more aggressive birds in the colony, if these latter (1) do not attack the smaller species and (2) are better at driving off potential predators than the smaller species. Mathematical models seem to be singularly lacking in this context, despite the fact that several of the above models could be adapted easily, by letting G depend on the density of the protector P, e.g.

$$G(X,Y) = \frac{gXY}{k_1 + X + k_2 P}\,, \tag{37}$$

which is yet again a form of competitive inhibition, similar to Eq. 15 or the decoy effect according to Eq. 34. Alternatively, the protector species effect may take a form similar to the Hassel and Varley (1969) or Arditi and Akçakaya

(1990) models, i.e.

$$G(X,Y) = \frac{gP^{-m}XY}{k_1 + P^{-m}X} . \tag{38}$$

I am not aware of any literature that describes this effect in microbes, and yet it is possible to imagine a similar effect happening in microbes. Suppose a third species produces a bacteriocin to which the predator is susceptible, but the prey is not. In this case the bacteriocin-producing species would act as protector species, albeit indirectly through the inhibitory or even bactericidal action of the bacteriocin. ODEs to model bacteriocins have been proposed by Frank (1994). Note that the phrase "protector species" is also used in a different context (Fisher and Freedman 1991), for which mathematical models do exist. In the case of Fisher and Freedman, no predator is modelled; rather, the protector species protects the environment of some other species, which in turn provides some sustenance to the protector.

5 Conclusions

Mathematical modelling of ecosystems, or even just single organisms, may seem a daunting task given the complexity of such systems compared to many systems in, e.g., physics. However, it is the very complexity of these biological systems which makes modelling an essential tool for their understanding. Highly complex systems consisting of many, much simpler, interacting units can be simulated with comparative ease on modern computers.

Fortunately, modelling predator–prey dynamics is a well-established field, and many effects have been studied. Furthermore, microbial predator–prey systems have many advantages compared to others, due to the short time scale at which dynamics such as oscillations occur, the small spatial extent and the degree of control, quite apart from the absence of ethical problems. As many authors have pointed out, chemostats offer an ideal system to observe the dynamics, and more importantly perform parameter estimation (Levin et al. 1977; Chao et al. 1977; Gerritse et al. 1992; Koch 1998; Kooi and Kooijmans 1994a). Many protist–bacterium, and bacteriophage–bacterium systems have been studied using such systems. Once parameters have been determined, they can be used to model the behaviour in real ecosystems with spatial extent (Jahnke et al. 1982; de Wit et al. 1995; Wilkinson 2002).

By contrast, mathematical modelling of predatory prokaryotes is in many ways still in its infancy. Very few articles provide models solely intended for these organisms (Marchand and Gabignon 1981; Dulos and Marchand 1984; Wilkinson 2001). On the other hand, the results from many other predator–prey models can be applied to these systems without major modifications.

Furthermore, many models already applied to bacteria, such as the bacteriocin model of Frank (1994) or Wilkinson (2002), can be adapted to model prokaryote predators.

One of the problems is the difficulty experienced in culturing many predatory prokaryotes in the laboratory (Martin 2002). Once these problems have been overcome, it should be possible to compare models, which are often easy enough to draw up, to the real dynamics observed in, e.g., a chemostat. Parameters estimated from such experiments could then be used to model the impact of these predators on, e.g., biofilm communities.

However, even without exact parameter estimates, strategic modelling can be used to gain some insight into the potential interactions. This is illustrated by the discussion on wolf-pack behaviour, modelled through Eqs. 24a–e. Even without real parameter estimates, we can determine that the two-species equilibrium in this system is so inherently unstable that the coexistence of two species is impossible. Therefore wolf-pack behaviour is unlikely to occur in obligate predators. In a way, we can consider mathematical modelling as a rigorous form of performing thought experiments in systems which are too complex to understand, or which exhibit counterintuitive behaviour. Many papers on microbial ecology describe the ecological effects of different parameters in the system qualitatively (Alexander 1981; Mallory et al. 1983; Shemesh and Jurkevitch 2004; Yair et al. 2003). I would not wish to claim these are at all wrong, especially when based on observation, or that we do not need a qualitative description. However, mathematical models can serve as a necessary "sanity check", if nothing else. They can also predict both the magnitude of the effects proposed and precisely under which conditions the effect should occur. Only with such quantitative predictions can we validate or invalidate our theories.

In this chapter a few new models have been put forward to model different types of predatory prokaryotes. Within the scope of this chapter it is impossible to analyse each of these models in detail, let alone provide a thorough validation. This must be left for future work. It might be objected that many of these models are somewhat speculative, and in some sense they are unashamedly so. However, the "speculations" made in this chapter do have a "mathematical backbone" which will help people to design experiments to prove the speculations right or wrong: if the latter, we will have to speculate anew.

I hope this chapter has served to illustrate some of the many factors which may complicate predator–prey dynamics. Many of these effects could occur in predatory prokaryotes, and drawing up suitable models is not very difficult. What is more difficult, and where many research opportunities lie, is in model validation and parameter estimation. Close collaboration between theoreticians and experimentalists in this area could lead to many new results and, of course, new questions.

References

Alander M, De Smet I, Nollet L, Verstraete W, von Wright A, Mattila-Sandholm T (1999) The effect of probiotic strains on the microbiota of the simulator of the human intestinal microbial ecosystem (SHIME). Int J Food Microbiol 46:71–79

Alexander M (1981) Why microbial predators and parasites do not eliminate their prey and hosts. Annu Rev Microbiol 25:113–133

Arditi R, Akçakaya HR (1990) Underestimation of mutual interference of predators. Oecologia 83:358–361

Arditi R, Callois JM, Tyutyunov Y, Jost C (2004) Does mutual interference always stabilize predator–prey dynamics? A comparison of models. C R Biol 327:1037–1057

Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2004) Bacterial persistence as a phenotypic switch. Science 305:1622–1625

Ballyk M, Smith H (1999) A model of microbial growth in a plug flow reactor with wall attachment. Math Biosci 158:95–126

Ballyk M, Jones DA, Smith HL (2001) Microbial competition in reactors with wall attachment: a mathematical comparison of chemostat and plug flow models. Microb Ecol 41:210–221

Ben-Jacob E, Cohen I, Shochet O, Tenenbaum A, Czirók A, Vicsek T (1995) Cooperative formation of chiral patterns during growth of bacterial colonies. Phys Rev Lett 75:2899–2902

Beddington JR (1975) Mutual interference between parasites or predators and its effect on search efficiency. J Anim Ecol 45:331–340

Beveridge TJ (1999) Structures of Gram-negative cell walls and their derived membrane vesicles. J Bacteriol 181:4725–4733

Bohannan BJM, Lenski RE (1997) Effect of resource enrichment on a chemostat community of bacteria and bacteriophage. Ecology 78:2303–2315

Bulmer M (1994) Theoretical evolutionary ecology. Sinauer, Sunderland, MA

Burnham JC, Collart SA, Highison BW (1981) Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of *Myxococcus xanthus* PCO2. Arch Microbiol 129:285–294

Button DK (1991) Biochemical basis for whole-cell uptake kinetics: specific affinity, oligotrophic capacity, and the meaning of the Michaelis–Menten constant. Appl Environ Microbiol 57:2033–2038

Cain CC, Lee D, Waldo RH, Henry AT, Casida EJ, Wani MC, Wall ME, Oberlies NH, Falkinham JO (2003) Synergistic antimicrobial activity of metabolites produced by a nonobligate bacterial predator. Antimicrob Agents Chemother 47:2113–2117

Campbell A (1961) Conditions for the existence of bacteriophage. Evolution 15:153–165

Canale RP (1969) Predator–prey relationships in a model for the activated sludge process. Biotech Bioeng XI:887–907

Casida LE Jr, Lukezic FL (1992) Control of leaf spot diseases of alfalfa and tomato with application of the bacterial predator *Pseudomonas* strain 679-2. Plant Dis 76:1217–1220

Chao L, Levin BR (1981) Structured habitats and the evolution of anticompetitor toxins in bacteria. Proc Natl Acad Sci USA 78:6324–6328

Chao L, Levin BR, Stewart FM (1977) A complex community in a simple habitat: an experimental study with bacteria and phage. Ecology 58:369–378

Christensen NO, Nansen P, Frandsen F (1976) Molluscs interfering with the capacity of *Fasciola hepatica* miracidia to infect *Lymnaea trunculata*. Parasitology 73:161–167

de Wit R, van den Ende FP, van Gemerden H (1995) Mathematical simulation of the interactions among cyanobacteria, purple sulfur bacteria, and chemotrophic sulfur bacteria in microbial mat communities. FEMS Microbiol Ecol 17:117–136

DeAngelis DL, Goldstein RA, O'Neill RV (1975) A model for trophic interaction. Ecology 56:881–892

DeAngelis DL (1992) Dynamics of nutrient cycling and food webs. Chapman & Hall, London

Deng B, Jessi S, Ledder G, Rand A, Srodulski S (2003) Biological control does not imply paradox—a case against ratio-dependent models. Discussion Paper, University of Nebraska-Lincoln

Dial BE, Fitzpatrick LC (1983) Lizard tail autotomy: function and energetics of postautotomy tail movement in *Scincella lateralis*. Science 219:391–393

Dockery J, Klapper I (2001) Finger formation in biofilm layers. SIAM J Appl Math 62:853–869

Donelson JE, Hill LH, El-Sayed NMA (1998) Multiple mechanisms of immune evasion by African trypanosomes. Mol Biochem Parasitol 91:51–66

Drutz DJ (1976) Response of *Neisseria gonorrhoea* to *Bdellovibrio* species. Infect Immun 13:247–251

Dulos E, Marchand A (1984) Oscillations des densites de population du couple bacterien proie–predateur *Escherichia coli–Bdellovibrio bacteriovorus*: etude experimentale et modele theorique. Ann Microbiol (Paris) 135A(2):271–295

Esteve I, Gaju N (1999) Bacterial symbioses: predation and mutually beneficial associations. Int Microbiol 2:81–86

Fisher ME, Freedman HI (1991) A model of environmental protection by a mutualist. Ecol Model 58:119–139

Ford RM, Cummings PT (1998) Mathematical models of bacterial chemotaxis. In: Koch AL, Robinson JA, Milliken GA (eds) Mathematical modeling in microbial ecology. Chapman & Hall, New York, pp 228–269

Forde SE, Thompson JN, Bohannan BJ (2004) Adaptation varies through space and time in a coevolving host–parasitoid interaction. Nature 431:841–844

Frank SA (1994) Spatial polymorphism of bacteriocines and other allelopathic traits. Evol Ecol 8:369–386

Fratamico PM, Whiting RC (1995) Ability of *Bdellovibrio bacteriovorus* 109J to lyse gram-negative food-borne pathogenic and spoilage bacteria. J Food Protect 58:160–164

Gerritse J, Schut F, Gottschal JC (1992) Modelling of mixed chemostat cultures of an aerobic bacterium *Comamonas testosteroni*, and an anaerobic bacterium *Veillonella alcalescens*: comparison with experimental data. Appl Environ Microbiol 58:1466–1476

Gibson GR, Wang X (1994) Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. FEMS Microbiol Lett 118:121–127

Gottschal JC (1993) Growth kinetics and competition—some contemporary comments. Antonie van Leeuwenhoek 63:299–313

Grover JP (1988) Dynamics and competition in a variable environment: experiments with two diatom species. Ecology 69:408–417

Grover JP (1990) Resource competition in a variable environment: phytoplankton growing according to Monod's model. Am Nat 136:771–89

Guerrero R, Pedros-Alio C, Esteve I, Mas J, Chase D (1986) Predatory prokaryotes: predation and primary consumption evolved in bacteria. Proc Natl Acad Sci USA 83:2138–2142

Harmon JP, Andow DA (2003) Alternative foods as a mechanism to enhance a generalist ladybird's predation of a target prey. Proceedings of the first international symposium on biological control of arthropods, Honolulu, Hawaii, pp 244–249

Hassel MP, Varley GC (1969) New inductive population model for insect parasites and its bearing on biological control. Nature 223:1133–1137

Hermanowicz SW (1998) Model of two-dimensional biofilm morphology. Water Sci Technol 37:219–222

Hofbauer J, Sigmund K (1998) Evolutionary games and population dynamics. Cambridge University Press, Cambridge, UK

Holling CS (1959) Some characteristics of simple types of predation and parasitism. Can Entomol 91:385–398

Itoh K, Freter R (1989) Control of *Escherichia coli* populations by a combination of indigenous clostridia and lactobacilli in gnotobiotic mice and continuous-flow cultures. Infect Immun 57:559–565

Iwasa Y, Nakamaru M, Levin SA (1998) Allelopathy of bacteria in a lattice population: competition between colicin-sensitive and colicin-producing strains. Evol Ecol 12:785–802

Jackson L, Whiting RC (1992) Reduction of an *Escherichia coli* K12 population by *Bdellovibrio bacteriovorus* under various in vitro conditions of parasite:host ratio, temperature, or pH. J Food Protect 55:859–861

Jahnke RA, Emerson SR, Murray JW (1982) A model for oxygen reduction, denitrification, and organic matter mineralization in marine sediments. Limnol Oceanogr 27:610–630

Jones DA, Smith H (2000) Microbial competition for nutrient and wall sites in plug flow. SIAM J Appl Math 60:1576–1600

Jost JL, Drake JF, Tsuchiya HM, Frederickson AG (1973) Microbial food chains and food webs. J Theor Biol 41:461–484

Jurkevitch E, Davidov Y (2006) Phylogenetic diversity and evolution of predatory prokaryotes. Micobiol Monogr, vol 4. Springer, Berlin Heidelberg New York

Kamerman DJ, Wilkinson MHF (2002) In silico modelling of the human intestinal microflora. In: Proceedings of the international conference on computational science (ICCS 2002). Springer, Berlin Heidelberg New York, pp 117–126

Kawasaki K, Mochizuki A, Matsushita M, Umeda T, Shigesada N (1997) Modelling spatiotemporal patterns generated by *Bacillus subtilis*. J Theor Biol 188:177–185

Koch AL (1982) Multistep kinetics: choice of models for growth of bacteria. J Theor Biol 98:401–417

Koch AL (1997) Microbial physiology and ecology of slow growth. Microbiol Mol Biol Rev 61:305–318

Koch AL (1998) The Monod model and its alternatives. In: Koch AL, Robinson JA, Milliken GA (eds) Mathematical modeling in microbial ecology. Chapman & Hall, New York, pp 62–93

Koch AL, Robinson JA, Milliken GA (1998) Mathematical modeling in microbial ecology. Chapman & Hall, New York

Kooi BW, Kooijman SALM (1994a) Existence and stability of microbial prey–predator systems. J Theor Biol 170:75–85

Kooi BW, Kooijman SALM (1994b) The transient behaviour of food chains in chemostats. J Theor Biol 170:87–94

Kooijman SALM (1993) Dynamic energy budgets in biological systems. Cambridge University Press, Cambridge, UK

Levin BR, Stewart FM, Chao L (1977) Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage. Am Nat 111:3–24

Levins D (1968) Evolution in a changing environment. Princeton University Press, Princeton

Lin D, McBride MJ (1996) Development of techniques for the genetic manipulation of the gliding bacteria *Lysobacter enzymogenes* and *Lysobacter brunescens*. Can J Microbiol 42:896–902

Lotka AJ (1925) Elements of physical biology. William and Wilkins, Baltimore

Mallory LM, Yuk CS, Liang LN, Alexander M (1983) Alternative prey: a mechanism for elimination of bacterial species by protozoa. Appl Environ Microbiol 46:1073–1079

Marchand A, Gabignon O (1981) Modèle théoretique de la cinétique d'interaction du couple proie–prédateur *Bdellovibrio bacteriovorus–Escherichia coli*. Ann Microbiol (Paris) 132B(3):321–326

Martin MO (2002) Predatory prokaryotes: an emerging research opportunity. J Mol Microbiol Biotechnol 4:467–477

Mashburn LM, Whiteley M (2005) Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature 437:422–425

McGlade J (1999) Advanced ecological theory. Blackwell Science, Oxford, UK

Mizoguchi K, Morita M, Fischer CR, Yoichi M, Tanji Y, Uno H (2003) Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. Appl Environ Microbiol 69:170–176

Monod J (1950) La technique de culture continue, théorie et applications. Ann Inst Pasteur 79:390–410

Neuhauser C, Fargione JE (2004) A mutualism–parasitism continuum model and its application to plant–mycorrhizae interactions. Ecol Model 177:337–352

Nisbet RM, Cunningham A, Gurney WSC (1983) Endogenous metabolism and the stability of microbial prey–predator systems. Biotech Bioeng XXV:301–306

Palm WJ III (2005) Introduction to MATLAB 7 for engineers. McGraw-Hill, Boston, pp 465–532

Payne RJH, Jansen VAA (2001) Understanding phage therapy as a density-dependent kinetic process. J Theor Biol 208:225–230

Pius SM, Leberg PL (1998) The protector species hypothesis: do black skimmers find refuge from predators in gull-billed tern colonies? Ethology 104:273–284

Press WH, Flannery BP, Teukolsky SA, Vetterling WT (1986) Numerical recipes. Cambridge University Press, Cambridge, UK

Punzo F (1997) Leg autotomy and avoidance behavior in response to a predator in the wolf spider, *Schizocosa avida* (Aranae, Lycosidae). J Arachnol 25:202–205

Ramasamy R (1998) Molecular basis for evasion of host immunity and pathogenesis in malaria. Biochim Biophys Acta 1406:10–27

Riley MA, Gordon DM (1996) The ecology and evolution of bacteriocins. J Ind Microbiol 17:151–158

Sarkar BL, Chakrabarti AK, Koley H, Chakrabarti MK, De SP (1996) Biological activity and interaction of *Vibrio cholerae* bacteriophages in rabbit ileal loop. Indian J Med Res 104:139–141

Shemesh Y, Jurkevitch E (2004) Plastic phenotypic resistance to predation by *Bdellovibrio* and like organisms in bacterial prey. Environ Microbiol 6:12–18

Smith HW, Huggins MB (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J Gen Microbiol 129:2659–2675

Tan Y, Wang Z-X, Marshall KC (1996) Modeling substrate inhibition of microbial growth. Biotech Bioeng 52:602–608

Tyson R, Lubkin SR, Murray JD (1999) A minimal mechanism for bacterial pattern formation. Proc R Soc Lond B 266:299–304

Van Loan CF (1997) Introduction to scientific computing. Prentice-Hall, Upper Saddle River, NJ, p 308–340

Volterra V (1926) Fluctuations in the abundance of species, considered mathematically. Nature 118:558–560

Vos M, Moreno-Berrocal S, Karamaouna F, Hemerik L, Vet LEM (2001) Plant-mediated indirect effects and the persistence of parasitoid–herbivore communities. Ecol Lett 4:38–45

Weld RJ, Butts C, Heinemann JA (2004) Models of phage growth and their applicability to phage therapy. J Theor Biol 227:1–11

Westergaard JM, Kramer TT (1977) *Bdellovibrio* and the intestinal flora of vertebrates. Appl Environ Microbiol 34:506–511

Wilder JW, Vasquez DA, Christie I, Colbert JJ (1995) Wave trains in a model of gypsy moth population dynamics. Chaos 5:700–706

Wilkinson MHF (2001) Predation in the presence of decoys: an inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems. J Theor Biol 208:27–36

Wilkinson MHF (2002) Model intestinal microflora in computer simulation: a simulation and modelling package for host–microflora interactions. IEEE Trans Biomed Eng 49:1077–1085

Wilkinson MHF (2003) Decoys in predation and parasitism. Comments Theor Biol 8:321–338

Yair S, Yaacov D, Susan K, Jurkevitch E (2003) Small eats big: ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator–prey systems. Agronomie 23:433–439

Yousif F, El-Emam M, El-Sayed K (1998) Effect of six non-target snails on *Schistosoma mansoni* miracidial host finding and infection of *Biomphalaria alexandrina* under laboratory conditions. J Egypt Soc Parasitol 28:559–568

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_2006_055/Published online: 11 November 2006

Bdellovibrio and Like Organisms: Potential Sources for New Biochemicals and Therapeutic Agents?

Eckhard Strauch (✉) · Sebastian Beck · Bernd Appel

Bundesinstitut für Risikobewertung (BfR), Federal Institute for Risk Assessment, Diedersdorfer Weg 1, 12277 Berlin, Germany
eckhard.strauch@bfr.bund.de

Abstract *Bdellovibrio* and like organisms (BALOs) are predatory bacteria capable of invading the periplasm of Gram-negative bacteria and of growing and replicating within this protected niche. Research dedicated to studying the sophisticated weaponry of these predators aims to find novel strategies for combating pathogenic bacteria as the worldwide increase of pathogens resistant to a wide range of antibiotics forces a search for alternative antimicrobial substances to counter this threat.

The physiology of BALOs will be the main focus of this chapter, and some potential applications for BALOs will be discussed. However, our current knowledge of the amazing

biology of these extraordinary prokaryotes that possess an astonishing predatory lifestyle and perform a well-organized deconstruction of prey bacteria is still rather limited. The great advances in proteomic and genomic techniques will allow the investigation of the interaction between predators and prey, lately supported by the availability of the genome sequence of one *B. bacteriovorus* reference strain. It seems likely that the exploitation of the unique weaponry of these bacteria will enable researchers to find new biochemicals and—perhaps—therapeutic agents.

1 Introduction

B. bacteriovorus was discovered in experiments designed for the isolation of bacteriophages from soil samples using the common double layer plate technique. In contrast to phage plaques that appear during the logarithmic growth of susceptible bacteria, plaques were discovered on a bacterial lawn that developed 2 to 3 d after the onset of the experiment and increased slowly in size during the course of 1 week (Stolp and Petzold 1962; Stolp and Starr 1963. See also the introductory chapter of this volume). The microscopic observation of these plaques revealed the presence of small motile bacteria, which were later shown to cause the lysis of the prey bacteria. Further predatory strains were obtained from soil, sewage and aquatic environments, e.g. the rhizosphere of plants, saltwater samples, freshwater habitats and, recently, even from the gut of animals (Taylor et al. 1974; Marbach et al. 1976; Williams and Falkler 1984; Richardson 1990; Ravenschlag et al. 1999; Jurkevitch et al. 2000; Schwudke et al. 2001; Snyder et al. 2002; Kleessen et al. 2003; Pineiro et al. 2004). These predators prey on a wide variety of Gram-negative bacteria, whereas they fail to grow on Gram-positive bacteria.

Originally, all isolates were included in the one genus *Bdellovibrio*. However, more detailed investigations revealed a taxonomically diverse group of bacteria: 16S rRNA analyses and DNA–DNA hybridization studies were performed and recently two new genera were introduced, *Bacteriovorax* and *Peredibacter* (Baer et al. 2000, 2004; Schwudke et al. 2001; Snyder et al. 2002; Davidov and Jurkevitch 2004). These two genera form the family Bacteriovoracaceae, while *B. bacteriovorus* belongs to the family Bdellovibrionaceae. The two families are found under the order Bdellovibrionales. Obligate predatory bacteria are now called the *Bdellovibrio* and like organisms (BALOs).

B. bacteriovorus is the best-characterized member of the Bdellovibrionales. Wild type strains possess an obligate predatory lifecycle consisting of a free living attack phase and of an intracellular growth and replication phase within the periplasm of prey bacteria, which is terminated by lysis of the latter and release of newly differentiated attack-phase bacteria. Based on microscopic investigations, the lifecycle of *B. bacteriovorus* has been divided into eight stages (Seidler and Starr 1969; Rendulic et al. 2004, see Fig. 1 in chapter by Tudor and McCann). At least one strain, *Bdellovibrio* sp. W, features

a variation of this theme, as this strain is able to produce resting cells termed bdellocysts within the bdelloplast. Bdellocysts possess enhanced resistance to high temperatures, desiccation and disruption (Burger et al. 1968; Hoeniger et al. 1971).

The recent publication of the genome sequence of a *B. bacteriovorus* strain (HD100) has opened the way to new research opportunities. Further genome-sequencing projects on *Bacteriovorax marinus* and *Bdellovibrio* sp. W are in progress (see chapter by Tudor and McCann). The majority of the early studies were performed on strain *B. bacteriovorus* 109J and its derivatives. As the 16S rRNA sequences of HD100 and 109J are identical, a close relationship between both strains seems likely.

With the availability of the genome sequence of *B. bacteriovorus* HD100, a discussion was initiated about the use of predatory bacteria as a kind of "living antibiotic" to reduce pathogenic bacteria within an infected mammalian host (Rendulic et al. 2004; Sockett and Lambert 2004). Based on the current knowledge of the biology of *B. bacteriovorus* and accounting for the many open questions concerning its lifestyle, this chapter will also address a therapeutic perspective.

2
Free-Living BALOs

In their free-living phase BALOs are small vibrioid to rod-shaped Gram-negative bacteria (0.2–0.5 μm wide, 0.5–2.5 μm long) that move with a high velocity, propelled by a single sheathed flagellum. The estimated speed, in the case of *B. bacteriovorus*, is about 100 cell lengths per second, which is about 10 times the speed of *E. coli* cells (Rittenberg 1983). BALOs are aerobic and mesophilic bacteria, which can respire a variety of compounds, including amino acids and acetate (Rittenberg 1983). However, growth and replication of wild type predators can only take place in the presence of prey bacteria. Thus, BALOs are obligate predatory prokaryotes.

2.1
Prey Location: Chemotaxis Versus Random Collision

The high metabolic activity caused by the rapid movement and the requirement for organic substrates for growth make it important that the predators find prey bacteria in order to avoid starvation and death. It has been estimated that free-living predators only have a few hours to locate prey bacteria before depletion of the limited energy reserves leads to cell death (Gray and Ruby 1991). The free-living bacteria are therefore always in search of putative victims and are permanently in an attack phase. For this reason, any mechanism for putative prey localization would be favourable for the preda-

tors. Research on chemotaxis as a mechanism of prey detection has been performed; however, the role of chemical detection in directing movement towards prey cells remains uncertain. Several studies conducted by one research group with attack-phase predators (*B. stolpii* and *B. bacteriovorus*) demonstrated relatively weak directed motility (in comparison to other bacteria) towards respirable substrates, specific amino acids and other organic compounds (Straley and Conti 1974; LaMarre et al. 1977; Straley et al. 1979). However, no evidence of specific motility towards prey bacteria was found (Straley and Conti 1977). It was proposed that positive chemotaxis of BALOs could enable the detection of ecological niches that might be rich in prey organisms, rather than the prey cells themselves. A minor role for chemotaxis in predation by *B. bacteriovorus* seems feasible, as a recent study indicated the involvement of a methyl-accepting chemotaxis protein (MCP) in *B. bacteriovorus* 109J prey cell attack (Lambert et al. 2003). Strain *B. bacteriovorus* HD100 has 20 MCP genes and a chemotatic machinery for signalling environmental changes to the flagellar motor. Aerotaxis of BALOs towards increased oxygen concentrations was clearly demonstrated (Straley et al. 1979). Genes coding for proteins possibly involved in oxygen sensing were found in the genome sequence of strain HD100 (Rendulic et al. 2004), but whether these findings can be linked to a general mechanism of prey location awaits experimental confirmation.

Most of the research addressing prey location performed in the first decades after isolation of these predatory bacteria concluded that the encounter between prey and predator resulted from random collisions (Varon and Shilo 1980). This means that the chances for collision between predator and prey are directly dependent on the cell density of both and can be explained by mathematical models (Wilkinson 2001, and his chapter in this book). By studying the predator–prey interaction using a marine *Bdellovibrio* strain and the luminous prey *Photobacterium leiognathi*, the extinction of bioluminescence was taken as an indicator for the number of successful encounters between the bacteria. The bioluminescence decay rate was dependent on the predator-to-prey ratio and the prey cell density. Upon dilution of the prey population, a higher predator-to-prey ratio was required to obtain the same decay ratio (Varon and Shilo 1980).

2.2 Cell-Wall Compounds

The first contact between predator and prey occurs through surface structures of the cell envelope. Therefore, the outer membrane of BALOs has been the focus of several studies. For a long time *B. bacteriovorus* was thought to reutilize unaltered and unmodified prey cell constituents for the integration into its own cell wall (Nelson and Rittenberg 1981; Guerrini et al. 1982; Diedrich et al. 1983, 1984; Talley et al. 1987; Diedrich 1988; Stein et al. 1992).

In these publications, the transfer of prey cell outer membrane components, such as lipid A, as well as complete lipopolysaccharides (LPS) and outer membrane proteins (Omps) was described. The relocation of cell components was thought to show the high metabolic efficiency of the predators, thus achieving a faster growth rate. Recent studies on the LPS and Omps of *B. bacteriovorus* strains have demonstrated that *B. bacteriovorus* strains possess cell walls with unusual structural features, which suggests that the predators synthesize their own innate membrane rather than reutilize prey components.

It was shown that the LPS anchor group lipid A possesses a unique structure among other lipid As in the microbial world (Schwudke et al. 2003). Lipid A from the wild type *B. bacteriovorus* HD100 and from the prey-independent strain HI100 consist of a β-(1→6)-linked 2,3-diamino-2,3-dideoxy-D-glucopyranose disaccharide carrying six hydroxylated, mostly branched fatty acids. In place of phosphate groups at the O-1 of the reducing and at the O-4′ of the non-reducing end, α-D-mannopyranose residues were found to be present in the sugar backbone (Fig. 1). Thus, both structures represent the first lipid As described so far completely missing negatively

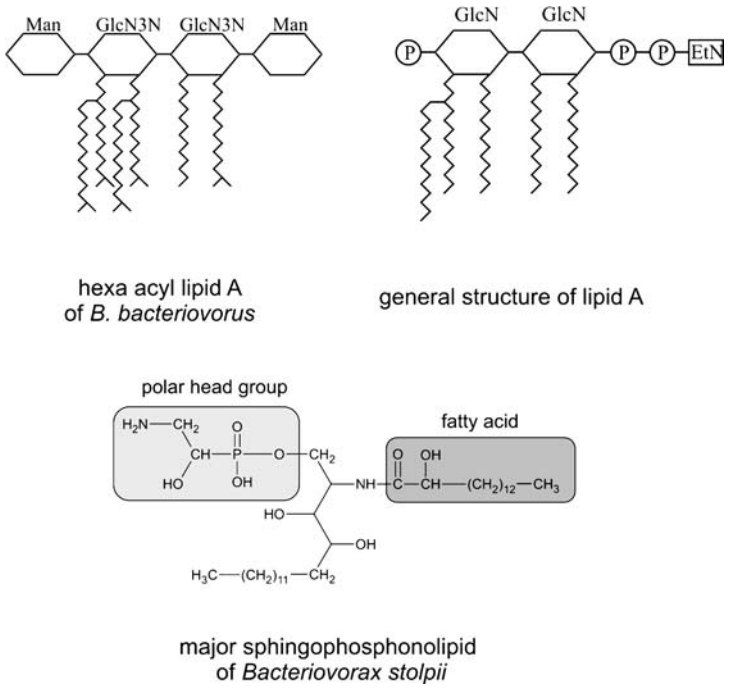


Fig. 1 *Top*: Uncharged lipid A of *B. bacteriovorus* HD100 compared to lipid A of Gram-negative bacteria. (Man: α-D-mannopyranose; GlcN3N: 2,3-diamino-2,3-dideoxy-D-glucopyranose; P: phosphate group, Glc: glucosamine; EtN: ethanolamine). *Bottom*: sphingophosphonolipid of *B. stolpii*

charged groups. Cytokine induction from human macrophages revealed that the *B. bacteriovorus* lipid A and LPS possessed a significantly reduced endotoxic activity compared to lipid A or LPS from other Gram-negative bacteria. Another conclusion of the detailed LPS analyses was that the wild type *B. bacteriovorus* synthesizes an innate LPS and does not integrate prey cell LPS into its outer membrane.

In earlier studies it was also claimed that *B. bacteriovorus* has the ability to reutilize prey cell porins. Relocation of *E. coli* OmpF into the predator's outer membrane was claimed (Diedrich 1988, and literature cited therein; Stein et al. 1992). However, another study refuted the transfer of OmpF to the outer membrane of the predator and showed that intraperiplasmic *B. bacteriovorus* synthesized its own OmpF-like membrane protein (Rayner et al. 1985). In later studies, translocation of the *B. bacteriovorus* OmpF-like protein into the cytoplasmic membrane of prey was reported (McCann et al. 1998; Tudor and Karp 1994). Two recent studies using mass spectrometry and reverse genetics clearly demonstrated that different *B. bacteriovorus* strains produce a highly abundant innate Omp, whereas no evidence for an Omp relocation was found (Beck et al. 2004; Barel et al. 2005). Protein data from these studies clearly suggested that the major Omp of *B. bacteriovorus* is the OmpF-like protein described in earlier studies (Rayner et al. 1985; Tudor and Karp 1994; McCann et al. 1998). The polypeptide was also found to be associated with membranes in prey ghosts (Barel et al. 2005). Analyses of outer membrane fractions of more BALOs outside the species *B. bacteriovorus* confirmed that related Omps are widely distributed in *Bdellovibrionales* (Beck et al. 2005; Schwudke et al. 2005).

2.3 Role of Shingolipids in Predation?

An interesting observation concerning cell-wall lipids was reported for *Bacteriovorax stolpii* UKi2. The predatory wild type strain produces the common bacterial phospholipids phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) as major glycerophosphatides and, additionally, three unexpected lipids (Diedrich et al. 1970), which turned out to be sphingophosphonolipids. One of these compounds was further characterized to be *N*-2′-hydroxypentadecanoyl-2-amino-3,4-dihydroxyheptadecan-1-phosphono-(1-hydroxy-2-aminoethane) in 2001 (Fig. 1, Watanabe et al. 2001). Sphingolipids are common to eukaryotic cells and are rarely found in bacteria. In mammalian cells they play an important role in transmembrane signalling (Waggoner et al. 1999). Furthermore, sphingolipids arranged in lipid rafts hold a key position for the invasion of mammalian cell by pathogens but also inhibit their invasion (Gulbins et al. 2004). It was suggested that the presence of sphingophosphonolipids may play a role in attack-phase *B. stolpii* Uki2 (Steiner et al. 1973), as saprophytic mutants of the strain able to grow

without prey bacteria lacked sphingolipids. However, as the phospholipid content of predators has only been investigated in *B. stolpii* so far, this suggestion is still speculative and further research on more strains must be performed.

Nevertheless, predatory BALOs need to possess special structures for prey recognition that are likely associated with the cell surface. So far, the cell-wall components investigated in detail have revealed novel structures, and it should be the aim of future research to assign functions for these structures in the predator–prey interaction.

3 Morphological and Biochemical Events During Attachment and Invasion

Predators attach to the prey via the pole opposite to the flagellum (Fig. 2a). The initial attachment is reversible and does not involve specific structures. This was shown by the fact that BALOs can attach to Gram-positive, non-prey bacteria and even abiotic surfaces (e.g. glass) (Gray and Ruby 1991). An irreversible, productive attachment was estimated to occur in only 3 per 100 collisions with prey bacteria (Varon and Shilo 1980). However, the nature of the interaction between prey and predator remains unclear, as the existence of specific receptors or sites could not be unequivocally demonstrated (Gray and Ruby 1991).

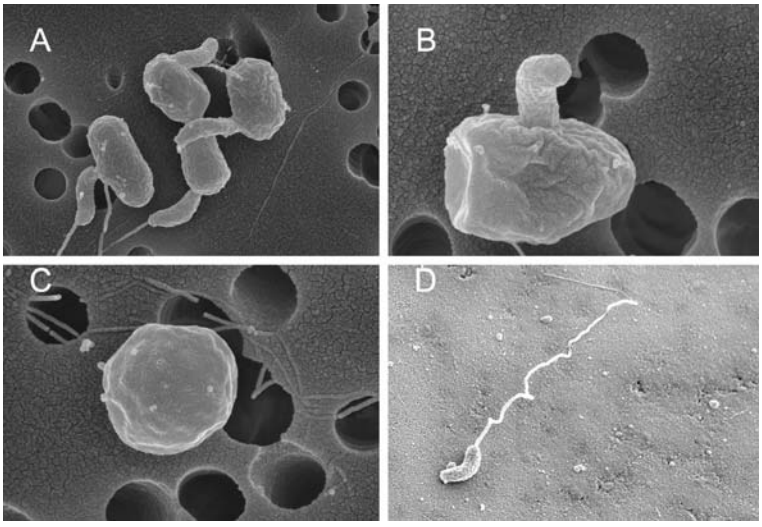


Fig. 2 Stages of predatory life cycle of *B. bacteriovorus* HD100 with prey *E. coli* K12: **a** Attack-phase predators. **b** Penetration of prey bacteria. **c** Bdelloplast containing intracellularly growing *B. bacteriovorus* **d** Released predator

3.1
Role of Prey Structures in Recognition

In one study, the attachment of predatory strains with a wide prey range (including *Enterobacteriaceae* and *Pseudomonadaceae*) to cell-wall mutants of *E. coli* K-12 and *Salmonella enterica* Typhimurium LT2 were investigated (Schelling and Conti 1986). As both rough and smooth strains of *Salmonella* were susceptible to predation, the receptor sites involved in attachment were thought to reside in the LPS core. Sequential deletion of sugar residues from the LPS core of *S. enterica* and inhibition studies with free sugars reduced the number of attached bacteria in the case of *B. bacteriovorus*. However, upon prolonged incubation, even the deep rough strain *S. enterica* SL1102 (heptoseless mutant) was penetrated. Attachment of *B. bacteriovorus* to wild type *E. coli* K-12 and mutants lacking several Omps occurred with the same efficiency. In contrast, *B. stolpii* was described to recognize the major outer membrane porins OmpF and OmpC to a certain degree but did not recognize differences in LPS.

So far, studies to determine receptor sites on the cell surface of prey bacteria did not give conclusive results. A study designed to use the prey range as taxonomic marker revealed that cultural conditions are critical for prey recognition and dismissed this phenotype as a taxonomic marker (Torrella et al. 1978). The wide prey range of most predators among Gram-negative bacteria indicates that there might be common motifs present on the surface triggering the change from a reversible to an irreversible attachment. In a review, the controversial results on the subjects of receptor/recognition sites were summarized, and it was concluded that '*bdellovibrios are capable of responding to a variety of cell surface characteristics as a means of identifying suitable prey. This reinforces the idea that bdellovibrios possess only very general, though highly adaptable, abilities of prey recognition that might more accurately be described as simple environmental sensing mechanisms.*' (Gray and Ruby 1991). This statement is still valid today.

3.2
Penetration by Hydrolytic Enzymes, Involvement of Pili?

When attachment becomes irreversible, the predator swivels violently and forms a pore in the envelope of the prey bacterium. It enters its prey within about 10 min and sheds the flagellum in the process (our Fig. 2b; Rittenberg 1983). A prerequisite for penetration seems to be that the prey cytoplasmic membrane comes into contact with the invading predator. Prey cells with a cytoplasmic membrane separated from the cell wall by plasmolysis were not successfully penetrated. Electron microscopy showed that the attachment pole of the invader remained in firm contact with the prey cytoplasmic membrane throughout the penetration phase (Abram et al. 1974). After in-

vasion the pore seems to reseal, thus generating a confined niche for the predator.

Invasion requires a number of hydrolytic enzymes, as the predators locally lyse the outer membrane and peptidoglycan layer of the prey (Shilo 1969; Tudor et al. 1990). The genomic sequence of *B. bacteriovorus* HD100 revealed numerous putative genes encoding hydrolytic enzymes (e.g. peptidases/proteinases, glycanases, lipases, etc.) (Rendulic et al. 2004) (also see chapter by Tudor and McCann). This large number of candidate genes makes it difficult to assess the individual importance of a hydrolase, but transcription profiling and proteomic approaches would be a suitable approach to address this question. The locally restricted degradative activity of the invading predator might be explained by enzymes anchored at the attachment site of the cell membrane or by a localized release of such enzymes (Sockett and Lambert 2004).

The genome sequence also revealed the presence of at least four potential pilus gene clusters and numerous dispersed *pil* genes coding for type IV pili that might play a role in attachment and during the penetration process (Rendulic et al. 2004). Rigid, filament-like structures that might correspond to pili were reported at the attachment site (Shilo 1969), but their existence has been questioned by others (Moulder 1985). A transcriptional analysis of a putative pilin gene (*flp1*) that is part of a cluster for pilus formation near the *hit* locus (see below) revealed a high transcriptional activity in attack-phase predators. However, a proteomic approach to detect Flp1-derived peptides by mass spectrometric methods failed (Schwudke et al. 2005). Therefore, pilus-mediated entry of BALOs remains an attractive idea that is still awaiting experimental proof.

3.3 Remodelling the Prey Cell

With the invasion of a suitable prey cell the predators start to remodel their 'home', as the modification of the prey envelope structures has been described. In this context *B. bacteriovorus N*-deacylates the prey peptidoglycan, removes diamino-pimelic acid from it and attaches long-chain fatty acids to the murein (Thomashow and Rittenberg 1978a–c; Tudor et al. 1990). Several putative genes for membrane-bound and soluble lytic murein transglycosylases have been found in the genome and have been implicated in the invasion process (Rendulic et al. 2004). Furthermore, the bonds between the outer membrane and the peptidoglycan are simultaneously breached, as the degradation of the prey OmpA and the murein-lipoprotein (Lpp) in the case of *E. coli* and the OprF in the case of *Pseudomonas* species has been reported (Rittenberg 1983; Cover et al. 1984; Beck et al. 2004). The result of these activities is the morphological change of the invaded prey cells to bdelloplasts that are rounded spherical, osmotically stable structures (Figs. 2c

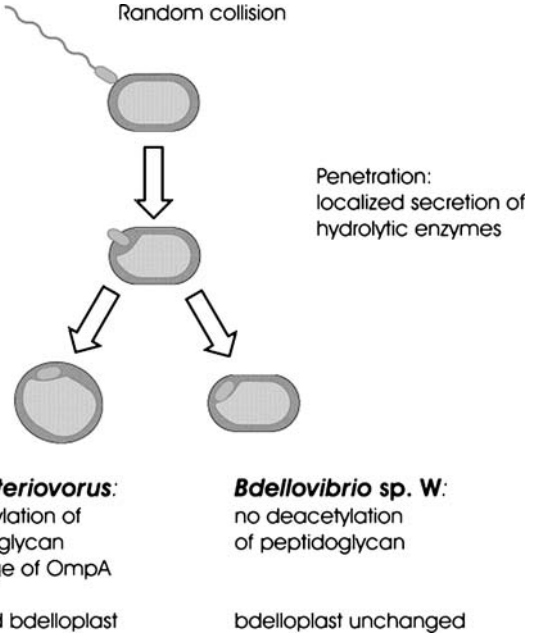


Fig. 3 Morphological and biochemical events in prey–predator interaction in attack and invasion phases

and 3), which provide the predator with an enlarged space for reproduction. In a synchronous culture it takes ca. 30 min to convert all substrate cells to bdelloplasts (Rittenberg, 1983). Based on the genome information aspartate, cysteine, serine and metallo proteases were suggested to be involved in prey cell penetration and establishment (Rendulic et al. 2004). The extensive modifications of the bdelloplast envelope were thought to prevent invasion by a second predator (Gray and Ruby 1991). In the cyst-forming *Bdellovibrio* sp. W deacetylation of the prey cell peptidoglycan does not occur and the invasion of prey cells does not lead to morphological alterations in bdelloplasts (Fig. 3). Nevertheless, the exclusion of secondary invaders was observed (Tudor et al. 1990; Gray and Ruby 1991).

4 Physiology of Intracellular Growth

Invasion of a prey bacterium reduces the oxygen supply of the predatory cell because conditions become microaerophilic. From the genome sequence data of *B. bacteriovorus* HD100, it seems likely that *B. bacteriovorus* can adapt well to this different situation, as the predator possesses cytochrome oxidases capable of oxygen binding under such conditions of reduced oxygen con-

centration. Probably also other substrates like nitric oxide or nitrite can be reduced for energy production (Sockett and Lambert 2004).

Within the bdelloplast a dramatic morphological change of the predator takes place. The short rod-shaped bacteria start to elongate into a filament that finally fills out the whole bdelloplast. This phase represents the actual growth of the predator cell. During this elongation phase, *B. bacteriovorus* incorporates prey cell components that have been digested by enzymatic activities. The incorporation of prey DNA and RNA nucleotides as well as fatty acids has been described (Matin and Rittenberg 1972; Hespell et al. 1975; Kuenen and Rittenberg 1975; Hespell and Odelson 1978). Interestingly, *B. bacteriovorus* HD100 only possesses biosynthesis pathways for 11 amino acids, while 10 amino acid degradation pathways are missing (Rendulic et al. 2004). However, alternative anabolic pathways are possible, at least for a few of these compounds (see chapter by Tudor and McCann). With such metabolic deficiencies, it is obvious that wild type BALOs are obligatorily prey dependent. The transport of substrates is probably achieved via the numerous predicted membrane transport systems: 244 such putative systems are found in the genome of *B. bacteriovorus* HD100. These either belong to the ATP-binding cassette or to the permease/major facilitator superfamily types. Utilization of prey compounds by bdellovibrios is astonishingly efficient. A mass balance shows that 50–55% of the substrate cell carbon is assimilated, 15% is respired and the remainder is discarded (Rittenberg 1983).

4.1 Prey Factor Requirement for Initiation of Growth and Elongation

The fact that wild type BALOs have an obligate requirement for a suitable prey in order to initiate growth has been addressed in a number of studies. Several experimental approaches have been taken to identify the growth initiation factors that induce reproduction. The first report was published in 1969 (Reiner and Shilo 1969) and described a prey-derived, heat-stable, DNAse-, RNAse- and pronase-resistant factor of a molecular weight of more than 50 kDa that was beneficial for extracellular growth of obligate prey-dependent strains of *B. bacteriovorus*. Further investigations described the growth of non-invading, prey-dependent predators by the addition of autoclaved Gram-negative as well as Gram-positive bacteria to the growth medium (Crothers et al. 1972; Huang and Starr 1973; Ross et al. 1974). Notably, Gram-positive cells are not suitable preys under predacious conditions. The contradictory results of various studies on this subject (summarized in Gray and Ruby 1991) left the nature of the prey-derived factors unclear. In a later study, axenic growth of *B. bacteriovorus* was stimulated by heat-shock, and it was concluded that heat shock had altered the transcription of one or more genes and had generated a signal normally derived from prey (Gordon et al. 1993). These observations have not been further pursued.

Additionally, elongation may require the continuous presence of prey-derived factors. Treatment with lytic enzymes normally produced by the predator at the end of a growth cycle induced the release of *B. bacteriovorus* from bdelloplasts at various stages of intracellular growth. This premature release prompted the predatory cells to differentiate into motile attack-phase cells upon completion of their previously initiated rounds of DNA replication, suggesting that certain substances are required to maintain intracellular growth (Ruby and Rittenberg 1983; Gray and Ruby 1989). As prey-derived factors are not limited to a single class of compounds, their identity remains unclear (Gray and Ruby 1990), and it is likely that more than one prey signal—perhaps a regulatory cascade—is necessary to commit predators to filamentous growth (Martin 2002).

4.2 Termination of Growth

Once the filament has reached a size several times that of the free-living predator, septation into daughter cells begins. The final length of the filament and, consequently, the number of progeny cells seem to be determined by the size of the prey cell. This was shown using *E. coli* K-12 Hfr strains that grow to variable lengths of up to 100 μm. The number of daughter cells obtained varied, from as few as 3 to 4 in small prey cells up to as many as 90 in filamentous, multinucleate *E. coli* (Kessel and Shilo 1976; Diedrich 1988). The dependence of growth duration on the size of the prey cell suggests that the filament extends until nutrient depletion and that differentiation into attack phase is initiated in response to starvation conditions.

After termination of elongation, the filament is multinucleate and cross-walls appear simultaneously and equidistantly in several places. The filament then septates into uniformly shaped progeny cells containing one nucleoid. Cell division has been explained by the accumulation of a division factor produced by the predator, which triggers septation after having reached a certain level—either in the filament or in the surrounding medium. This division factor, which was described as a small cyclic peptide, was not further characterized (Eksztejn and Varon 1977). The concept of an endogenously produced signal initiating the division into progeny cells, therefore, remains unproven.

With the onset of cell division, the morphological differentiation into attack phase predators and the production of lytic enzymes are initiated (Gray and Ruby 1991). Daughter cells are finally equipped with a flagellum, ready to be released to hunt down their next victims (Fig. 2d). It was proposed that the release is caused by hydrolytic enzymes produced by the predator that degrade the modified peptidoglycan layer from the inside of the infected prey cell (Rittenberg 1983; Tudor et al. 1990). Although candidate genes encoding such enzymes were identified (Rendulic et al. 2004), the presence of large amounts of prey cell LPS in *B. bacteriovorus* cultures (Schwudke et al.

2003) and the fact that prey cell envelopes can still be isolated after release (Beck et al. 2004; Barel et al. 2005) suggest that a putative LPSase activity is rather limited. This is in agreement with conclusions from early studies that LPSase activity is only locally expressed and is timely confined to the early attachment phase, when *B. bacteriovorus* drills a hole into the prey cell wall (Rittenberg 1983).

5
Growth in Natural Environments

The physiological deficiencies of BALOs and their high metabolic activity in the free-living state support that rapid encounters with prey are essential for survival. Most of the work on predatory bacteria has been performed under laboratory conditions optimized for research. It is clear that the commonly used physiological growth conditions, e.g. a temperature of 30 °C, high numbers of 'well-prepared' stationary-phase prey bacteria and optimal aerobic conditions, are very different from the natural environment. What happens if attack-phase predators are not supplied with prey and instead face starvation?

5.1
Prey Independency as a Survival Strategy

Under laboratory conditions, it was reported that spontaneously appearing derivatives were found that were able to grow on rich media in the absence of living prey (Stolp and Starr 1963). Such prey-independent mutants were reported to occur with a frequency of 10^{-6} to 10^{-7}, suggesting that they resulted from a single mutational event (Thomashow and Cotter 1992). Prey-independent mutants are difficult to interpret because their nutritional requirements vary. Some mutants form small colonies on heat-killed prey bacteria or on agar supplemented with cell extract. Other mutants grow on standard rich media and develop into larger colonies that are often yellow pigmented (Varon and Shilo 1980; Thomashow and Cotter 1992). Most of the saprophytic mutants are initially facultative predators, although prey lysis occurs with a lower frequency. Maintaining these mutants under prey-free conditions induces them to develop into non-predatory forms (Thomashow and Cotter 1992). However, co-cultivation of a predatory strain and a prey-independent strain on the same prey always led to elimination of the axenic mutants. Cultivation of axenic mutants alone in presence of living prey soon led to the development of prey-dependent revertants (Varon and Shilo 1980).

What is the molecular basis of prey independency? Is the development of prey independency reversible under natural conditions and/or is it part of a survival strategy? In a pioneering work, Cotter and Thomashow (1992) showed that a short open reading frame (ORF) within a small genetic region,

the host-interaction locus (*hit*), was mutated in different prey-independent clones, leading to major changes in the resulting gene product. The *hit* locus in two closely related *B. bacteriovorus* strains (HD100 and HD114) is probably part of a genetic cluster responsible for pilus formation and adherence that might play an important role in the attachment and invasion process (Rendulic et al. 2004; Schwudke et al. 2005). Interestingly, all saprophytic mutants analysed in the original study (Cotter and Thomashow 1992) had mutations in the *hit* ORF, whereas a recent study reported prey-independent mutants with an unchanged *hit* locus (Barel and Jurkevitch 2001). Southern hybridization experiments indicated that the *hit* locus is only present in *B. bacteriovorus* strains (Jurkevitch et al. 2000; Schwudke et al. 2001), although it is conceivable that related genetic sequences in other predators exist that are too different to be detected by this method. On the other hand, it may also indicate that the predator–prey interactions are quite different for other predators.

The processes leading to prey independency remain a major open question. The development of prey independency under natural conditions seems to be advantageous only if this capability is restricted in time and is reversible. The metabolic deficiencies of BALOs make it likely that the prey-dependent lifestyle is superior to the axenic lifestyle (Varon and Shilo 1980).

5.2 Bdelloplasts as Resting Stages

The ecological role of BALOs has mostly been studied in aquatic environments, but it seems rather obvious that a balance between prey and predator populations exists in all habitats. As the initial encounter between prey and predator appears to occur by random collision, the survival of predators in an aquatic environment requires more than 10^4 prey cells ml^{-1}. The actual number of prey bacteria in such environments (water, sediment, oyster-shell surfaces) was found to vary from 10^3 to 10^5 cells ml^{-1} (lowest in water), and 73% to 85% of all bacteria were found to be susceptible to predation (Rice et al. 1998). However, it was suggested that the actual prey population is 100 to 1000 times higher, as only a small fraction of the indigenous bacteria is cultivable. The highest population density of prey and predators was found on the surface of oyster shells, supporting the idea that BALOs may survive best in biofilms (Williams et al. 1995). Recent studies have confirmed the ability of *B. bacteriovorus* to prey successfully on biofilms (Kadouri and O'Toole 2005; Nunez et al. 2005).

The intracellular growth phase, in which the predators are enclosed in a nutrient-rich sheltered environment, might represent a prolonged resting period under natural conditions, but not under laboratory conditions. To survive starvation, marine BALOs form stable bdelloplasts containing viable progeny of the predator in a state of reduced metabolic activity (Sanchez-

Amat and Torrella 1990). Interestingly, *B. bacteriovorus* bdelloplasts also protect the predator from xenobiotic substances (Markelova 2002). In times when prey populations are reduced, the predators might take refuge in specific niches, preferably on submerged surfaces where the concentration of prey cells may be higher (Varon and Shilo 1980). Alternatively, bdelloplasts may not mature until conditions better.

In *Bdellovibrio* sp. strain W, the resting cells are termed 'bdellocysts' and are produced within the bdelloplast. These bdellocysts have an enhanced resistance to high temperatures, desiccation and disruption (Burger et al. 1968; Hoeniger et al. 1971). The encysted resting cells of *Bdellovibrio* sp. W represent a stage with low metabolic activity during which the predator is able to outlast nutrient depletion while remaining inside its prey.

6
Predatory Prokaryotes as Therapeutic Agents

The worldwide spread of pathogenic bacteria resistant to antibiotics makes it necessary to seek alternatives for treatment of infectious diseases. Many researchers believe that natural biological systems, like bacteriophages, hold a promising therapeutical potential for treatment of bacterial diseases (reviews: Sulakvelidze et al. 2001; Summers 2001; Duckworth and Gulig 2002). BALOs were shown to decrease the number of viable Gram-negative bacteria in polluted waste-water sewage plants (Lambina et al. 1987). A successful reduction of a number of foodborne pathogenic (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*) and spoilage bacteria (*Pseudomonas* spp.) was reported using *B. bacteriovorus* (Fratamico and Whiting 1995). Additionally, as biofilms turn out to provide a protected environment for pathogenic bacteria, it is of relevance that BALOs were shown to be associated with biofilms in nature (Williams et al. 1995) and are able to kill susceptible bacteria in biofilms (Kadouri and O'Toole 2005; Nunez et al. 2005).

With the elucidation of the complete genome sequence for one *B. bacteriovorus* strain, the idea was put forward to use *B. bacteriovorus* directly as therapeutic agents, as a kind of 'living antibiotic' (Rendulic et al. 2004; Sockett and Lambert 2004). The isolation of *B. bacteriovorus* strains from the intestinal tract of animals (Schwudke et al. 2001) and the low endotoxic potential of the predator LPS (Schwudke et al. 2003) were used as arguments to support this concept.

6.1
Use of *B. bacteriovorus* as a Living Antibiotic

The use of predatory bacteria to combat of pathogenic bacteria is an intriguing idea. Their capability to prey on biofilms led to suggest the following

therapeutic approaches: (1) topical application of *B. bacteriovorus* on burn wounds, (2) inhalation of predator-containing aerosols targeted to pathogenic *Burkholderia* and *Pseudomonas* in lungs of cystic fibrosis patients and (3) use in controlling urinary-tract infections (Sockett and Lambert 2004). However, caution for the concept of a 'living antibiotic' should be exercised.

The concept parallels in some aspects the reemerged discussion on phage therapy. Bacteriophages are physically very stable particles, which can survive in a mammalian environment—at least temporarily—to be effective. Phage treatments were successfully performed by either direct injections into the body (intravenous, subcutaneous or intraperitoneal) or by oral route. In case of the predatory prokaryotes the situation is less clear. *B. bacteriovorus* does not possess the weaponry of pathogenic bacteria for establishing in a eukaryotic host and for resisting the immune response. How should predatory bacteria escape the attack of the immune system without being equipped with appropriate virulence factors or cross barriers within the mammalian host; e.g. gastrointestinal barriers, to follow enteropathogens into deeper tissues through M-cells?

Bacteriophages possess a high specificity for target bacteria, while predatory *B. bacteriovorus* have a wide prey range among Gram-negative bacteria. Resistance to bacteriophages may develop rather rapidly in target bacteria, while resistance to *B. bacteriovorus* seems to be only transient (Shemesh and Jurkevitch 2004; Sockett and Lambert 2004), and upon new cultivation such strains regain susceptibility. Transient resistance, termed 'plastic resistance' (Shemesh and Jurkevitch 2004), is an obstacle to the living antibiotic concept.

Therefore, the biology of the predatory lifestyle calls the concept of a 'living antibiotic' into question. In nature, predator–prey interaction requires a balance between the two populations. A successful predator does not eradicate its prey, as this means that it would extinguish its own food supply and, therefore, eliminate itself. The fact that predator and prey coexist in nature is a priori evidence that mechanisms have developed during evolution to allow coexistence. This coexistence is often a steady-state condition over the long term, whereas in the short term, large fluctuations or oscillations in the populations of feeders and substrate cells are common (Alexander 1981) (see chapter by Wilkinson). Studies with continuous cultures, conducted to determine the predator–prey population dynamics, revealed an oscillation of the two populations. The *B. bacteriovorus* titre increased as the prey titre decreased, followed by a decrease in the number of *B. bacteriovorus* as the prey number increased again (Varon and Shilo 1980). What does this mean for a 'living antibiotic'? A high prey density is necessary for the predatory lifestyle and—because of the wide prey range—predator strains used in therapeutic applications would prey on commensal bacteria as soon as the pathogen concentration dropped. A prerequisite for a therapeutic use is therefore a limitation of the predator's prey range. How this could be accomplished is completely unsolved. The research performed so far does not give

any clue as to how predators could be coerced into a purposive attack on a single target bacterium.

6.2 *B. bacteriovorus* as Probiotic?

The presence of predatory bacteria in the gut of animals and humans may indicate that they have a potential to be used as probiotics. Probiotics are viable non-pathogenic microorganisms that, when administered to man or animals, confer health benefits to the host by improving the microbial balance of the indigenous microflora. In an applied veterinary study, *B. bacteriovorus* was found as part of the natural intestinal population of chicken (Kleessen et al. 2003). In this study, the influence of a special diet (feeding with Jerusalem artichoke, *Helianthus tuberosus*) on the gut microflora was investigated, and it was reported that a decreased level of aerobic bacteria and *Enterobacteriaceae* was accompanied by an increased level of *B. bacteriovorus*. The observation was interpreted as meaning that higher levels of *B. bacteriovorus* protect the mucosal epithelium from adherence to or invasion by Gram-negative pathogenic bacteria and, therefore, improve the state of health of animals. As the veterinary study is—so far—the only published work on the role of predators in the intestinal tract, more research must be conducted to elucidate their roles in the intestinal flora.

Before a probiotic application becomes feasible, applied research should focus on the isolation of predators from the intestine of mammals and on the characterization of their function in this environment. At present it is not clear if such strains contribute to the establishment of a stable beneficial population in the mammalian gut or if the retrieval of *B. bacteriovorus* from faeces of domestic animals and humans (Edao 2000; Schwudke et al. 2001) is only the result of a steady input of the predatory bacteria via nutrition.

6.3 BALOs as Sources of Therapeutical Compounds

The most promising use for BALOs arises from the exploitation of their vast arsenal of degradative enzymes. The genome sequence has opened the field for research of the sophisticated weaponry of these prokaryotic predators. It might be promising to identify and isolate those components of attack-phase BALOs that are involved in the recognition and successive killing of the prey. *B. bacteriovorus* possesses a wide range of genes coding for hydrolytic/degradative enzymes; hence these are potential tools for targeting pathogenic bacteria. The analysis of the genome sequence of strain *B. bacteriovorus* HD100 revealed the presence of at least 150 proteases/peptidases, 15 lipases, 10 glycanases, 89 other hydrolases, 20 DNAses and 9 RNAses (Rendulic et al. 2004). The first candidate enzymes for therapeutic use may be

found within the numerous proteases that are secreted into the medium by attack-phase BALOs. These proteases may have the potential to degrade the matrices surrounding bacteria in biofilms (Sockett and Lambert 2004). The degradative enzymes of the predator used to gain access to the prey's periplasm might help identify prey cell-wall targets that could offer clues on the design of antimicrobial agents. With the genome sequence available it should be straightforward to obtain expression profiles by proteomic studies and RNA transcription analyses to identify the involved factors (see chapter by Tudor and McCann).

7 Perspectives and Conclusions

BALOs open a large field of research activities. Determining the nature of the requirements that make a cell a prey for BALOs is fundamental for the analysis of the prey–predator interaction. The understanding of this interaction may be helpful for the analyses of other cell-to-cell interactions. The development pathway from small rods to long filaments and the successive septation of this filament into flagellated motile cells is a fascinating model for studying differentiation in prokaryotes. Finally, for applied research, the exploitation of the predatory weaponry for therapeutic purposes seems to be an extraordinarily enthralling research area, which may lead to novel, effective and specific antimicrobial agents, avoiding the application of intact bacteria.

References

Abram D, Castro e Melo J, Chou D (1974) Penetration of *Bdellovibrio bacteriovorus* into host cells. J Bacteriol 118:663–680

Alexander M (1981) Why microbial predators and parasites do not eliminate their prey and hosts. Annu Rev Microbiol 35:113–133

Baer ML, Ravel J, Chun J, Hill RT, Williams HN (2000) A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax* gen. nov. as *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* comb. nov., respectively. Int J Syst Evol Microbiol 50:219–224

Baer ML, Ravel J, Pineiro SA, Guether-Borg D, Williams HN (2004) Reclassification of saltwater *Bdellovibrio* sp. as *Bacteriovorax marinus* sp. nov. and *Bacteriovorax litoralis* sp. nov. Int J Syst Evol Microbiol 54:1011–1016

Barel G, Jurkevitch E (2001) Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch Microbiol 176:211–216

Barel G, Sirota A, Volpin H, Jurkevitch E (2005) Fate of predator and prey proteins during growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* and *Pseudomonas syringae* prey. J Bacteriol 187:329–335

Beck S, Schwudke D, Strauch E, Appel B, Linscheid M (2004) *Bdellovibrio bacteriovorus* strains produce a novel major outer membrane protein during predacious growth in the periplasm of prey bacteria. J Bacteriol 186:2766–2773

Beck S, Schwudke D, Appel B, Linscheid M, Strauch E (2005) Characterization of outer membrane protein fractions of Bdellovibrionales. FEMS Microbiol Lett 243:211–217

Burger A, Drews G, Ladwig R (1968) Host range and infection cycle of a newly isolated strain of *Bdellovibrio bacteriovorus*. Arch Microbiol 61:261–279

Cotter TW, Thomashow MF (1992) Identification of a *Bdellovibrio bacteriovorus* genetic locus, hit, associated with the host-independent phenotype. J Bacteriol 174:6018–6024

Cover WH, Martinez RJ, Rittenberg SC (1984) Permeability of the boundary layers of *Bdellovibrio bacteriovorus* 109J and its bdelloplasts to small hydrophilic molecules. J Bacteriol 157:385–390

Crothers SF, Fackrell HB, Huang JC, Robinson J (1972) Relationship between *Bdellovibrio bacteriovorus* 6–5-S and autoclaved host bacteria. Can J Microbiol 18:1941–1948

Davidov Y, Jurkevitch E (2004) Diversity and evolution of *Bdellovibrio*- and -like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax-Peredibacter* clade as Bacteriovoracaceae fam. nov. Int J Syst Evol Microbiol 54:1439–1452

Diedrich DL (1988) Bdellovibrios: recycling, remodelling and relocalizing components from their prey. Microbiol Sci 5:100–103

Diedrich DL, Denny CF, Hashimoto T, Conti SF (1970) Facultatively parasitic strain of *Bdellovibrio bacteriovorus*. J Bacteriol 101:989–996

Diedrich DL, Portnoy CA, Conti SF (1983) *Bdellovibrio* possesses a prey-derived OmpF protein in its membrane. Curr Microbiol 8:54–56

Diedrich DL, Duran CP, Conti SF (1984) Acquisition of *Escherichia coli* outer membrane proteins by *Bdellovibrio* sp. strain 109D. J Bacteriol 159:329–334

Duckworth DH, Gulig PA (2002) Bacteriophages: potential treatment for bacterial infections. BioDrugs 16:57–62

Edao A (2000) Untersuchungen zum Vorkommen und zur Bedeutung von *Bdellovibrio bacteriovorus* im Magen-Darm-Trakt von Tieren und Menschen sowie in der Umwelt. PhD thesis, Universitity of Leipzig, Germany

Eksztejn M, Varon M (1977) Elongation and cell division in *Bdellovibrio bacteriovorus*. Arch Microbiol 114:175–181

Fratamico PM, Whiting RC (1995) Ability of *Bdellovibrio bacteriovorus* 109J to lyse gram-negative food-borne pathogenic and spoilage bacteria. J Food Prot 58:160–164

Gordon RF, Stein MA, Diedrich DL (1993) Heat shock-induced axenic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 175:2157–2161

Gray KM, Ruby EG (1989) Unbalanced growth as a normal feature of development of *Bdellovibrio bacteriovorus*. Arch Microbiol 152:420–424

Gray KM, Ruby EG (1990) Prey-derived signals regulating duration of the developmental growth phase of *Bdellovibrio bacteriovorus*. J Bacteriol 172:4002–4007

Gray KM, Ruby EG (1991) Intercellular signalling in the *Bdellovibrio* developmental cycle. In: Dworkin M (ed) Microbial Cell-Cell Interactions. American Society for Microbiology, Washington, DC, pp 333–366

Guerrini F, Romano V, Valenzi M, Di Giulio M, Mupo MR, Sacco M (1982) Molecular parasitism in the Escherichia coli-*Bdellovibrio bacteriovorus* system: translocation of the matrix protein from the host to the parasite outer membrane. Embo J 1:1439–1444

Gulbins E, Dreschers S, Wilker B, Grassme H (2004) Ceramide, membrane rafts and infections. J Mol Med 82:357–363

Hespell RB, Miozzari GF, Rittenberg SC (1975) Ribonucleic acid destruction and synthesis during intraperiplasmic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 123:481–491

Hespell RB, Odelson DA (1978) Metabolism of RNA-ribose by *Bdellovibrio bacteriovorus* during intraperiplasmic growth on Escherichia coli. J Bacteriol 136:936–946

Hoeniger JFM, Ladwig R, Moor H (1971) The fine structure of resting bodies of *Bdellovibrio* sp. strain W developed in *Rhodospirillum rubrum*. Can J Microbiol 18:87–100

Huang JC, Starr MP (1973) Effects of calcium and magnesium ions and host viability on growth of bdellovibrios. Antonie Van Leeuwenhoek 39:151–167

Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere Bdellovibrio spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66:2365–2371

Kadouri D, O'Toole GA (2005) Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 71:4044–4051

Kessel M, Shilo M (1976) Relationship of *Bdellovibrio* elongation and fission to host cell size. J Bacteriol 128:477–480

Kleessen B, Elsayed NA, Loehren U, Schroedl W, Krueger M (2003) Jerusalem artichokes stimulate growth of broiler chickens and protect them against endotoxins and potential cecal pathogens. J Food Prot 66:2171–2175

Kuenen JG, Rittenberg SC (1975) Incorporation of long-chain fatty acids of the substrate organism by *Bdellovibrio bacteriovorus* during intraperiplasmic growth. J Bacteriol 121:1145–1157

LaMarre AG, Straley SC, Conti SF (1977) Chemotaxis toward amino acids by *Bdellovibrio bacteriovorus*. J Bacteriol 131:201–207

Lambert C, Smith MC, Sockett RE (2003) A novel assay to monitor predator-prey interactions for *Bdellovibrio bacteriovorus* 109 J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol 5:127–132

Lambina VA, Ledova LA, Churkina LG (1987) Importance of *Bdellovibrio* in regulating microbial cenoses and self-purification processes in domestic sewage. Mikrobiologiia 56:860–864

Marbach A, Varon M, Shilo M (1976) Properties of marine Bdellovibrios. Microb Ecol 2:284–295

Markelova NY (2002) Effect of toxic pollut ants on Bdellovibrio. Proc Biochem 37:1177–1181

Martin MO (2002) Predatory prokaryotes: an emerging research opportunity. J Mol Microbiol Biotechnol 4:467–477

Matin A, Rittenberg SC (1972) Kinetics of deoxyribonucleic acid destruction and synthesis during growth of *Bdellovibrio bacteriovorus* strain 109D on Pseudomonas putida and Escherichia coli. J Bacteriol 111:664–673

McCann MP, Solimeo HT, Cusick F Jr, Panunti B, McCullen C (1998) Developmentally regulated protein synthesis during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. Can J Microbiol 44:50–55

Moulder JW (1985) Comparative biology of intracellular parasitism. Microbiol Rev 49:298–337

Nelson DR, Rittenberg SC (1981) Incorporation of substrate cell lipid A components into the lipopolysaccharide of intraperiplasmically grown *Bdellovibrio bacteriovorus*. J Bacteriol 147:860–868

Nunez ME, Martin MO, Chan PH, Spain EM (2005) Predation, death, and survival in a biofilm: Bdellovibrio investigated by atomic force microscopy. Colloids Surf B Biointerfaces 42:263–271

Pineiro SA, Sahaniuk GE, Romberg E, Williams HN (2004) Predation pattern and phylogenetic analysis of Bdellovibrionaceae from the Great Salt Lake, Utah. Curr Microbiol 48:113–117

Ravenschlag K, Sahm K, Pernthaler J, Amann R (1999) High bacterial diversity in permanently cold marine sediments. Appl Environ Microbiol 65:3982–3989

Rayner JR, Cover WH, Martinez RJ, Rittenberg SC (1985) *Bdellovibrio bacteriovorus* synthesizes an OmpF-like outer membrane protein during both axenic and intraperiplasmic growth. J Bacteriol 163:595–599

Reiner AM, Shilo M (1969) Host-dependent growth of *Bdellovibrio bacteriovorus* in microbial extracts. J Gen Microbiol 59:401–410

Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans KJ, Goesmann A, Meyer F, Sockett RE, Schuster SC (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692

Rice TD, Williams HN, Turng BF (1998) Susceptibility of bacteria in estuarine environments to autochthonous bdellovibrios. Microb Ecol 35:256–264

Richardson IR (1990) The incidence of *Bdellovibrio* spp. in man-made water systems: coexistence with legionellas. J Appl Bacteriol 69:134–140

Rittenberg SC (1983) *Bdellovibrio*: attack, penetration, and growth on its prey. ASM News 49:435–439

Ross EJ, Robinow CF, Robinson J (1974) Intracellular growth of *Bdellovibrio bacteriovorus* 6-5-S in heat-killed *Spirillum serpens* VHL. Can J Microbiol 20:847–851

Ruby EG, Rittenberg SC (1983) Differentiation after premature release of intraperiplasmically growing *Bdellovibrio bacteriovorus*. J Bacteriol 154:32–40

Sanchez-Amat A, Torrella F (1990) Formation of stable bdelloplasts as a starvation-survival strategy of marine bdellovibrios. Appl Environ Microbiol 56:2717–2725

Schelling M, Conti S (1986) Host receptor sites involved in the attachment of *Bdellovibrio bacteriovorus* and *Bdellovibrio stolpii*. FEMS Microbiol Lett 36:319–323

Schwudke D, Strauch E, Krueger M, Appel B (2001) Taxonomic studies of predatory bdellovibrios based on 16S rRNA analysis, ribotyping and the hit locus and characterization of isolates from the gut of animals. Syst Appl Microbiol 24:385–394

Schwudke D, Linscheid M, Strauch E, Appel B, Zahringer U, Moll H, Muller M, Brecker L, Gronow S, Lindner B (2003) The obligate predatory *Bdellovibrio bacteriovorus* possesses a neutral lipid A containing alpha-D-Mannoses that replace phosphate residues: similarities and differences between the lipid As and the lipopolysaccharides of the wild type strain B. bacteriovorus HD100 and its host-independent derivative HI100. J Biol Chem 278:27502–27512

Schwudke D, Bernhardt A, Beck S, Madela K, Linscheid MW, Appel B, Strauch E (2005) Transcriptional activity of the host-interaction locus and a putative pilin gene of *Bdellovibrio bacteriovorus* in the predatory life cycle. Curr Microbiol 51:1–8

Seidler RJ, Starr MP (1969) Factors affecting the intracellular parasitic growth of *Bdellovibrio bacteriovorus* developing within *Escherichia coli*. J Bacteriol 97:912–923

Shemesh Y, Jurkevitch E (2004) Plastic phenotypic resistance to predation by *Bdellovibrio* and like organisms in bacterial prey. Environ Microbiol 6:12–18

Shilo M (1969) Morphological and physiological aspects of the interaction of *Bdellovibrio* with host bacteria. Curr Top Microbiol Immunol 50:174–204

Snyder AR, Williams HN, Baer ML, Walker KE, Stine OC (2002) 16S rDNA sequence analysis of environmental *Bdellovibrio*- and -like organisms (BALO) reveals extensive diversity. Int J Syst Evol Microbiol 52:2089–2094

Sockett RE, Lambert C (2004) *Bdellovibrio* as therapeutic agents: a predatory renaissance? Nat Rev Microbiol 2:669–675

Stein MA, McAllister SA, Torian BE, Diedrich DL (1992) Acquisition of apparently intact and unmodified lipopolysaccharides from Escherichia coli by *Bdellovibrio bacteriovorus*. J Bacteriol 174:2858–2864

Steiner S, Conti SF, Lester RL (1973) Occurrence of phosphonosphingolipids in *Bdellovibrio bacteriovorus* strain UKi2. J Bacteriol 116:1199–1211

Stolp H, Petzold H (1962) Untersuchungen über einen obligat parasitischen Mikroorganismus mit lytischer Aktivität für Pseudomonas-Bakterien. Phytopathol Z 45:364–390

Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie Van Leeuwenhoek 29:217–248

Straley SC, Conti SF (1974) Chemotaxis in *Bdellovibrio bacteriovorus*. J Bacteriol 120:549–551

Straley SC, Conti SF (1977) Chemotaxis by *Bdellovibrio bacteriovorus* toward prey. J Bacteriol 132:628–640

Straley SC, LaMarre AG, Lawrence LJ, Conti SF (1979) Chemotaxis of *Bdellovibrio bacteriovorus* toward pure compounds. J Bacteriol 140:634–642

Sulakvelidze A, Alavidze Z, Morris JG Jr (2001) Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659

Summers WC (2001) Bacteriophage therapy. Annu Rev Microbiol 55:437–451

Talley BG, McDade RL Jr, Diedrich DL (1987) Verification of the protein in the outer membrane of *Bdellovibrio bacteriovorus* as the OmpF protein of its *Escherichia coli* prey. J Bacteriol 169:694–698

Taylor VI, Baumann P, Reichelt JL, Allen RD (1974) Isolation, enumeration, and host range of marine bdellovibrios. Arch Microbiol 98:101–114

Thomashow MF, Rittenberg SC (1978a) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: solubilization of *Escherichia coli* peptidoglycan. J Bacteriol 135:998–1007

Thomashow MF, Rittenberg SC (1978b) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: N-deacetylation of *Escherichia coli* peptidoglycan amino sugars. J Bacteriol 135:1008–1014

Thomashow MF, Rittenberg SC (1978c) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: attachment of long-chain fatty acids to *Escherichia coli* peptidoglycan. J Bacteriol 135:1015–1023

Thomashow MF, Cotter TW (1992) *Bdellovibrio* host dependence: the search for signal molecules and genes that regulate the intraperiplasmic growth cycle. J Bacteriol 174:5767–5771

Torrella F, Guerrero R, Seidler RJ (1978) Further taxonomic characterization of the genus *Bdellovibrio*. Can J Microbiol 24:1387–1394

Tudor JJ, Karp MA (1994) Translocation of an outer membrane protein into prey cytoplasmic membranes by bdellovibrios. J Bacteriol 176:948–952

Tudor JJ, McCann MP, Acrich IA (1990) A new model for the penetration of prey cells by bdellovibrios. J Bacteriol 172:2421–2426

Varon M, Shilo M (1980) Ecology of aquatic bdellovibrios. Academic, New York

Waggoner DW, Xu J, Singh I, Jasinska R, Zhang QX, Brindley DN (1999) Structural organization of mammalian lipid phosphate phosphatases: implications for signal transduction. Biochim Biophys Acta 1439:299–316

Watanabe Y, Nakajima M, Hoshino T, Jayasimhulu K, Brooks EE, Kaneshiro ES (2001) A novel sphingophosphonolipid head group 1-hydroxy-2-aminoethyl phosphonate in *Bdellovibrio stolpii*. Lipids 36:513–519

Wilkinson MH (2001) Predation in the presence of decoys: an inhibitory factor on pathogen control by bacteriophages or bdellovibrios in dense and diverse ecosystems. J Theor Biol 208:27–36

Williams HN, Falkler WAJ (1984) Distribution of bdellovibrios in the water column of an estuary. Can J Microbiol 30:971–974

Williams HN, Kelley JI, Baer ML, Turng BF (1995) The association of bdellovibrios with surfaces in the aquatic environment. Can J Microbiol 41:1142–1147

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_056/Published online: 14 October 2006

Genomic Analysis and Molecular Biology of Predatory Prokaryotes

John J. Tudor (✉) · Michael P. McCann

Department of Biology, Saint Joseph's University, 5600 City Avenue,
Philadelphia, PA 19131, USA
jtudor@sju.edu

Abstract *Bdellovibrio* and *Bdellovibrio*-like organisms (BALOs) are defined by their unique intraperiplasmic developmental cycle, which is an essential part of their predatory activity on other Gram-negative bacteria. The genome sequence of the type strain of the genus *Bdellovibrio* is the first of a predatory bacterium to be completed, and will serve as a good model for genomic analysis of predation. Many putative genes have already been identified that could encode products that play important roles in predation. Much work has been done in the past to elucidate the biochemistry and physiology of the BALO predatory life cycle, and the genomic information will permit this wealth of information to be connected with the genetic basis of predation in these unique organisms. As sequence data from other predatory bacteria becomes available, comparative genome analysis will provide important insights into the evolution of genes involved in predatory mechanisms. Clearly, we are on the threshold of a more complete understanding of the BALO developmental cycle, which can serve to increase our understanding of not only predation but also cell–cell interaction. Additionally, the knowledge provided through genome analysis could lead to the potential use of the BALOs as biocontrol, or even biotherapeutic, agents.

1 Introduction

There are a variety of bacteria that are predatory on other prokaryotes, the majority of which are found among the Proteobacteria group of Gram-negative bacteria. These predatory representatives can be found in each of the α, β, γ, and δ subdivisions of the Proteobacteria. They exhibit varying mor-

phologies, lifestyles, and predatory strategies. Martin (2002) has identified four basic strategies for predation by these bacteria:

1. Wolf pack, exemplified by *Myxococcus*;
2. Epibiotic, as seen with *Vampirococcus*;
3. Direct invasion, as demonstrated by *Daptobacter*; and
4. Periplasmic, as defined by *Bdellovibrio*.

(A broad description of predatory bacteria and of their predatory modes can be found in the chapter by Jurkevitch and Davidov in this volume).

The most extensively studied category of predation is occupied by the δ-proteobacteria *Bdellovibrio* and related organisms, referred to as *Bdellovibrio*-and-like organisms (BALOs, Snyder et al. 2002), and classified in the order *Bdellovibrionales* (Garrity et al. 2002). Based on the analysis of 16S rRNA genes (Baer et al. 2000; Davidov and Jurkevitch 2004), the *Bdellovibrionales* include at least three genera, *Bdellovibrio*, *Bacteriovorax* (mostly marine forms), and the recently named *Peredibacter*; there are also a number of other periplasmic predators that remain uncharacterized, although they are all closely related morphologically and behaviorally to the *Bdellovibrio*. Almost all members of the *Bdellovibrionales* have been shown to be periplasmic predators. This predatory lifestyle is advantageous for these very small bacterial predators in that, upon establishment of residence within the periplasmic space of the prey cell, there can be no other competitors for the rich nutrients available from the cytoplasm of the invaded cell. In this chapter, the common name "bdellovibrio" will be used interchangeably with BALO.

This chapter is intended to provide an analysis of the genomic data on predatory bacteria. Only three predatory bacterial genomes have been sequenced to date, and all are from the *Bdellovibrio* or *Bacteriovorax* genera. The genome of *Bdellovibrio bacteriovorus* HD 100 was the first of this group to be sequenced, followed by the marine form *Bacteriovorax marinus* SJ. The last to be sequenced was the unclassified *Bdellovibrio* sp. strain W, which is of particular interest because it is the only BALO that has been found capable of producing dormant cysts, termed bdellocysts (Tudor and Conti 1977a). We will summarize what is known about these genomes, identifying peculiarities and potential important loci for predation, and present an initial comparative analysis of the three genomes. From this we hope to supply information that will aid in understanding the predatory behavior of these periplasmic predators, and provide a framework for future investigations.

After a brief description of the predatory life cycle, we will attempt to enumerate some of the putative genes and gene clusters identified in the BALO genomes that may be involved in predacious growth. We will also point out methods that are available to elucidate the regulatory networks controlling gene expression during attack phase and intraperiplasmic growth.

2 The BALO Predatory Life Cycle

This genomic information must first be put into context with the intricate life cycle of these unique, obligately predatory bacteria. Regardless of the degree of relatedness among these periplasmic predators, they all follow a rather uniform developmental cycle, which is outlined in Fig. 1. The bdellovibrios exhibit a complex two-phase developmental life cycle that involves an extracellular free-living form, termed an attack-phase cell, and an intraperiplasmic form that undergoes growth and reproduction within the periplasmic space of the invaded bacterial cell. A typical cycle of an in vitro culture is completed within 3–4 h following initial attachment to a prey cell by an attack-phase BALO. This developmental process is complex and must involve multiple signaling pathways that give rise to temporally regulated gene expression enabling the predator to find, invade, and devour its prey. Although the mechanisms of direct invasion and degradation of prey contents have been studied, many of the processes and their genetic controls are not understood.

The bdellovibrios are highly motile as attack-phase cells, moving at rates of up to 160 μm per second (Sockett and Lambert 2004), and powered by a single polar sheathed flagellum. Although they are metabolically active and capable of synthesizing macromolecules, such as RNA, protein, and peptidoglycan (Thomashow and Rittenberg 1979), these cells do not replicate DNA and are not capable of cell division. Their rapid motility while in search of susceptible Gram-negative prey comes at a high price in terms of energy expenditure. The endogenous rate of respiration for bdellovibrios has been

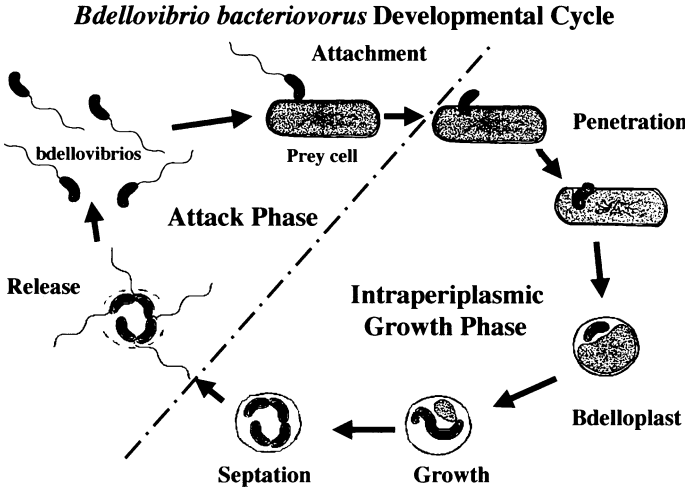


Fig. 1 The predatory life cycle of *B. bacteriovorus*. See text for explanation

shown to be some seven times that of *E. coli* (Hespell et al. 1973). This high rate of energy usage leads to rapid loss of viability in the absence of prey. Whether the mechanism for finding prey cells is purely random chance due to the predator's extreme mobility, or is dependent on chemical signals from the prey cells or the environment, is still open to debate. The fact that some of the bdellovibrios are chemotactic is indisputable (Straley and Conti 1974; 1977; Lamarre et al. 1977; Straley et al. 1979), but whether this capability is involved in locating prey cells still remains unknown.

Regardless of the mechanism of prey location, once the predator encounters prey it attaches to the prey cell envelope, at first reversibly, followed rapidly by an irreversible attachment. The mechanism of this interaction remains a mystery although there is suggestive evidence that it involves the core polysaccharide of the prey lipopolysaccharide (Schelling and Conti, 1986). This irreversible attachment is followed by the invasion of the prey cell periplasm as the bdellovibrio penetrates the outer layers of the prey cell envelope. During this early stage of invasion, the attacking BALO sheds its flagellum. Following entry into the periplasm, the penetration pore is sealed, and the attacking predator takes up residence without breaching the cytoplasmic membrane of the prey cell. Prey cells are rapidly immobilized, followed within a few minutes by cessation of RNA and protein synthesis and cellular respiration (Rittenberg and Shilo 1970; Thomashow and Rittenberg, 1978a). This invasion process results in the periplasmic predator sitting inside an isolated "food bag", the contents of which are made available by the many degradative enzymes that are part of the predator's arsenal.

In order to keep the prey cell intact during the intraperiplasmic growth phase, the prey cell envelope is modified, producing an osmotically stable spheroplast, termed a bdelloplast (Thomashow and Rittenberg 1978a, Tudor et al. 1990, Ruby 1991). The bdelloplast serves as a unique niche for the invader, where it has sole access to the rich nutrients provided by the prey cell cytoplasm. The remainder of the bdellovibrio growth phase takes place in this structure, with the predator systematically degrading the cytoplasmic contents of the prey and incorporating these nutrients into the growing filament. It remains unclear how these nutrients cross what appears to be an intact prey membrane, but there is evidence that a porin or porin-like protein is translocated from the bdellovibrio to the cytoplasmic membrane of the prey immediately following invasion (Tudor and Karp 1994). Kinetic studies of protein synthesis utilizing ^{35}S-labeled methionine indicate that a protein of similar molecular weight and isoelectric point is synthesized soon after the bdellovibrio resides in the periplasm of the prey (McCann et al. 1998). The translocation of such an outer membrane protein would explain earlier observations on *Bdellovibrio* attack, such as increased permeability of the prey cytoplasmic membrane (Rittenberg and Shilo 1970; Rittenberg and Thomashow 1979), and the rapid loss of cytoplasmic potassium following an attack (Galdiero 1975).

When the initial invasion process is completed, the intraperiplasmic bdellovibrio shifts into a growth phase, systematically degrading the cellular contents of the prey and synthesizing and assembling its own cellular structures. Many degradative enzymes, transport proteins, and biosynthetic pathways are required during this rapid growth phase. It is during this phase that DNA synthesis takes place, as the bdellovibrio grows into a filament, several cell lengths long. The length of the filament, and ultimately the number of progeny produced, is directly related to the size of the invaded prey cell (Seidler and Starr 1969). Once the filament reaches its maximum length, it fragments into multiple progeny predatory cells, each of which synthesizes its own flagellum. Lytic enzymes are then released that lyse what remains of the bdelloplast, and new attack-phase cells emerge.

3 *Bdellovibrio bacteriovorus* HD 100 as a Model for Genomic Analysis of Predation

The genome of the type strain of *B. bacteriovorus*, strain HD 100 has been determined to be circular, containing 3 782 950 base pairs, and is predicted to code for approximately 3580 proteins (the exact number varies based on prediction method; see Integr8 at http://www.ebi.ac.uk/integr8, microbesonline at http://www.microbesonline.org and the PEDANT genome database at http://www.pedant.gsf.de). Two complete rRNA operons have been identified, that also contain 36 genes encoding tRNAs for all 20 translationally used amino acids (Rendulic et al. 2004). The genome contains 50.7% G+C, and no plasmids have been found. Only one other genome from a BALO, *Bacteriovorax marinus* SJ, has been sequenced and completely assembled at the time of this writing. Sequence data for this organism were produced by the *Bacteriovorax marinus* Sequencing Group at the Sanger Institute (*B. marinus* sequence data can be obtained at: ftp://ftp.sanger.ac.uk/pub/pathogens/bm). It consists of a single chromosome of 3 435 933 bp with a G+C content of 36.75%, and a plasmid of 1973 bp having a G+C content of 36.19%. Our analysis of the Sanger sequence data suggests that, like *B. bacteriovorus* HD 100, the genome of *B. marinus* SJ contains two rRNA operons and 36 tRNA genes. The predicted *B. marinus* proteome is some 13% smaller than that of *B. bacteriovorus* HD 100, at approximately 3100 protein-coding genes (PEDANT). The genome of a third BALO, *Bdellovibrio* sp. strain W has also been sequenced (http://www.micro-gen.ouhsc.edu/), but at the time of writing it had not yet been assembled into a complete sequence. Preliminary analysis has found it to be very similar in composition and sequence to the genome of *B. bacteriovorus* HD 100, but dissimilar to that of *B. marinus* SJ.

The increasing numbers of bacterial genes and whole genomes that have been sequenced have facilitated the development of robust computer pro-

grams that use sequence similarity at both the nucleotide and the deduced amino acid sequence level to identify gene orthologs and paralogs. While this is a powerful technique, it is naturally limited to the identification of genes that are present in many different organisms. While some of the most interesting genes in BALO predators may not have orthologs in other bacteria, this approach can be used to identify many of the genes essential for the predatory life style. Genes unique to bacterial predators would not be expected to have orthologs in non-predatory bacteria. Thus a group of candidate predation genes can be identified by exclusion. Similarly, the functions of the products encoded by these genes (and other not essential for predatory growth) may well be deduced based on the presence of conserved domains or other predicted motifs in the predicted polypeptide products.

It is important to note at this point that, other than the genomic DNA sequences, there is very little genetic, molecular or biochemical information regarding specific genes or gene products in any of these BALO-type predators. Much of what follows regarding "genes" in this chapter is therefore speculative and largely driven by computer-based analysis of homology to genes and proteins of known or suspected function. Thus, most of the "genes" to which we refer should be considered putative or probable genes until there is experimental evidence addressing the function of their products. However, to avoid needless repetition we will typically omit these terms in the descriptions that follow.

Until the sequencing of the *B. bacteriovorus* HD 100 genome in 2004 (Rendulic et al. 2004) research on the predatory life cycle of the BALOs had been almost exclusively based on physiological and morphological studies. The results of all of these past studies can now serve as a framework on which the genomic data can be evaluated and interpreted. Since all wild-type BALOs that have been isolated from nature have an absolute requirement for prey cells, comprehending the nature of this cell–cell interaction, and the intricate genetic regulation of the predatory life cycle is fundamental to our understanding of the BALOs and the role they play in the environment. Increasing our knowledge of the genetic and molecular basis of predation could lead to much broader biological implications for other cell–cell interactions that are at present medically and environmentally relevant. The remainder of this section will use the annotated *B. bacteriovorus* HD 100 genome as a basis for describing possible molecular and genetic components of predation.

It is assumed that motility is a prerequisite for the predatory capability of the BALOs as a means of efficiently encountering and attaching to susceptible prey bacteria. Early work demonstrated that when bdellovibrios were rendered non-motile, they were incapable of attaching to prey cells (Varon and Shilo, 1968). *Bdellovibrio* motility is achieved using a single polar sheathed flagellum. The genome of *B. bacteriovorus* HD 100 contains six clusters of genes responsible for flagellar synthesis and motility, as well as six genes coding for the flagellin protein located at multiple loci. Expression of one of the

motility genes, *mot*A is required for predation and for efficiently exiting the prey cell following the growth phase. Flannagan et al. (2004) have shown that inhibiting the expression of the *mot*A gene with antisense RNA delayed bdellovibrio escape from the invaded prey cell.

Twenty genes encoding putative methyl-accepting chemotaxis proteins (MCPs) have been identified in the *Bdellovibrio* genome. It has long been known that the bdellovibrios exhibit chemotaxis (Straley and Conti 1974, 1977; Lamarre et al. 1977; Straley et al. 1979). However, all of these studies were performed using strain UKi2, now classified as *Bacteriovorax stolpii*, whereas only a couple of experiments demonstrated chemotaxis by other strains, with *B. bacteriovorus* strain 109J exhibiting no chemotactic behavior against the compounds tested (Straley et al. 1979). The *B. bacteriovorus* HD 100 genome contains all the required genes for a functional chemotactic system (Fig. 2). The role that CheD would play in chemotaxis is not known for the bdellovibrios, but has been shown to be necessary for a chemotactic response in other prokaryotes (Kristich and Ordal 2004). It is interesting to note that no *che*Z homolog has been found in the *B. bacteriovorus* HD 100 genome. An unknown protein must therefore carry out its usual function of dephosphorylating CheY. Whether chemotaxis plays an important role in location of prey is still open to speculation. Lambert et al. (2003) have shown that disruption of an *mcp* gene of *B. bacteriovorus* led to a decrease in efficiency of predation. Determining which chemotaxis genes, if any, are important or essential for either prey location and/or predation efficiency should now be possible because of the genomic data available. Insight into their importance may also be gleaned from a comparative examination of

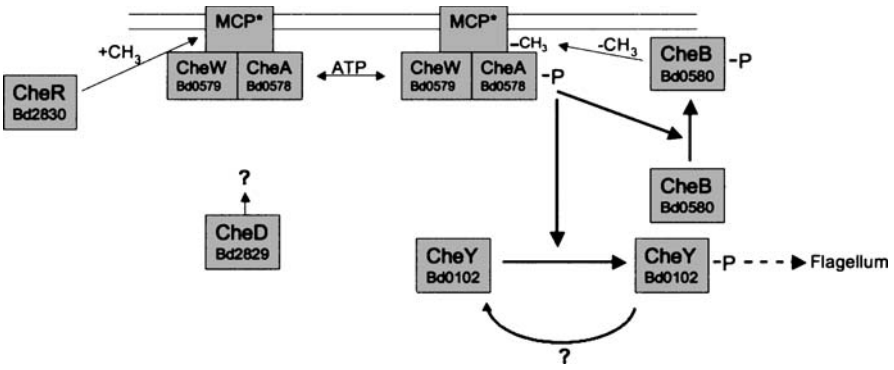


Fig. 2 Putative chemotaxis proteins encoded in the *B. bacteriovorus* HD 100 genome. Numbers beneath the protein designations represent genomic loci. This schematic is based on the model proposed by KEGG (Kyoto encyclopedia of genes and genomes; www.genome.jp). Note that an ortholog of the gene for CheZ (dephosphorylates CheY) has not been identified based on sequence comparison *There are potentially 20 MCP genes in the *Bdellovibrio* genome

the MCP genes found in available BALO genomes (see discussion below). *B. stolpii* has also been shown to be tactic in regard to oxygen concentrations (Straley et al. 1979) and therefore presumably should have the machinery for aerotaxis. The *B. bacteriovorus* HD 100 genome contains a gene that codes for a putative aerotaxis sensor (Aer, protein accession #CAE77867), that could function in sensing oxygen concentrations in the environment. This could be important for BALOs in finding their potential prey. Aerotaxis experiments showed that *B. stolpii* accumulated below the surface of the medium, indicating a preference for lower oxygen tension and that, therefore, they are potentially microaerophilic.

Irreversible attachment closely follows after the collision of a predator with a susceptible prey cell. The mechanism of adhesion between the two cells is still unknown. Many attempts have been made to isolate predator-resistant prey, and to date only one success has been reported (Varon 1979) using continuous culture over several days. BALOs are capable of preying on a wide range of Gram-negative bacteria, but the identification of the receptor molecule(s) on the prey remains elusive. Bacterial adhesion to substrates and other cells many times involves fimbriae. Electron microscopy has revealed the presence of fimbriae, especially on the pole of bdellovibrios opposite the flagellum (Abram and Davis 1970), which may be involved in attachment to the prey cell envelope. Several clusters of *pil* genes are present in the *B. bacteriovorus* HD 100 genome. The number of *pil* genes is greater than found in most bacteria to date. These genes could be involved in a number of functions (i.e., secretion, uptake, adherence, etc.), but also may also be the means by which these predators establish irreversible attachment.

In synchronous cultures of *B. bacteriovorus* growing on *Escherichia coli* as prey, penetration of the outer envelope of the prey by the predator commences within minutes of attachment, resulting in the bdellovibrio cell invading the prey periplasm (Thomashow and Rittenberg, 1978a; Tudor et al. 1990). This breach of the outer layers of the prey cell is the result of a localized coordinated attack on the prey cell wall by hydrolytic enzymes produced by the predator. The *B. bacteriovorus* HD 100 genome reveals the presence of the second highest density of genes encoding hydrolytic enzymes that has been seen in bacterial genomes, including ten glycanases, 150 protease/peptidases (the highest number ever reported), 20 DNAases, nine RNAases, and 15 lipases. A number of enzymatic activities are released during the initial stages of bdellovibrio attack, which not only result in penetration of the prey envelop, but in extensive modification of the outer prey layers. Thomashow and Rittenberg (1978a,c) presented data for several degradative enzyme activities that would affect the outer layers of the prey, such as glycanase, peptidase/protease, and LPSase. The outer membrane of the prey is extensively modified, including the solubilization of up to 25% of LPS glucosamine during the initial stages of bdellovibrio attack (Thomashow and Rittenberg 1978a), with the prey cell's OmpA and Braun's lipoprotein getting degraded (Cover et al. 1984; Barel et al.

2005; Beck et al. 2004), and a subsequent increase in hydrophobicity (Cover and Rittenberg 1984). The protein degradation observed in the outer membrane of the prey can be easily be accounted for by the extensive number of proteases/peptidases encoded by genes found in the predatory genome. Several secreted proteases have been identified that could fulfill this role during predation. Sockett and Lambert (2004) identified a secreted protease of $\sim$ 90 kDa that could be the product of any one of three different protease genes in the *B. bacteriovorus* HD 100 genome.

These extensive modifications of the prey cell envelope apparently lead to an immunity to subsequent invasion by a second predator. Two potential explanations for this exclusion mechanism have been presented. Thomashow and Rittenberg (1978b,c) suggested that deacetylation and acylation of the peptidoglycan inhibit further solubilization of this macromolecule following penetration, and result in the bdelloplast being resistant to superinfection. An alternative explanation has been presented by Tudor et al. (1990), which suggests that changes in the topography of the outer membrane could lead to masking of receptor sites, excluding subsequent irreversible attachment. In any case, once a predator has established itself in the periplasmic space, and the prey is converted into a rounded bdelloplast, subsequent invasions by other bdellovibrios are prevented, making the bdelloplast an exclusive, protected environment for growth and reproduction of the intraperiplasmic predator.

Diedrich et al. (1983, 1984) reported that bdellovibrios pick up intact outer membrane proteins from their prey cell during the invasion of the periplasm and incorporate them into their own outer membrane. However, subsequent reports have presented data that question these earlier conclusions (Barel et al. 2005; Beck et al. 2004; 2005). The latter have demonstrated that BALOs produce an Omp that, while it is similar to *E. coli* OmpF, is unique to the predators (for more details, see chapter by Strauss et al. in this volume). These data confirm the earlier report by Rayner et al. (1985) that *B. bacteriovorus* 109J produces its own Omp similar to OmpF of *E. coli*. The genes coding for this Omp are conserved across diverse groups of BALOs, showing sequence similarities ranging in value from 34% to 89% at the amino acid level (Beck et al. 2005).

The exact mechanism by which the predator successfully breaches the outer layers of the prey cell is still not understood. Whether the enzymatic degradation of a portion of the outer membrane of the prey is required for the movement of the predator through this envelop, or if movement of the predator, due either to flagellar motion (so called drilling) or by twitching motility due to Type IV pili, is sufficient, is not known. The *B. bacteriovorus* HD 100 genome possesses two copies of genes that code for Type IV pili that could be involved in penetration (Rendulic et al. 2004). The actual means of penetration may well be a combination of both enzymatic and mechanical activities. Whatever the mechanism for breaching the outer membrane,

in order to penetrate the rigid peptidoglycan, the predator must break covalent bonds to produce a penetration pore large enough to squeeze through. Several reports (Tudor et al. 1990; Thomashow and Rittenberg 1978a,c) have demonstrated that both glycan and peptide moieties of the prey peptidoglycan are released into solution during the first few minutes of invasion as the result of glycanase and peptidase activities. Up to 15% of the prey cell peptidoglycan is solubilized during penetration, with glycanase activity ceasing almost immediately following invasion, but peptidase/protease activity continuing throughout intraperiplasmic growth. The glycanase activity is inhibited through the modification of the substrate rather than direct inhibition of the enzyme (Thomashow and Rittenberg 1978c). A gene coding for a putative peptidoglycan GlcNAc deacetylase (#CAE78449) is present in the *B. bacteriovorus* HD 100 genome. This could be the enzyme responsible for this modification. The amount of glycanase activity recorded, however, may be sufficient to cause the peptidoglycan layer to lose rigidity, resulting in the formation of a rounded structure called a bdelloplast. There is one glycanase gene identified in the *B. bacteriovorus* HD 100 genome that codes for an extracellular enzyme that could be a good candidate for this activity (Rendulic et al. 2004). Glycanase, however, may not be necessary for invasion, as heat-killed cells are penetrated without any detectable release of glycan units (Tudor et al. 1990). An examination of the temporal activity of these hydrolytic enzymes during invasion would be necessary to determine the exact role of each in these early events in predation.

Peptidase/protease activity continues throughout the intraperiplasmic growth phase, and this continuous peptidase activity could be attributed to any number of the protease/peptidase genes found in the *B. bacteriovorus* HD 100 genome. Indeed, 12 of these genes encode putative extracellular enzymes that could be secreted into the periplasm of the prey following invasion. However, the activity responsible for the production of the penetration pore most likely is not an excreted enzyme, but rather a peptidase that is localized and anchored in the outer membrane of the anterior pole of the predator. Bd0168 is a gene in the *B. bacteriovorus* HD 100 genome encoding a putative peptidase (#CAE77837) that is predicted to be an integral membrane protein. If it is localized at the anterior pole of the cell, it could function to produce localized hydrolysis of prey peptide cross links, permitting the predator to squeeze through the prey's peptidoglycan layer into the periplasmic space. This mechanism for penetration has been suggested by the work of Tudor et al. (1990), based on the study of *B. bacteriovorus* 109J and strain W versus live and heat-killed *E. coli*.

These extensive modifications to the prey envelope result in the formation of an osmotically stable rounded bdelloplast, in which the predator is safely nestled in the periplasmic space. The work of systematically degrading the prey cytoplasmic contents for nutrient acquisition begins, and the predator is now faced with two challenges:

1. *The extensive hydrolytic arsenal must be exported* from the predator cytoplasm across its two membranes into the prey periplasm, and then across the prey cytoplasmic membrane. Therefore, if products of *Bdellovibrio* genes are to serve as the agents responsible for hydrolysis of prey cell macromolecules, then there must be mechanisms present in the predator to move these products across the three membranes. A number of pathogenic bacteria have very specialized Type III secretory complexes that are capable of moving important virulence factors across both their cytoplasmic and outer membranes and directly across the host cell membrane into the cytoplasm of a host cell. Because of the close proximity of the bdellovibrio cell outer membrane and the cytoplasmic membrane of the prey (Abram et al. 1974), it would be reasonable to suspect a similar process for the bdellovibrios. However, no homologs of genes coding for either Type III or Type IV secretory pathways have been found in any of the sequenced genomes of BALOs, so the bdellovibrios must make use of alternate secretory pathways.
   There are a number of genes found in the *B. bacteriovorus* HD 100 genome that correspond to Type I and Type II secretion systems, as well as twin arginine translocation (TAT) systems, which could code for translocation complexes capable of moving hydrolytic enzymes into the prey periplasm. For example, gene Bd0887 codes for a putative outer membrane protein (#CAE78831) as part of an ABC transporter for protein export across both the cytoplasmic and outer membranes of the predator. The genome also contains genes coding for Type IV pili capable of translocating proteins, and a number of genes that code for putative translocators of the TAT system, which could translocate native folded proteins across both predator membranes. Such export systems could be used to secrete the predator's hydrolytic arsenal into the bdelloplast periplasm. However, since the prey's cytoplasmic membrane does not appear to be physically disrupted by the predator, the mechanism by which specific bdellovibrio hydrolytic enzymes are then introduced into the prey cytoplasm remains unknown. One proposal involves the secretion of predator enzymes from the periplasm into the prey cytoplasm using the prey's own secretory systems, but operating in a reverse fashion (Saier 1994). Saier also suggested the need for molecular chaperons in the process of moving proteins from the predator to the prey cytoplasm. The *B. bacteriovorus* HD 100 genome contains three genes encoding proteins of the GroELES system (#CAE77776, #CAE77777, #CAE78154), two for the DnaJK system (#CAE79192, #CAE79194), and one gene encoding a putative SugE chaperone protein (#CAE80306). Whether these chaperons are essential for predation and whether they could function in the prey periplasm and/or the cytoplasm has yet to be elucidated.
   Many studies have demonstrated that the BALOs produce a wide variety of hydrolytic enzymes that could be used for degrading their prey cy-

toplasmic contents, and that the sequenced genomes of these predators contain a high concentration of genes encoding this hydrolytic arsenal. In addition to the plethora of proteases/peptidases synthesized by the predator, there are a full range of hydrolytic enzymes that could be responsible for degradation of the prey cytoplasmic contents. Specifically, polysaccharides, lipids, proteins, and nucleic acids of the prey would need to be hydrolyzed to produce precursors for bdellovibrio biosynthesis to fuel the predator's growth. There are 12 candidate protease/peptidase genes in the *B. bacteriovorus* HD 100 genome that code for predicted extracellular enzymes. Protease activity was shown to increase throughout the intraperiplasmic growth phase (Romo et al. 1992). Two putative DNase genes (Bd0934 and Bd3507) that code for proteins (#CAE78875 and #CAE78299) are predicted to be secreted by the predator and could be responsible for the controlled degradation of the prey DNA (Matin and Rittenberg 1972; Rosson and Rittenberg 1979), resulting in the production of a variety of nucleosides and nucleotides, as well as ribose sugars. There are nine genes predicted to code for RNases and 15 genes encoding lipases, which would complete the degradative potential of the predator for producing biosynthetic precursors.

2. *Hydrolyzed products must be imported* from the prey cytoplasm into its own cytoplasm to serve as nutrients. This latter process involves movement of small molecules from the prey cytoplasm, across the cytoplasmic membrane into the periplasm, and then transport of these molecules across the predator cytoplasmic membrane. As the bdellovibrio enzymes hydrolyze prey macromolecules, there must be a means of moving the resultant hydrolyzate across the prey cytoplasmic membrane and into the periplasmic space, where the predator would have access to it. The exact mechanism employed for this function is not known,. However, as noted earlier, the invading bdellovibrio is capable of inserting a porin-like protein into the cytoplasmic membrane of the prey (Tudor and Karp 1994). Recent reports confirm that the bdellovibrios make an outer membrane protein with similar molecular mass and isoelectric point as that reported by Tudor and Karp (Barel et al. 2005; Beck et al. 2004). Subsequent work by Beck et al. (2004; 2005) presented data showing that a number of BALOs (*B. bacteriovorus* HD 114, HD W, *B. stolpii*, and *B. starrii*) possess homologs of this major outer membrane protein, suggesting that this function of translocating a porin into the prey cytoplasmic membrane is presumably conserved among all the *Bdellovibrionales*. The gene Bd0427 encodes a protein (#CAE78413) in *B. bacteriovorus* HD 100 of 36 kDa with a pI of 4.75, which matches the physical characteristics of this Omp. This major predator Omp is unique among porin-like proteins in that it has extensive α-helices. This is in contrast to the β-sheets of most Gram-negative porins, which are organized as β-barrel channels. The unique structure of this Omp explains how it may undergo translocation from the *Bdellovib-*

rio outer membrane to the prey cytoplasmic membrane, when no other Omps have been shown to be capable of such movement. It follows that the hydrolyzed products of prey macromolecular breakdown could diffuse into the periplasmic space through this uniquely placed porin, giving the intraperiplasmic bdellovibrio access to these nutrients.

After the prey cell molecules have leaked into the periplasm, the predator must have a full complement of transport systems to import the nutrients. The *B. bacteriovorus* HD 100 genome contains 244 ORFs that could correspond to genes coding for membrane transporters (Rendulic et al. 2004). Of these ORFs, 147 potentially code for proteins of the ABC type that could be used to produce 40 ABC transport complexes, about half of which are putative exporters and half importers. The remaining number of transport ORFs represent primary transporters of the major facilitator superfamily (MFS).

Since the bdellovibrios have not been shown to utilize sugars as major sources of energy, it is not surprising that there is a paucity of sugar transporter genes found in the *B. bacteriovorus* HD 100 genome. For example, only genes encoding periplasmic binding proteins and permeases for ribose (#CAE78424 and #CAE78425) and maltose (#CAE79129 and #CAE79131) have been identified. The genome sequence also predicts that bdellovibrios can transport phosphorylated glycerol utilizing a glycerol-3-phosphate transporter (#CAE79991 and #CAE80692).

Previous work (Hespell et al. 1973; Ruby et al. 1985) has shown that although BALOs cannot use sugars as sources of energy production, they do use amino acids as a major source of energy as well as for protein synthesis. Rendulic et al. (2004) reported that the *B. bacteriovorus* HD 100 genome shows that genes for the biosynthesis of nine amino acids are absent, suggesting that the predator must obtain them from the substrate cell. However, we have identified genes coding for biosynthetic pathways for both asparagine and alanine, suggesting that *B. bacteriovorus* HD 100 may not be auxotrophic for these amino acids. The *B. bacteriovorus* HD 100 genome contains three genes that convert deliberately mischarged asp-trn(asn) to asn-trn(asn), thus forming asparagine:*gat*A (Bd0059) that codes for glutamyl-tRNA(Gln) amidotransferase, subunit A (CAE77740), *gat*B coding for glutamyl-tRNA(Gln) amidotransferase, subunit B (CAE77741) and *gat*C coding for glutamyl-tRNA(Gln) amidotransferase, subunit C (CAE77739) (Min et al. 2002). A gene (Bd1194) encoding the enzyme cysteine desulfurase (CAE79103), which catalyzes the conversion of L-cysteine to L-alanine, is present in the genome of *B. bacteriovorus* HD 100. Whether this pathway would be used to produce significant amounts of alanine is questionable, since its main function is the formation of sulfane sulfur. It is of interest to note that the genome of *B. marinus* SJ does not appear to have any of the *gat* genes, and contains a poorly aligned sequence for the cysteine desulfurase gene (46% similarity).

The genome of *B. bacteriovorus* HD 100 shows that the bdellovibrios possess the genes necessary to transport three of the nine amino acids considered essential by Rendulic et al. (2004). The remainder are apparently moved across the predator's membranes as peptides. The HD 100 genome has genes coding for transporters and permeases for di-, tetra-, penta-, and oligopeptides (Rendulic et al. 2004). Once transported into the bdellovibrio, the many cytoplasmic proteases/peptidases can convert the peptides into individual amino acids to be used for biosynthesis of proteins and other cellular molecules, and for energy production. Interestingly, no genes for the enzymes of the urea cycle have been identified, while they have in other closely related δ proteobacteria, such as *Geobacter* and *Desulfovibrio*. Other essential nutrients, such as lipids, phosphates, nitrates, metal ions, anions, and ribonucleotides appear to be transported by dedicated ABC transporters or transporters of the MFS system. Although it has been reported that the bdellovibrios are capable of transporting ATP directly via a dedicated permease system (Ruby and McCabe 1986), the genome annotation of *Bdellovibrio bacteriovorus* HD 100 failed to identify any homologs of known ATP transport systems (Rendulic et al. 2004). However, there are a number of uncharacterized putative transporter genes in the genome that could serve this function. The repertoire of genes coding for transporters in the bdellovibrios includes the capability to transport multiple drugs, organic solvents, lipoproteins, polyamines, and, interestingly, two antimicrobial peptides (#CAE79032 and #CAE79033). The role these genes play in predation remains unknown, but it is interesting to speculate that they may be involved in the killing of the prey, or in the subsequent termination of prey cellular functions. How these antimicrobial peptides could be delivered to the prey cell is not understood at this time.

Information from the sequenced genome of the *B. bacteriovorus* HD 100 shows that it should have full metabolic capability for glycolysis, the TCA cycle, fatty acid β-oxidation, and oxidative respiration (Rendulic et al. 2004; KEGG). This is in agreement with previous studies showing that *B. bacteriovorus* 109J contains glycolytic enzymes (Hespell 1976) and a functional TCA cycle and electron transport chain (Rittenberg and Hespell, 1975). Even though they have the genes encoding for all of the glycolytic enzymes, these prior studies have shown these enzymes to be present at reduced levels in intraperiplasmically growing bdellovibrios (Hespell et al. 1973), suggesting that this pathway is probably not utilized by the BALOs for energy production via substrate-level phosphorylation, but for synthesis of organic precursors for biosynthesis. Evidence suggests that there is a preference for amino acids and components of nucleic acids for fueling energy production in the bdellovibrios (Hespell 1976). Production of ATP must proceed via oxidative phosphorylation, utilizing electron transport. The *B. bacteriovorus* HD 100 genome contains genes coding for cytochrome aa3 (*cta*A; #CAE80048), the

terminal aerobic electron acceptor, as well as for alternate cytochromes that may function under microaerophilic conditions (two cytochrome c types – #CAE78847 and #CAE80606; and a cytochrome bb3 type – #CAE80404). The presence of these alternate cytochromes may reveal that the bdellovibrios can change the composition of their electron transport chain based on oxygen tension, or perhaps use terminal acceptors other than oxygen. This functionality may be important for these predators, especially intraperiplasmically, where the oxygen tension may be low. Inorganic electron acceptors may also be made available during the breakdown of prey cellular material. The genome sequence reveals the presence of both *nrf* and *nor* genes, coding for a nitric oxide reductase (#CAE80395) and a nitrite reductase (#CAE80607), respectively. The biosynthesis of these electron transport components requires that the bdellovibrios have the capability of scavenging iron and other metals. Putative genes have been identified in the *B. bacteriovorus* HD 100 genome that would code for a TonB-like siderophore-mediated iron transporter and a receptor (#CAE80640 and #CAE79283), as well as iron transporters FeoA and B (#CAE79719 and #CAE79720) and Fut A (#CAE79001), an iron ABC transporter. There also appear to be genes encoding proteins for heme and aerobactin-like siderophore synthesis and for bacterioferritin (Rendulic et al. 2004). It is clear that the bdellovibrios have the potential for acquiring metals, transporting them, and synthesizing electron-transport components. It is assumed that these metals are being scavenged from the degraded prey cell constituents that then leak into the periplasm.

It is well known that BALOs cannot initiate DNA replication as attack phase cells, and that synthesis of DNA is limited to the intraperiplasmic growth phase. Although the BALOs are known to be capable of taking up and incorporating a number of different prey molecules intact (Kuenen and Rittenberg 1975; Rittenberg and Langley 1975; Ruby et al. 1985), there is still a need for de novo macromolecular synthesis. Significant *de novo* synthesis must occur in order to synthesize four to five copies of a genome the size of the *Bdellovibrio*'s. Sockett and Lambert (2004) suggested that a considerable amount of the prey's cytoplasmic sugars and amino acids would need to be used simply to synthesize the predator's nucleic acids. An analysis of the BALO genomes shows clearly that they have the genetic content necessary for the synthesis of nucleotides and for coding the proteins needed for synthesis of all the macromolecular components of the cell. The genome of *B. bacteriovorus* HD 100 contains genes homologous to those of other prokaryotes that encode polymerases and the accessory proteins necessary to replicate DNA.

The processes of cell growth and DNA replication are limited to a particular phase of the bacterium's life cycle, the intraperiplasmic growth phase. They represent an unusual reproductive strategy among the prokaryotes in that the intraperiplasmic cell grows into a filament, elongating into a cell that is several times unit length. The replicated DNA is then distributed within this filament prior to septum formation and fragmentation of the filament into

individual progeny. Even though the genome of *B. bacteriovorus* HD 100 contains genes coding for proteins that are typically involved in cell wall growth (eg. a full complement of peptidoglycan biosynthesis proteins), cell shape (MreB/Mrl homologs; #CAE79605 and #CAE77874), septum formation (eg. FtsZ; #CAE80944), and in genome segregation (e.g., homolog of SMC protein, #CAE79072), how they may function in positioning of multiple septa, and segregation of genomes in the elongated predator is not at all clear. Understanding this very unusual reproductive strategy could potentially provide important insights into the fundamental cellular mechanisms required for determining how growing cells establish symmetry, and how genomes are distributed in growing cells.

During, or immediately following, septation new flagella are synthesized and assembled just prior to the release of progeny cells from the bdelloplast. Since all of the BALOs require flagella for motility, and as a requirement for predation, it was expected that they would possess the genes required for both the synthesis of the flagellar components and its biogenesis. Using homology-based searches, almost all of the required flagellar genes have been identified. This includes the genes for structural components such as flagellin and the motor and basal body, as well as signaling systems to connect chemotaxis sensors to flagellar rotation (see below). There are also a number of open reading frames (ORFs) that have weaker homology to known genes related to motility. While some of these may be involved in functions specific to locating and attacking prey, it is likely that many (perhaps all) of them are simply less-well conserved forms of the "missing" flagellar genes (i.e., *flh*C and *flh*D) that encode transcriptional regulators of some flagellar operons that have yet to be identified.

Along with the identification of most of the genes encoding components of the flagellum, some interesting gene duplications have been identified. The *B. bacteriovorus* HD 100 genome contains six (or possibly seven) paralogous copies of the flagellin gene. These ORFs all encode polypeptides with greater than 70% similarity to each other over their entire length. One of these flagellin genes is predicted to be part of an operon with a gene encoding a flagellar-hook protein (FlgK) and a conserved hypothetical, transmembrane protein (#CAE78516). Four of the other six predicted flagellin genes are in two clusters with two genes each, which are not predicted to be part of an operon. One of these clusters is located within 6 kb of a *fli*DLS operon (2 *hag* genes, Bd0604 and Bd0606, coding for proteins #CAE78570 and #CAE78572). The remaining two paralogs are relatively distant from the other flagellin genes, but one is located within 11 kb of an operon containing 12 predicted *fli* genes. The polypeptides encoded by the six ORFs most similar to flagellin are all predicted to be just over 29 kD in size. This value agrees very well with the SDS-PAGE analysis of purified *B. bacteriovorus* 109J flagella by Thomashow and Rittenberg (1985), in which a large band of ca. 29.5 kD was observed. The seventh potential paralog (Bd0542), is predicted to encode a much larger polypeptide of nearly 40 kD (#CAE78515). No polypeptide of this size was

detected in their analysis, suggesting that it is either a very minor component of the flagellum or that it is involved in some unrelated function. These paralogous copies may make versions of flagellin that are used at different locations within the *B. bacteriovorus* flagellum, analogous to the situation found in *Caulobacter crescentus* (Leclerc et al. 1998). It is also worth noting that at least two other members of the δ-proteobacteria, *Desulfovibrio vulgaris* Hildenborough (Heidelberg et al. 2004) and *Geobacter sulfurreducens* (Methe et al. 2003), also have multiple paralogous copies of the flagellin gene (possibly five and three copies, respectively), which may be a common feature of this group.

Following flagellar biogenesis and septation of the growing filament into progeny predators, a new burst of glycanase activity breaks open the bdelloplast, freeing the newly formed attack-phase bdellovibrios (Thomashow and Rittenberg 1978c) to search out new prey. The identification of the specific gene or genes that code for this lytic enzyme among the ten predicted glycanases must await experiments that will study timed gene expression during the predatory cycle.

The switch, or signal, that initiates the processes of cellular growth and replication remains an unknown. Gray and Ruby (1990) have speculated that there are signal molecules from the prey that somehow regulate these processes. They suggest a two-signal model, the first signal regulating the switch from attack phase to growth phase, and the second initiating DNA replication. Prey-dependent BALOs can be made to elongate, and in some cases undergo fragmentation, when cellular extracts from prey cells are added to cultures. This appears to support their suggestion that there are signal molecules from the prey (Friedberg 1978; Horowitz et al. 1978). However, regulation of the predatory life cycle of the BALOs must involve additional regulatory systems, some of which presumably include two-component regulatory systems.

One of the problems in studying the genetics of the developmental process of the bdellovibrios has been their obligately predatory nature. All attempts to isolate BALOs from nature and grow them axenically on laboratory media have been unsuccessful (Friedberg 1978; Horowitz et al. 1974, Reiner and Shilo, 1969). Mutations in genes essential for predation would be lethal, and therefore undetectable. A number of prey-independent mutants have been isolated that have gained the ability to grow on complex media in the absence of prey. Although their growth pattern is similar to that seen intraperiplasmically and they are more easily manipulated, most have lost the ability to efficiently engage in predatory growth on living cells (Seidler and Starr 1969; Varon and Seijffers 1975). This conversion of prey-dependence (PD) to prey-independence (PI) appears to occur with single-hit kinetics (Seidler and Starr 1969), implying the mutation may be in a regulatory gene. Cotter and Thomashow (1992b), using a *Bdellovibrio* genomic library cloned into a suicide vector, were able to identify a single locus, called *hit*, that apparently controls the ability of PI bdellovibrios to grow predaceously. Rendulic et al.

(2004) located the *hit* locus as putative gene Bd0108, which is part of a transcripton coding for cell wall-associated proteins. They also suggest that this region may have been inserted into the *B. bacteriovorus* HD 100 genome between putative genes coding for chemotaxis and MCP proteins. What role this region may play in switching from PI to PD is, however, still open to question. Barel and Jurkevitch (2001) isolated seven independent axenic mutants, only three of which had mutations located in the *hit* region. Additionally, all seven PI mutants exhibited diverse morphologies and growth characteristics. The *hit* locus apparently codes for a small hypothetical polypeptide of 10.6 to 11 kDa (Cotter and Thomashow 1992b; Schwudke et al. 2005). The role that this small polypeptide plays in predation is unknown, but its size suggests that its function might be regulatory, perhaps being secreted. Expression analysis using RT-PCR has shown that transcripts for this peptide are highest in attack-phase and very early and late intraperiplasmic-phase cells, suggesting a role in attack and/or penetration of the prey (Schwudke et al. 2005). Clearly, the waters are still quite muddy when it comes to understanding what genes are involved in the change from PD to PI bdellovibrios, or vice versa. Certainly there is much work still to be done in order to understand the function(s) of these mutated sequences.

4 Genomics and Proteomics of *Bdellovibrio* and like Organisms

Since the only annotated BALO genome available at the time of this writing is that of *B. bacteriovorus* HD 100, a detailed comparison of several BALO genomes and proteomes is not yet possible. One interesting possibility of such an analysis would be the definition of a minimum "*Bdellovibrio*/BALO predator-specific genome/proteome". Our initial comparisons of the *B. bacteriovorus* and *B. marinus* genome sequences indicate that they are rather different in terms of their gene complement, yet, since both organisms are intraperiplasmic bacterial predators, a common set of orthologous genes required for this predatory lifestyle may exist between them (Fig. 3, stippled region). Such genes may well encode products involved in prey detection, attachment, penetration, bdelloplast formation and the mobilization of prey components for use by the growing predatory cell, as described above. These genes should easily be distinguished from the other set of common genes encoding proteins involved in central metabolic processes, DNA replication, transcription, etc. (Fig. 3, hatched region), based on the homology between these housekeeping type genes and known genes in other bacteria. Any remaining genes that are unique to either *B. bacteriovorus* or *B. marinus* would likely encode proteins involved in the specific environments that these bacterial predators encounter in their rather different niches. This section is intended to provide a preliminary analysis of the relationships between the

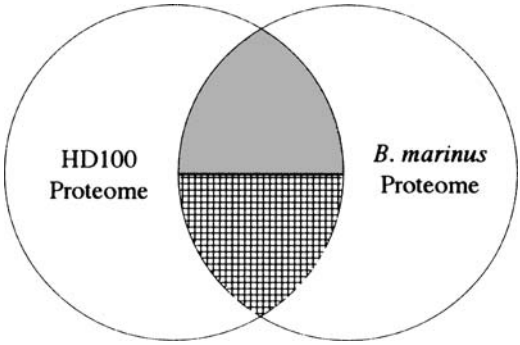


Fig. 3 Venn diagram showing the potential functional relationships between the proteomes of *Bdellovibrio bacteriovorus* HD 100 and *Bacteriovorax marinus* SJ. While there are a number of predicted polypeptides that are not shared between these two predators (*unshaded regions*; see text) there is a large common set (*stippled and hatched regions*). Of these common proteins, some are expected to be "housekeeping" in nature (*hatched region*) and thus similar to those found in other bacteria, while others may be required for the BALO lifestyle (*stippled region*). If this is the case, these common "predatory" proteins may define a minimum predatory proteome. The diagram is not to scale and areas do not reflect the abundance of different types of genes

genomes and proteomes of *B. bacteriovorus* HD 100, *Bdellovibrio* sp. strain W and *B. marinus* SJ. For simplicity, all comparisons will be made to the *B. bacteriovorus* HD 100 sequence and proteome.

Until the *B.* sp. strain W genome sequence is closed, a large-scale analysis is not possible, but comparisons of the larger contigs from the assembly (http://www.micro-gen.ouhsc.edu/b_bacter/b_bacter_home.htm) with the *B. bacteriovorus* HD 100 genome using BLASTN, shows that they are almost completely syntenic. Synteny refers to a region of similarity in terms of gene sequence, location, and orientation between two or more genomes (Bently and Parkhill 2004). This is quite surprising given their differences in morphology, prey-range, rDNA sequences, and the apparently unique ability of strain W to encyst (Tudor and Conti 1977a,b). Given the very high level of sequence identity observed between the *B. bacteriovorus* HD 100 and the *Bdellovibrio* sp. strain W genomes, it is expected that they will be almost completely syntenic with most orthologous genes in similar/identical locations, and in the same orientation. This was confirmed by TBLASTN (Altschul 1990) comparisons of the *B bacteriovorus* HD 100 genome against several of the largest available *B.* sp. strain W contigs (totaling more than 200 000 bp). Not only were almost all of the predicted ORFs conserved between both species, the deduced amino acid sequences were nearly identical over their entire length, with identities typically greater than 99%.

The very high conservation of both nucleotide and protein sequence between *B. bacteriovorus* HD 100 and *B.* sp. strain W is surprising since, as stated above, they differ in many ways, notably the ability of strain W to

encyst inside prey cells. We had presumed that these morphological and developmental differences would be reflected in substantial differences in genome and proteome composition, but there are several other possible explanations as to why this is not the case. First, it may be that other BALOs are capable of encystment, but that the frequency at which this occurs under laboratory conditions is extremely low. It is important to note that the signal(s) that trigger cyst formation are not known, and procedures to induce an attack-phase culture to encyst do not result in a consistently high percentage of cells encysting. An ability to encyst would help to explain how these predatory cells with such high metabolic rates manage to persist in environments with very low prey cell densities. If *B. bacteriovorus* HD 100 is actually capable of encystment, then comparative genomics/proteomics will probably not provide much insight into this process, as orthologs of the genes involved will be present in both species. The second possible explanation is that the ability to encyst is unique to *B.* sp. strain W, and was either recently acquired by this strain (perhaps through horizontal gene transfer in a manner analogous to that of the transfer of pathogenicity islands; Hacker and Kaper 2000), or that encystment evolved early in the *Bdellovibrio* lineage but was only retained in the line of descent, leading to strain W. If either of these occurred, a comparative genomics/proteomics approach should identify the genes/proteins involved since some of them should be unique to strain W. The third possibility is that the ability to encyst is unique to strain W and that it evolved by modification of genes involved in predatory growth. In this case, a comparative approach will again probably not be fruitful unless the evolution of this process has been driven by the formation of multiple paralogous genes in *B.* sp. strain W. Whatever mechanism is responsible for controlling encystment, genomics and proteomics can only provide candidate genes for examination by genetic and biochemical techniques (see below).

While *B. bacteriovorus* HD 100 and *B.* sp. strain W are quite similar in terms of their genomic sequence, they both differ significantly from *B. marinus* SJ. Only a comparatively few, short regions of similarity at the nucleotide sequence level could be found by BLASTN comparison of segments of the *B bacteriovorus* HD 100 and *B marinus* SJ genomes. A preliminary analysis comparing 1000 predicted proteins from the *B bacteriovorus* HD 100 proteome (the first 1000 polypeptides in the FASTA formatted proteome set available from the EMBL–EBI Integr8 database: http://www.ebi.ac.uk/integr8/) with the *B. marinus* sequence using TBLASTN (Altschul 1990) revealed some interesting similarities and differences. Nearly 25% of the tested HD 100 polypeptide sequences had a very good match (E value of less than 10^{-40}) with a predicted SJ gene product, and almost half of the *B bacteriovorus* HD 100 sequences returned a match with an E value of 10^{-10} or lower. On the other hand, 29% of the tested protein sequences returned no match to the *B. marinus* SJ genome by this method. Of these, more than 80% (233 out of 290) are ORFs encoding "hypothetical" proteins with no significant homology to

known proteins. In the absence of transcriptome data it is not possible to draw any conclusions regarding the nature of these hypothetical ORFs and it is likely that at least some of them are not actual genes. The verification of "real" genes (those that are actually transcribed into RNA) will be an important step in the comparison of the proteomes of these species (see below).

Given the observed differences between the proteomes of *B. marinus* SJ and *B. bacteriovorus* HD 100, it is not surprising that there appears to be little large-scale synteny between their genomes. While a full genome comparison is beyond the scope of this work, we were able to assess synteny by manually comparing assigned ORF identities and locations at several dozen randomly selected locations throughout the genomes. In a few instances, short areas of synteny were identified; however, all but one of these regions was restricted to predicted operons. The one exception is a region of ca. 35 kb (Fig. 4) on either side of the region that is predicted to contain *oriC* in *B. bacteriovorus* HD 100, based on GC skew (Rendulic, et al. 2004). We have identified four potential DnaA boxes in the region immediately upstream of *dnaA*, in both genomes, consistent with this being *oriC* (Mackiewicz et al. 2004).

Synteny in the *oriC* region is not uncommon; in fact, in many eubacteria the origin is flanked by *gidA, trmE, yidC, rnpA, rnpA, dnaA, dnaN, recF*, and *gyrB*, with *oriC* upstream of *dnaA* (Mackiewicz et al. 2004). Putative matches to all of these genes were found in this region, as well as ORFs for 17 others, including several encoding proteins involved in cell division (*parA* and *parB*) and the *atpFDAGBC* operon. One segment within this syntenic region in the *B. marinus* SJ genome, containing *risA–ribA–ribH*, is located more than 830 kb counterclockwise from this region, and is in an inverted orientation in *B. bacteriovorus* HD 100 and *B.* sp. strain W, relative to *B. marinus* (Fig. 4b). While apparent orthologs of most of the genes in this region that were not syntenic were identified in the other strains, their locations were scattered around the chromosome.

While it is likely that other regions of synteny exist between the *B. marinus* SJ and *B. bacteriovorus* HD 100 genomes, the observation that there are many non-syntenic regions argues for a relatively distant evolutionary relationship between them. This is consistent with the recent taxonomic change in which the genus *Bacteriovorax* was created and moved out of the genus *Bdellovibrio*, based on rDNA sequence analysis (Baer et al. 2000). The high level of sequence identity and synteny between *B. bacteriovorus* HD 100 and *B.* sp. strain W argues for a very close evolutionary relationship, which has been supported by rDNA analysis.

Another approach for comparing the *B. bacteriovorus* HD 100 and *B. marinus* SJ proteomes is to examine the predicted polypeptide sequences for regions of similarity to conserved protein domains and structures. A comparison of potential ORFs in both genomes against the protein family database (Pfam; Bateman et al. 2004) and to the clusters of orthologous groups of proteins database (COG; Tatusov et al. 2001) has been performed as part of

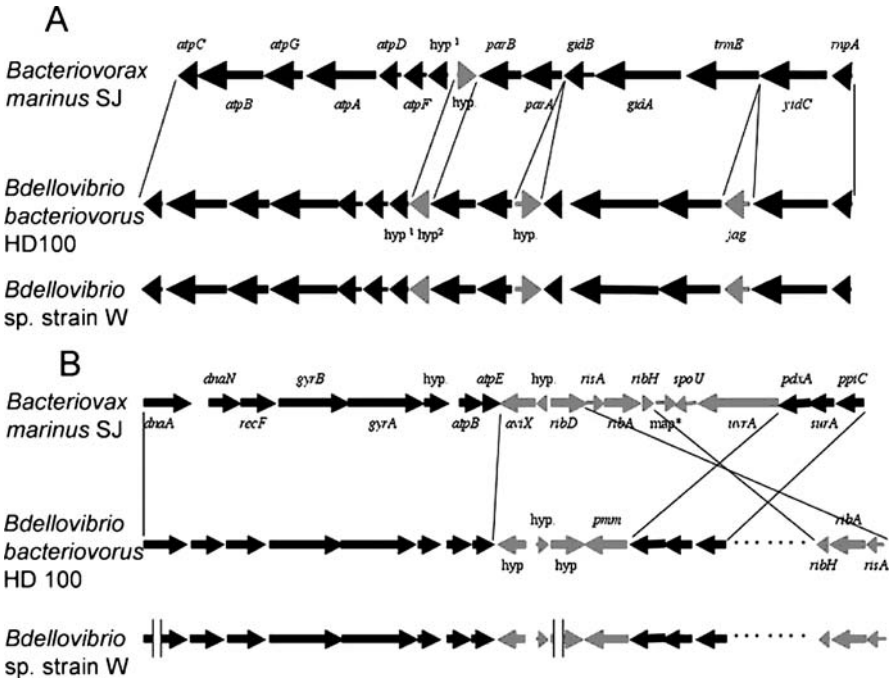


Fig. 4 A region of synteny between the chromosomes of *Bacteriovorax marinus* SJ, *Bdellovibrio bacteriovorus* HD 100 and *Bdellovibrio* sp. strain W in the region containing *oriC*. A region spanning nearly 35 kb, roughly centered on the predicted location of *oriC*, is syntenic between all three BALOs whose genomes have been sequenced. In both panels, orthologous genes are designated by *black arrows* while non-orthologous genes are shown as *gray arrows*. ORF positions for HD 100 and SJ are based on the auto-called ORF locations in PEDANT (Riley, et al. 2005). **a** shows a region of ca. 16 kb while **b** represents some 19 kb of sequence. The predicted *oriC* regions for all three chromosomes lies between *rnpA* in **a** and *dnaA* in **b**. The sequences for HD 100 and strain W are nearly identical throughout this region (BLASTN identity of 99%) except where the strain W sequence is broken at the end of several contigs (in **b** indicated by *hatch marks*). ORFs labeled "hyp". encode hypothetical proteins. In **a**, hyp^1 is an ORF found in all three genomes, which is similar to the amino terminal region of *atpF*, encoding the b subunit of ATP synthase (Walker, et al. 1982) which is leftward; hyp^2 refers to an ORF (*grey*) found only in HD 100 and strain W, which has similarity to a conserved hypothetical protein. In **b**, the ORF labeled "map*" in the SJ genome aligns by BLAST to putative methionine aminopeptidase genes. The region containing *risA–ribA–ribH* is inverted in *B. marinus* SJ relative to HD 100 and strain W. In these strains these three genes are located some 832 kb counterclockwise away from the predicted *oriC*

the PEDANT (protein extraction, description, and analysis tool; Riley et al. 2005) project at the Munich Information Center for Protein Sequences. Pfam and COG can be highly informative in terms of inferring protein function by matching a deduced amino acid sequence to existing protein families and, since their basis for detecting matches is somewhat different, they can

serve as complementary tools in genome/proteome analysis. The Pfam and COG analysis of *B. bacteriovorus* HD 100 and *B. marinus* SJ were undertaken for two reasons: first, to obtain an initial estimate of the possible number of genes/proteins from each of these predators that are involved in several key areas (chemotaxis, two-component signal transduction systems, alternate sigma factors, and transcriptional regulators); and secondly to compare the gene/protein complements of these two predators and other selected bacteria. Two other members of the δ-proteobacteria, *Geobacter sulfurreducens* and *Desulfovibrio vulgaris* subspecies *vulgaris*, were chosen for comparison, along with two bacteria with complex developmental cycles, *Bacillus subtilis* and *Caulobacter crescentus*, and two pathogens, *Escherichia coli* O157:H7 and *Bacteroides fragilis*, since the predatory lifestyle of the BALOs shares some common features with the parasitism of pathogens.

The comparison of the components of the chemotaxis systems (Table 1) is not meant to be an exhaustive one, but simply a basis for comparison in terms of the potential breadth of the chemotactic or, in the case of the BALOs, potential prey-taxis systems. One interesting observation from this comparison is that *B. bacteriovorus* HD 100 has potentially 2.5 times as many methyl-accepting chemotaxis proteins than *B. marinus* SJ. Unfortunately none of the published studies on chemotaxis in BALOs (see above) were conducted with either of these predators, so our ability to assign function to these systems is very limited. It has been suggested that BALOs may use chemotaxis systems to locate and move towards regions of high prey cell density, but this idea has not been thoroughly examined. Given the rapid loss of viability of attack-phase cells in culture, the presence of a prey-taxis system would help explain how BALOs can be found in environments where the prey cell density seems to be too low to support them (Varon and Ziegler 1978). It is also worth noting that the other two members of the δ-proteobacteria included in the comparison may have a considerably larger number of MCP genes than do either of the BALOs studied here.

A similar situation was found when potential members of two-component signaling systems were examined. As was the case with potential MCPs, *B. bacteriovorus* HD 100 appears to have a larger array (by about one third) of sensory transduction histidine kinases than does *B. marinus* SJ, yet they have about the same number of response regulator proteins that are the targets of histidine kinases. These systems serve as a primary mechanism for bacteria to respond to changes in environmental conditions (Stock et al. 2000), providing an excellent link between the extracellular environment and patterns of gene expression. As *B. bacteriovorus* 109J has been shown to undergo a complex, temporally-controlled pattern of gene expression during intraperiplasmic growth (McCann et al. 1998), we suspected that the BALOs might possess a large suite of these systems. While both *B. bacteriovorus* HD 100 and *B marinus* SJ have more predicted sensory kinases and response regulators than do *E. coli* and *B. subtilis* (Table 2), they are fairly comparable

Table 1 Comparison of predicted chemotaxis system protein complements among eight different bacterial species

	Bdellovibrio bacteriovorus	*Bacteriovorax marinus* SJ HD 100	*Geobacter sulfurreducens* PCA	*Desulfovibrio vulgaris*	*Bacillus subtilis* 168	*Caulobacter crescentus* CB15	*Escherichia coli* O157 H7 EDL933	*Bacteroides fragilis* NCTC 9434
Chemotaxis								
MCP	20	8	32	28	10	18	5	0
CheB	2	1	3	3	1	2	1	0
CheC	1	1	1	1	1	0	0	0
CheD	6	3	10	9	2	0	0	0
CheR	2	2	4	3	1	3	1	0
CheW	5	3	14	10	3	6	2	0
	36/0.010%	18/0.006%	64/0.0186%	54/0.0153%	18/0.004%	29/0.008%	9/0.002%	0/0%

The presence or absence of various proteins involved in chemotaxis was assessed in *Bdellovibrio bacteriovorus* HD 100, *Bacteriovorax marinus* SJ and six other bacteria base on sequence comparisons to both the protein family database (Pfam; Bateman, et al. 2004) and to the clusters of orthologous groups of proteins database (COG; Tatusov, et al. 2001). The other bacteria were chosen for their membership in the δ-proteobacteria along with BALOs (*Geobacter sulfurreducens* and *Desulfovibrio vulgaris*), because they have complex life cycles (*Caulobacter crescentus* and *Bacillus subtilis*) or because they are pathogens (*Escherichia coli* O157 and *Bacteroides fragilis*). The numbers at the bottom of each column are the total number of predicted genes encoding proteins involved in chemotaxis in that species and the percentage of the total proteome this represents

Table 2 Comparison of predicted two-component regulatory system complements among eight different bacterial species

	Bdellovibrio bacteriovorus	*Bacteriovorax marinus* SJ HD 100	*Geobacter sulfurreducens* PCA	*Desulfovibrio vulgaris*	*Bacillus subtilis* 168	*Caulobacter crescentus* CB15	*Escherichia coli* O157 H7 EDL933	*Bacteroides fragilis* NCTC 9434
COG0642 Sensory transduction histidine kinases[1]	63	47	105	78	42	60	31	64
COG0745 Response regulators [1]	37	43	59	52	34	28	38	24
	100/0.028%	90/0.029%	164/0.048%	130/0.037%	76/0.019%	88/0.024%	69/0.013%	88/0.021%

The number of proteins involved in signal transduction as part of a two-component system was assessed in *Bdellovibrio bacteriovorus* HD 100, *Bacteriovorax marinus* SJ and six other bacteria base on sequence comparisons to the clusters of orthologous groups of proteins database (COG; Tatusov, et al. 2001). The other bacteria were chosen for their membership in the δ-proteobacteria along with BALOs (*Geobacter sulfurreducens* and *Desulfovibrio vulgaris*), because they have complex life cycles (*Caulobacter crescentus* and *Bacillus subtilis*) or because they are pathogens (*Escherichia coli* O157 and *Bacteroides fragilis*). The numbers at the bottom of each column are the total number of predicted genes encoding proteins involved in chemotaxis in that species and the percentage of the total proteome this represents

[1] The numbers reported include all of the ORFs assigned to the specified COG, even when they may encode a protein involved in another signal transduction system (i.e., members of the chemotaxis signaling pathway)

Table 3 Comparison of predicted Sigma factor regulatory system complements among eight different bacterial species

	Bdellovibrio bacteriovorus	*Bacteriovorax marinus* SJ HD 100	*Geobacter sulfurreducens* PCA	*Desulfovibrio vulgaris*	*Bacillus subtilis* 168	*Caulobacter crescentus* CB15	*Escherichia coli* O157 H7 EDL933	*Bacteroides fragilis* NCTC 9434
Sigma factors								
σ54 type	1	1	1	1	1	1	1	1
σ70 type	5	5	6	3	13	2	4	16
ECF type	1	1	0	0	3	11	1	26
Anti-σ factors	1	0	1	1	0	0	0	0
	8/0.002%	7/0.002%	8/0.002%	5/0.001%	17/0.004%	14/0.004%	6/0.001%	43/0.010%

The number of predicted sigma factors and anti-sigma factors was assessed in *Bdellovibrio bacteriovorus* HD 100, *Bacteriovorax marinus* SJ and six other bacteria base on sequence comparisons to both the protein family database (Pfam; Bateman, et al. 2004) and to the clusters of orthologous groups of proteins database (COG; Tatusov, et al. 2001). The other bacteria were chosen for their membership in the δ-proteobacteria along with BALOs (*Geobacter sulfurreducens* and *Desulfovibrio vulgaris*), because they have complex life cycles (*Caulobacter crescentus* and *Bacillus subtilis*) or because they are pathogens (*Escherichia coli* O157 and *Bacteroides fragilis*). The numbers at the bottom of each column are the total number of predicted genes encoding proteins involved in chemotaxis in that species and the percentage of the total proteome this represents

to the numbers predicted for *C. crescentus* and *B. fragilis*. This does not support a specialized function for these systems during intraperiplasmic growth in the BALOs. It is worth noting that, once again, the other two members of the δ-proteobacteria studied have a substantially greater potential array of these genes than both of these intraperiplasmic predators, the significance of which is unclear.

Another mechanism for coordinating the temporal pattern of gene expression observed during intraperiplasmic growth would be the use of alternate sigma factors in a cascade, such as observed in *B. subtilis* during sporulation (Piggot and Hilberty 2004). Indeed, this has already been suggested as a potential mechanism used by the BALOs (McCann et al. 1998; Rendulic et al. 2004). Unfortunately, this does not seem to be the case. Both *B. bacteriovorus* HD 100 and *B. marinus* SJ appear to have sigma factor complements very similar to those found in *E. coli* (Table 3) with one σ54-type, an RpoN ortholog, presumably regulating transcription of genes involved in nitrogen limitation (Reitzer 2003). They also appear to have five paralogous σ70-type genes and one extracytoplasmic function (ECF; Helmman 2002). As the ECF-type sigma factors have been shown to control such processes as cell wall biosynthesis, protein folding, and pathogenesis (Paivio 2001), we suspected that the BALOs might possess multiple sigma factors for control of gene expression during intraperiplasmic growth. Unfortunately only one match to an ECF was found in both predatory genomes by COG analysis. Comparing the total number of potential sigma factors identified in the BALOs by this approach (seven each) with the number of known or predicted alternate sigma factors in *C. crescentus* (14), *B. subtilis* (17), and *B. fragilis* (43) suggests that these BALOs do not make extensive use of alternate sigma factors during their developmental cycle. Indeed, the predicted sigma factor complements for both *B bacteriovorus* HD 100 and *B. marinus* SJ are basically the same as those in *E. coli* with one σ54-type and one ECF-type (presumably an ortholog of the *E. coli* σE; Erickson and Gross 1989) involved in responding to envelop/periplasmic stresses, and five σ70/32 class members. Given the need for sigma factors in "housekeeping" gene expression, heat-shock, etc., the genomes of these predators do not seem to possess the genes required in order to use an alternate sigma factor cascade to direct differential expression governing intraperiplasmic growth.

Matches to known and putative transcription regulatory proteins in the COG database were also examined (Table 4). Similar to what we had anticipated regarding the two-component regulatory systems and sigma factor families, we had expected to find a large number of transcriptional regulators. However, this was not the case. In fact, with the exception of *B. fragilis*, *B. bacteriovorus* HD 100 and *B. marinus* SJ have the fewest number of ORFs (72 and 67, respectively) matching known transcription regulatory proteins among the eight bacteria we studied. When these ORFs are considered as a percentage of the total number of predicted ORFs (taking into considera-

Table 4 Comparison of predicted transcriptional regulators among eight different bacterial species

	Bdellovibrio bacteriovorus	*Bacteriovorax marinus* SJ HD 100	*Geobacter sulfurreducens* PCA	*Desulfovibrio vulgaris*	*Bacillus subtilis* 168	*Caulobacter crescentus* CB15	*Escherichia coli* O157 H7 EDL933	*Bacteroides fragilis* NCTC 9434
COG0583 LysR	22	17	10	10	26	11	50	4
COG0640 ArsR	5	11(1)	13	8	25(1)	8	2	4
COG0789 SoxR	5	7	2	2	13	4	6	4
COG1167 Aro8	1	4(2)	10	10(1)	25	5	4	6
COG1221 PspF	18	13	35	41	8	1	7	10
COG1309 AcrR	7	3	9	7	20	19	13	4
COG1316 LytR	0	0	0	0	3	0	0	0
COG1321 TroR	2(1)	(4)	1(1)	2(2)	4(4)	0	1	0
COG1329 CarD	1	1	0	1	1	1	0	0
COG1339 FadR	0	0	0	0	1(1)	0	0	0
COG1349 GlpR	(2)	(4)	2	0	6(9)	0	10	1
COG1414 IclR	(2)	(2)	1(7)	(3)	(3)	1	5	0
COG1420 HrcA	(1)	(1)	1	1	(1)	1	0	0
COG1522 Lrp	(2)	2(5)	(8)	2(3)	10(6)	8	3	3(1)
COG1609 PurR	3(1)	2	4(1)	4	15(8)	13(1)	25	5(2)
COG1737 RpiR	1	3	1	1	5	1	6	2
COG1802 GntR	0	0	(5)	(3)	1(10)	6(2)	11	(3)

Table 4 continued

	Bdellovibrio bacteriovorus	*Bacteriovorax marinus* SJ HD 100	*Geobacter sulfurreducens* PCA	*Desulfovibrio vulgaris*	*Bacillus subtilis* 168	*Caulobacter crescentus* CB15	*Escherichia coli* O157 H7 EDL933	*Bacteroides fragilis* NCTC 9434
COG1846 MarR	1(5)	1(4)	3(4)	4(2)	17(17)	11(1)	4	4(3)
COG1940 NagC	1	(2)	1(2)	(1)	4(1)	3	6	2
COG1974 LexA	1	1(3)	2(2)	2(5)	1(1)	1	4	1
COG2186 FadR	1	(2)	(4)	(3)	(17)	1(6)	(11)	(3)
COG2188 PhnF	0	(2)	(4)	(3)	(15)	2(3)	6	(3)
COG2808 PaiB	1	1	0	0	1	1	0	0
COG2909 MalT	2	1	0	0	5	1	2	1
COG3283 TyrR	(9)	(8)	(5)	(14)	(3)	0	1(1)	(1)
Total ORFs	72/0.020%	67/0.022%	95/0.028%	95/0.027%	191/0.047%	99/0.027%	166/0.031%	51/0.012%

The number of proteins predicted to be transcriptional regulators was assessed in *Bdellovibrio bacteriovorus* HD 100, *Bacteriovorax marinus* SJ and six other bacteria base on sequence comparisons to the clusters of orthologous groups of proteins database (COG; Tatusov, et al. 2001). The other bacteria were chosen for their membership in the δ-proteobacteria along with BALOs (*Geobacter sulfurreducens* and *Desulfovibrio vulgaris*), because they have complex life cycles (*Caulobacter crescentus* and *Bacillus subtilis*) or because they are pathogens (*Escherichia coli* O157 and *Bacteroides fragilis*). Numbers in parentheses indicate that those ORFs also belong to another COG previously listed on the table. The numbers at the bottom of each column are the total number of predicted genes encoding proteins involved in chemotaxis in that species and the percentage of the total proteome this represents

tion the differences in genome sizes) the BALOs again are at the bottom of the list (0.02% for HD 100 and 0.022% for SJ), with only *B. fragilis* (0.012%) having a lower percentage of its genome/proteome dedicated to transcription factors. But the case of *B. fragilis* must be considered in light of its very large number of predicted sigma factors (43; Table 2), nearly seven times the number predicted for *B. bacteriovorus* HD 100 and *B. marinus* SJ. One group of transcription factors, the PspF family, is of particular interest. PspF was identified in *E. coli* as a regulator of the phage-shock operon (Brissette et al. 1990) and subsequently found to be a member of the AAA (ATPases Associated with various cellular Activities) class of regulators of σ54-RNAP (Jovanovic et al. 1996). PspF-regulated genes have been shown to be involved in protein secretion (Nishiyama et al. 1999), and virulence in the intracellular parasites *Yersinia enterocolitica* (Darwin and Miller 2001) and *Salmonella enterica* (Eriksson et al. 2003). The possible roles of these σ54-RNAP regulators becomes even more interesting (and less clear) when one considers that about half of the genes known to be transcribed by σ54-RNAP are not involved with nitrogen limitation but are instead involved in responses to other environmental stresses (Reitzer 2003). While the number of PspF-family members predicted for both *B. bacteriovorus* HD 100 and *B. marinus* SJ is less than that predicted for either *G. sulfurreducens* or *D. vulgaris* (Table 4), it is greater than the number predicted for all of the other bacteria examined in this analysis. Perhaps this family of transcription factors may be playing a role in controlling the genes encoding proteins secreted into the prey cell periplasm and cytoplasm during intraperiplasmic growth. Additionally, the presence of a relatively large number of predicted LysR-type transcription factors is not surprising as they are thought to be the largest family of prokaryotic DNA-binding proteins (Zaim and Kierzek 2003). This is one of the few instances where the predatory BALO genomes actually have more predicted genes of this type than do either *G. sulfurreducens* or *D. vulgaris*. Unfortunately, little insight can be gleaned as to the possible function of the genes these factors might regulate in the BALOs, since the LysR-type transcription factors regulate a wide variety of operons involved in nitrogen fixation, oxidative stress, and virulence (Schell 1993).

5 Conclusions and Perspectives

As powerful as genomic and proteomic analyses can be, there are obvious limitations to them in attempting to pinpoint predatory genes and their regulation in the BALOs. Some of these problems will be overcome when more predatory genomes have been sequenced and thoroughly annotated. A comparative genome analysis of multiple genomes from BALOs should yield more insights into the strategies of these obligate intraperiplasmic predators. This

type of comparative analysis could perhaps identify a shared set of genes that are involved in predation, and also help resolve the question of the evolutionary relationship within this unique group of prokaryotes.

Although there are limitations to the usefulness of genomic analysis, experimental methods are available and should be utilized to address some of the questions pertaining to the BALO predatory life style. One obvious approach is to systematically examine transcriptional activity (the transcriptome; Velculescu et al. 1997) throughout the entire life-cycle. The ease with which synchronously attacking and developing cultures of many of the BALOs can be prepared (Thomashow and Rittenberg 1978a) will permit isolation of total RNAs at different points in the life cycle. Further, the availability of several genomic sequences will permit the fabrication of DNA microarrays of all potential ORFs. This temporal analysis of gene expression, analogous to the protein synthesis profile already done for *B. bacteriovorus* 109J (McCann et al. 1998), will provide an essential starting point in assigning specific functions related to the predatory life style to individual genes. One of the first pieces of information it will provide will be a demarcation between genes transcribed at high levels in attack-phase cells versus genes transcribed at high levels during intraperiplasmic growth. Genes expressed at high levels only during the attack phase will likely produce proteins involved in finding and attacking prey (chemotaxis, motility, prey cell receptors, etc.) while those expressed at high levels only during the intraperiplasmic growth phase will likely encode products involved in utilization of prey cell components, growth of the bdellovibrio filament, formation of progeny cells, etc. It is likely that gene expression in both of these phases will be further temporally defined with genes expressed early in the intraperiplasmic phase encoding proteins involved in prey cell penetration and bdelloplast formation, while genes expressed later will encode proteins involved in the mobilization and utilization of prey components for growth. For example, at least one glycanase and one peptidase are expected to be expressed either during or before prey cell penetration, as these enzymatic activities have been shown to be involved in penetration of the cell wall (Thomashow and Rittenberg 1978a; Tudor et al. 1990). A genomics approach has already identified ten potential glycanase genes and more than 150 proteases/peptidase genes (see above). The microarray analysis will show which (if any) of these putative glycanase and peptidase genes have a pattern of expression consistent with the known time course of enzymatic activity. Similarly, other genes producing proteins involved in bdelloplast formation, prey cell degradation, utilization of prey cell components, etc., would be expected to be maximally expressed later in the growth phase.

While the use of genomic analysis and global expression patterns as described above are powerful tools, they are limited in defining gene function, especially in regard to predicted genes with little or no detectable similarity to known genes. Developing methods for the generation and isolation of

mutants that are deficient in predation would be a direct way of identifying predation-specific genes. A number of attempts have been made to move exogenous DNA into bdellovibrios, but most without success. In 1992, Cotter and Thomashow (1992a) were the first to demonstrate that conjugative plasmids could be introduced into both prey-dependent and prey-independent bdellovibrios by conjugation with *E. coli*. More recently, Martin (2002) reported that a variety of transposons could be conjugatively transferred into *Bdellovibrio* and inserted into the predator's genome by transposition. Reporter genes, such as antibiotic resistance markers and β-galactosidase, were shown to be expressed in the resultant transconjugants. However, Martin's work focused on the use of prey-independent mutants and simply demonstrated that transposition could occur in BALOs.

A handful of facultative mutants of BALOs, with the capacity to grow when cultivated either on prey or on complex media, have been isolated over the years (Diedrich et al. 1970; Pritchard et al. 1975). Most of these mutants appear inefficient in switching from one form of growth to the other. This defect has hampered attempts to use mutagenesis to search for genes that are essential for predation. Our laboratory has recently developed a protocol that permits the introduction of transposons into facultative bdellovibrios, and the subsequent screening of large numbers of transposon-insertion mutants for predation using two different facultative strains of *B. bacteriovorus* 109J (manuscript in preparation). Expression of suspected predatory genes can easily be analyzed by looking at temporal gene expression using the real-time polymerase chain reaction (RT-PCR) or Northern blots. These facultative predators thus have the potential for use as a system for identifying genes essential for predatory-growth but dispensable for prey-independent growth. A facultative mutant defective in a gene required only for predatory growth (such as a mutant unable to express the enzymes required for prey cell penetration) would still be viable and could be grown in complex medium, but not on prey cells. This could be a very fruitful approach for the identification of genes necessary for predacious growth only.

The opportunities afforded by the availability of the genomic sequences of these BALOs, along with the already developed molecular genetic tools, are enormous. We are on the threshold of a new wave of exploration into the genetics, biochemistry, and physiology of these unique bacterial predators. The potential applications of this understanding are far reaching and only begin with the exploration of this system in terms of its behavior and developmental biology. The BALOs will provide a point of comparison for the other types of bacterial predators and it seems likely that many similarities and differences will be found. Further, the potential applications for BALOs and the possible utilization of their predation systems is only beginning to be realized. BALOs (especially those capable of encystment like *Bdellovibrio species* strain W) may well have utility in limiting or eradicating bacterial pathogens in food processing and industrial settings. They may

similarly be effective against Gram-negative plant pathogens in the field, as many of the previously isolated BALOs have prey ranges that include common pathogens such as *Pseudomonas* and *Erwinia*. BALOs may also have potential as biocontrol agents in the event of a bacterial pathogen being used as a bioterrorism weapon. It also seems likely, by exploring the way in which the BALOs manipulate prey cell membranes and constituents, that useful tools for modifying and controlling the structure and behavior of other Gram-negative bacteria may be developed, which could lead to potential use as therapeutic agents (Sockett and Lambert 2004). While much of this remains speculative, we obviously have a great deal to learn from these fascinating creatures.

References

Abram D, Davis BK (1970) Structural properties and features of parasitic *Bdellovibrio bacteriovorus*. J Bacteriol 104:948–965

Abram D, Castro e Melo J, Chou D (1974) Penetration of *Bdellovibrio bacteriovorus* into host cells. J Bacteriol 118:663–680

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Baer M, Ravel J, Chun J, Hill R, Williams HN (2000) A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax* gen. nov. as *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* com. nov., respectively. Int J Syst Evol Microbiol 50:219–224

Barel G, Jurkevitch E (2001) Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch Microbiol 176:211–216

Barel G, Sirota A, Volpin H, Jurkevitch E (2005) Fate of predator and prey proteins during growth of *Bdellovibrio bacteriovorus* on*Escherichia coli* and *Pseudomonas syringae* prey. J Bacteriol 187:329–335

Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S, Khanna A, Marshall M, Moxon S, Sonnhammer EL, Studholme DJ, Yeats C, Eddy SR (2004) The Pfam protein families database. Nucleic Acids Res 32:D138–D141

Beck S, Schwudke D, Strauch E, Appel B, Linscheid M (2004) *Bdellovibrio bacteriovorus* strains produce a novel major outer membrane protein during predacious growth in the periplasm of prey bacteria. J Bacteriol 186:2766–2773

Beck S, Schwudke D, Appel B, Linscheid M, Strauch E (2005) Characterization of outer membrane protein fractions of *Bdellovibrionales*. FEMS Microbiol Lett 243:211–217

Bentley SD, Parkhill J (2004) Comparative genomic structure of prokaryotes. Ann Rev Genet 38:771–791

Brissette JL, Russel M, Weiner L, Model P (1990) Phage shock protein, a stress protein of *Escherichia coli*. Proc Nat Acad Sci USA 87:862–866

Cotter TW, Thomashow MF (1992a) A conjugation procedure for *Bdellovibrio bacteriovorus* and its use to identify DNA sequences that enhance the plaque-forming ability of a spontaneous host-independent mutant. J Bacteriol 174:6011–6017

Cotter TW, Thomashow MF (1992b) Identification of a *Bdellovibrio bacteriovorus* genetic locus, *hit*, associated with the host-independent phenotype. J Bacteriol 174: 6018–6024

Cover WH, Rittenberg SC (1984) Change in the surface hydrophobicity of substrate cells during bdelloplast formation by *Bdellovibrio bacteriovorus* 109J. J Bacteriol 157:391–397

Cover WH, Martinez RJ, Rittenberg SC (1984) Permeability of the boundary layers of *Bdellovibrio bacteriovorus* 109J and its bdelloplasts to small hydrophobic molecules. J Bacteriol 157:385–390

Darwin A, Miller V (2001) The *psp* locus of *Yersinia enterocolitica* is required for virulence and for growth in vitro when the Ysc type III secretion system is produced. Mol Microbiol 39:429–444

Davidov Y, Jurkevitch E (2004) Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax–Peredibacter* clade as *Bacteriovoracaceae* fam. nov. Int J Syst Evol Microbiol 54:1439–1452

Diedrich DL, Denny CF, Hashimoto T, Conti SF (1970) Facultatively parasitic strain of *Bdellovibrio bacteriovorus*. J Bacteriol 101:989–996

Diedrich DL, Duran CP, Conti SF (1984) Acquisition of *Escherichia coli* outer membrane proteins by *Bdellovibrio* sp. strain 109D. J Bacteriol 159:329–334

Diedrich DL, Portnoy CA, Conti SF (1983) *Bdellovibrio* possesses a prey-derived OmpF protein in is outer membrane. Current Microbiol 8:51–56

Erickson JW, Gross CA (1989) Identification of the σE subunit of *Escherichia coli* RNA polymerase: a second alternate factor involved in high-temperature gene expression. Genes Dev 3:146271

Eriksson S, Lucchini S, Thompson A, Rhen M, Hinton JC (2003) Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. Mol Microbiol 47:103–118

Flannagan RS, Valvano MA, Koval SF (2004) Downregulation of the *mot*A gen delays the escape of the obligate predator *Bdellovibrio bacteriovorus* 109J from bdelloplasts of bacterial prey cells. Microbiol 150:649–656

Friedberg D (1978) Growth of host-dependent *Bdellovibrio* in host cell-free system. Arch Microbiol 116:185–190

Galdiero F (1975) Membrane damage and incorporation of *Escherichia coli* components into *Bdellovibrio bacteriovorus*. Zbl Bakt Hyg I Abt 230:203–209

Garrity GM, Johnson KL, Bell JA, Searles DB (2002) Taxonomic outline of the prokaryotes. In: Bergey's manual of systemic bacteriology, 2nd edn. Springer, Berlin Heidelberg New York

Gray KM, Ruby EG (1990) Prey-derived signals regulating duration of the developmental growth phase of *Bdellovibrio bacteriovorus*. J Bacteriol 172:4002–4007

Hacker J, Kaper JB (2000) Pathogenicity islands and the evolution of microbes. Ann Rev Microbiol 54:641–679

Heidelberg JF, Seshadri R, Haveman SA, Hemme CL, Paulsen IT, Kolonay JF, Eisen JA, Ward N, Methe B, Brinkac LM, Daugherty SC, Deboy RT, Dodson RJ, Durkin AS, Madupu R, Nelson WC, Sullivan SA, Fouts D, Haft DH, Selengut J, Peterson JD, Davidsen TM, Zafar N, Zhou L, Radune D, Dimitrov G, Hance M, Tran K, Khouri H, Gill J, Utterback TR, Feldblyum TV, Wall JD, Voordouw G, Fraser CM (2004) The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. Nat Biotechnol 22:554–559

Helman JD (2002) The extracytoplasmic function (ECF) sigma factors. Adv Microb Physiol 46:47–51

Hespell RB (1976) Glycolytic and tricarboxylic acid cycle enzyme activities during intraperiplasmic growth of *Bdellovibrio bacteriovorus* on *Escherichia coli*. J Bacteriol 128:677–680

Hespell RB, Rosson RA, Thomashow MF, Rittenberg SC (1973) Respiration of *Bdellovibrio bacteriovorus* strain 109J and its energy substrates for intraperiplasmic growth. J Bacteriol 113:1280–1288

Horowitz AT, Kessel M, Shilo M (1974) Growth cycle of predaceous bdellovibrios in a host-free extract system and some properties of the host extract. J Bacteriol 117:270–282

Jovanovic G, Weiner L, Model P (1996) Identification, nucleotide sequence, and characterization of PspF, the transcriptional activator of the *Escherichia coli* stress-induced *psp* operon. J Bacteriol 178:1936–1945

Kristich CJ, Ordal GW (2004) Analysis of chimeric chemoreceptors in *Bacillus subtilis* reveals a role for CheD in the function of the McpC HAMP domain. J Bacteriol 186:5950–5955

Kuenen JG, Rittenberg SC (1975) Incorporation of long-chain fatty acids of the substrate organism by *Bdellovibrio bacteriovorus* during intraperiplasmic growth. J Bacteriol 121:1145–1157

LaMarre AG, Straley SC, Conti SF (1977) Chemotaxis toward amino acids by *Bdellovibrio bacteriovorus*. J Bacteriol 131:201–207

Lambert C, Smith MCM, Sockett RE (2003) A novel assay to monitor predator–prey interactions for *Bdellovibrio bacteriovorus* 109J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol 5:127–132

Leclerc G, Wang SP, Ely B (1998) A new class of *Caulobacter crescentus* flagellar genes. J Bacteriol 180:5010–5019

Mackiewicz P, Zakrzewska-Czerwinska J, Zawilak A, Dudek MR, Cebrat S (2004) Where does bacterial replication start? Rules for predicting the oriC region. Nucleic Acids Res 32:3781–3791

Martin M (2002) Predatory prokaryotes: an emerging research opportunity. J Mol Microbiol Biotechnol 4:467–477

Matin A, Rittenberg SC (1972) Kinetics of deoxyribonucleic acid destruction and synthesis during growth of *Bdellovibrio bacteriovorus* strain 109D *on Pseudomonas putida* and *Escherichia coli*. J Bacteriol 111:664–673

McCann MP, Solimeo HT, Cusick F, Panunti B, McCullen C (1998) Developmentally regulated protein synthesis during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. Can J Microbiol 44:50–55

Methe BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, Heidelberg JF, Wu D, Wu M, Ward N, Beanan MJ, Dodson RJ, Madupu R, Brinkac LM, Daugherty SC, DeBoy RT, Durkin AS, Gwinn M, Kolonay JF, Sullivan SA, Haft DH, Selengut J, Davidsen TM, Zafar N, White O, Tran B, Romero C, Forberger HA, Weidman J, Khouri H, Feldblyum TV, Utterback TR, Van Aken SE, Lovley DR, Fraser CM (2003) Genome of *Geobacter sulfurreducens:* metal reduction in subsurface environments. Science 302:1967–1969

Min B, Kitabatake M, Polycarpo C, Pelaschier J, Raczniak G, Ruan B, Kobayashi H, Namgoong S, Soll D (2003) Protein synthesis in *Escherichia coli* with mischarged tRNA. J Bacteriol 185:3524–3526

Nishiyama K, Fukuda A, Morita K, Tokuda H (1999) Membrane deinsertion of SecA underlying proton motive force-dependent stimulation of protein translocation. EMBO J 18:1049–1058

Piggot PJ, Hilbert DW (2004) Sporulation of *Bacillus subtilis*. Curr Opin Microbiol 7:579–586

Pritchard MA, Langley D, Rittenberg SC (1975) Effects of methotrexate on intraperiplasmic and axenic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 121:1131–1136

Raivio TL, Silhavy TJ (2001) Periplasmic stress and ECF sigma factors. Ann Rev Microbiol 55:591–624

Rayner JR, Cover WH, Martinez RJ, Rittenberg SC (1985) *Bdellovibrio bacteriovorus* synthesizes an OmpF-like outer membrane protein during both axenic and intraperiplasmic growth. J Bacteriol 163:595–599

Reiner AM, Shilo M (1969) Host-independent growth of *Bdellovibrio bacteriovorus* in microbial extracts. J Gen Microbiol 59:401–410

Reitzer L (2003) Nitrogen assimilation and global regulation in *Escherichia coli*. Ann Rev Microbiol 57:155–176

Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans KJ, Goesmann A, Meyr F, Sockett RE, Schuster SC (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692

Riley ML, Schmidt T, Wagner C, Mewes HW, Frishman D (2005) The PEDANT genome database in 2005. Nucleic Acids Res 33:D308–310

Rittenberg SC, Hespell RB (1975) Energy efficiency of intraperiplasmic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 121:1158–1165

Rittenberg SC, Langley D (1975) Utilization of nucleoside monophosphates per se for intraperiplasmic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 121:1137–1144

Rittenberg SC, Shilo M (1970) Early host damage in the infection cycle of *Bdellovibrio bacteriovorus*. J Bacteriol 102:149–160

Rittenberg SC, Thomashow M (1979) Intraperiplasmic growth – life in a cozy environment. In: Schlessinger D (ed) Microbiology 1979. American Society for Microbiology, Washington, DC, pp 80–85

Romo AJ, Ruby EG, Saier MH (1992) Effect of *Bdellovibrio bacteriovorus* infection on the phosphoenolpyruvate: sugar phosphotransferase system in *Escherichia coli*: evidence for activation of cytoplasmic proteolysis. Res Microbiol 143:5–14

Rosson RA, Rittenberg SC (1979) Regulated breakdown of *Escherichia coli* deoxyribonucleic acid during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. J Bacteriol 140:620–633

Ruby EG (1991) The genus *Bdellovibrio*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer, Berlin Heidelberg New York

Ruby EG, McCabe JB, Barke JI (1985) Uptake of intact nucleoside monophosphates by *Bdellovibrio bacteriovorus* 109J. J Bacteriol 163:1087–1094

Ruby EG, McCabe JB (1986) An ATP transport system in the intracellular bacterium *Bdellovibrio bacteriovorus*. J Bacteriol 167:1066–1070

Saier MH (1994) Protein uptake into *E. coli* during *Bdellovibrio* infection. A process of reverse secretion? FEBS Lett 337:14–17

Schelling M, Conti SF (1986) Host receptor sites involved in the attachment of *Bdellovibrio bacteriovorus* and *Bdellovibrio stolpii*. FEMS Microbiol Lett 36:319–323

Schell MA (1993) Molecular biology of the LysR family of transcriptional regulators. Ann Rev Microbiol 47:597–626

Schwudke D, Bernhardt A, Beck S, Madela K, Linscheid MW, Appel B, Strauch E (2005) Transcriptional activity of the host-interaction locus and a putative pilin gene of *Bdellovibrio bacteriovorus* in the predatory life cycle. Curr Microbiol 51:310–316

Seidler RJ, Starr MP (1969) Factors affecting the intracellular parasitic growth of *Bdellovibrio bacteriovorus* developing within *Escherichia coli*. J Bacteriol 97:912–923

Snyder AR, Williams HN, Baer ML, Walker KE, Stine OC (2002) 16S rDNA sequence analysis of environmental *Bdellovibrio* and like organisms (BALO) reveals extensive diversity. Int J Syst Evol Microbiol 52:2089–2094

Sockett RE, Lambert C (2004) *Bdellovibrio* as therapeutic agents: a predatory renaissance? Nature Rev Microbiol 2:669–675

Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Ann Rev Biochem 69:183–215

Straley SC, Conti SF (1974) Chemotaxis in *Bdellovibrio bacteriovorus*. J Bacteriol 120:549–551

Straley SC, Conti SF (1977). Chemotaxis by *Bdellovibrio bacteriovorus* toward prey. J Bacteriol 132:628–640

Straley SC, LaMarre AG, Lawrence LJ, Conti SF (1979) Chemotaxis of *Bdellovibrio bacteriovorus* toward pure compounds. J Bacteriol 140:634–642

Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV (2001) The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res 29:22–8

Thomashow MF, Rittenberg SC (1978a) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: solubilization of *Escherichia coli* peptidoglycan. J Bacteriol 135:998–1007

Thomashow MF, Rittenberg SC (1978b) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: attachment of long-chain fatty acids to *Escherichia coli* peptidoglycan. J Bacteriol 135:1015–1023

Thomashow MF, Rittenberg SC (1978c) Intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J: N-deacetylation of *Escherichia coli* peptidoglycan amino sugars. J Bacteriol 135:1008–1014

Thomashow MF, Rittenberg SC (1979) The intraperiplasmic growth cycle – the life style of the bdellovibrios, pp 115–138. In: Parish JH (ed) Developmental biology of prokaryotes. Blackwell, Oxford

Thomashow LS, Rittenberg SC (1985) Isolation and composition of sheathed flagella from *Bdellovibrio bacteriovorus* 109J. J Bacteriol 163:1047–1054

Tudor JJ, Conti SF (1977a) Characterization of bdellocysts of *Bdellovibrio* sp. J Bacteriol 131:314–322

Tudor JJ, Conti SF (1977b) Ultrastructural changes during encystment and germination of *Bdellovibrio* sp. J Bacteriol 131:323–330

Tudor JJ, Karp MA (1994) Translocation of an outer membrane protein into prey cytoplasmic membranes by bdellovibrios. J Bacteriol 176:948–952

Tudor JJ, McCann MP, Acrich IA (1990) A new model for the penetration of prey cells by bdellovibrios. J Bacteriol 172:2421–2426

Varon M, Shilo M (1968) Interaction of *Bdellovibrio bacteriovorus* and host bacteria. I. Kinetic studies of attachment and invasion of *Escherichia coli* B by *Bdellovibrio bacteriovorus*. J Bacteriol 95:744–753

Varon M (1979) Selection of predation-resistant bacteria in continuous culture. Nature 277:386–388

Varon M, Seijffers J (1975) Symbiosis-independent and symbiosis-incompetent mutants of *Bdellovibrio bacteriovorus* 109J. J Bacteriol 124:1191–1197

Varon M, Zeigler BP (1978) Bacterial predator–prey interaction at low prey density. Appl Environ Microbiol 36:11–17

Velculescu VE, Zhang L, Zhou W, Vogelstein J, Basrai MA, Bassett DE Jr, Hieter P, Vogelstein B, Kinzler KW (1997) Characterization of the yeast transcriptome. Cell 88:243–251

Walker JE, Saraste M, Gay NJ (1984) The *unc* operon: nucleotide sequence, regulation and structure of ATP synthase. Biochim Biophys Acta 768:164–200

Zaim J, Kierzek AM (2003) The structure of full-length LysR-type transcriptional regulators. Modeling of the full-length OxyR transcription factor dimer. Nucl Acids Res 31:1444–1454

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_057/Published online: 27 October 2006

The Search for Hunters: Culture-Dependent and -Independent Methods for Analysis of *Bdellovibrio* and Like Organisms

Susan F. Koval

Department of Microbiology and Immunology, University of Western Ontario, London, ON N6A 5C1, Canada
skoval@uwo.ca

Abstract The aim of this chapter is to review the methods available for the isolation, cultivation and identification of *Bdellovibrio* and like organisms (BALOs) from freshwater and terrestrial environments. Culture-dependent methods are discussed with a focus on the selection of appropriate dilute media and prey cells for direct isolation, enrichment cultures, and pure cultures. The preparation of cocultures for physiological and genetic studies is outlined, with a discussion of the use of buffer vs. dilute nutrient medium and the monitoring of predation. Two approaches for characterizing BALOs are discussed. Cell morphology and the stages in the life cycle can be analyzed by light microscopy and transmission or scanning electron microscopy. Methods for determining the prey range of an isolate are described, as well as appropriate techniques for enumeration of predators. The potential use of the fluorescence in situ hybridization (FISH) and of PCR-based

techniques for tracking and identification of BALOs in environmental samples is then discussed, as these are excellent tools for those researchers unfamiliar with the subtleties of growing and maintaining BALOs.

1 Introduction

The first obligate predatory bacterium to be discovered, *Bdellovibrio bacteriovorus*, owes its place in bacteriology to serendipity (see introductory chapter) and the use of a classical microbiological technique: the double-layer agar method for viral plaque formation. Just as the development of the field of microbiology was dependent upon technological advancements, mainly in the development of the light microscope and the agar culture method, so our introduction to and understanding of the first predatory bacterium (Stolp and Starr 1963) was dependent upon the use of appropriate methods to isolate and characterize these novel bacteria. These methods were well used in the next 25 years or so of research and documented by Starr and Stolp (1976) and Jurkevitch (2006). With the availability of molecular techniques in the past few years, we are now able to use nonculture methods to identify predatory prokaryotes in various habitats and to study their evolution and phylogenetic relationships (Davidov and Jurkevitch 2004; Baer et al. 2000). The recent genome sequencing of the type strain of *B. bacteriovorus* (Rendulic et al. 2004) has provided new insights into the physiology and life cycle of these organisms (see chapter by Tudor and McCann). However, biochemical and physiological experiments to characterize specific functions of genes still have to be done. Thus the researcher still has a need to grow the organism in culture to understand the life cycle and to enumerate progeny cells under various growth conditions. This chapter will discuss both classical and molecular techniques for use in studies of predatory prokaryotes, with the emphasis on *Bdellovibrio* and like organisms (BALOs) from freshwater and terrestrial environments. Methods for studies on BALOs from halophilic environments are discussed in the chapter by Williams.

2 Culture-Dependent Methods

2.1 Direct Isolation

The basic approach to the isolation of BALOs from environmental samples utilizes techniques fundamental to the study of bacteriophages. Dilutions of soil, sewage, or water are mixed with a susceptible prey bacterium in soft

agar (0.6%), plated, and incubated at a temperature appropriate to the source of the sample. Plaques produced by BALOs are slower to appear (3 to 4 d) than plaques from bacteriophages (ca. 24 h), and thus care must be taken to avoid overgrowth of BALO plaques by contaminating organisms from the sample that are not susceptible to attack by predators. Soil and sewage are particularly rich in microbial composition, which may include other predatory microorganisms. The solution to this potential problem is to physically separate BALOs from other microorganisms in the sample.

The technique first described by Stolp and Starr (1963) for the direct isolation of *Bdellovibrio* from soil, and subsequently explained by Starr and Stolp (1976), Stolp (1981), and Ruby (1992), still works effectively. A 50- to 500-g soil sample is suspended in 500 ml of tap water or buffer. This suspension is shaken vigorously for 1 h and centrifuged for 5 min at 2000 g, and the supernatant is passed through a series of decreasing pore-size membrane filters (3.0, 1.2, 0.8, 0.65, and 0.45 μm). Samples of the final filtrate are plated using the conventional double-layer agar technique. Presumably the large sample size (50–500 g) is used because some bdellovibrios are lost during the successive filtration steps.

The composition of the agar medium for isolation can vary. It was noted in the original description of *Bdellovibrio* that reduced growth of prey cells favors development of predators (Stolp and Starr 1963). YP medium (0.3% yeast extract, 0.06% peptone, pH 7.2) and YP medium diluted tenfold (YP/10) were used to isolate bdellovibrios. Seidler and Starr (1969) introduced the use of dilute nutrient broth (DNB) for propagation of BALOs (0.08% Difco nutrient broth, 2 mM $CaCl_2 \cdot 2H_2O$, 3 mM $MgCl_2 \cdot 6H_2O$), and this medium is the most widely used today.

A critical step in any isolation technique for predatory prokaryotes is the selection of the prey cell. Most BALOs can utilize a wide range of Gram-negative prey bacteria, but it is not inclusive. Cell surface components need to be taken into consideration. *B. bacteriovorus* can penetrate the capsule of *E. coli* cells and complete its life cycle (Koval and Bayer 1997), but paracrystalline surface layers (S-layers) are a barrier for predation (Koval and Hynes 1991). If one is interested in the numbers of BALOs in general in a particular habitat, the selection of one kind of prey cell for plaque formation may skew the data, as one may not account for BALOs that do not use that prey cell, i.e., only a subset of BALOs will be enumerated. As most bacteria are not amenable to cultivation, this bias may be large. Herein lies the value of direct microscopic counts (Sect. 4) for estimation of BALO populations. The selection of prey cell will depend upon the objective of the research. A "representative" prey cell may be chosen for isolation of predatory prokaryotes, but it must be chosen carefully, as it will be used in the subsequent preparation of pure cultures of predators. Or if the focus of research is on a particular Gram-negative bacterium, this species can be used as the prey cell for ecological studies on the distribution of BALOs that use that organism as a prey cell.

Stolp and Starr (1963) in their initial description of *Bdellovibrio* used many different Gram-negative bacteria to isolate these predators from soil and sewage. They obtained isolates on lawns of *Erwinia amylovora*, *Pectobacterium carotovorum* subsp. *carotovorum, Escherichia coli* B, *Aerobacter aerogenes*, *Proteus mirabilis*, *Serratia marcescens*, *Ralstonia solanacearum*, *Enterobacter cloacae*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *tabaci*, and *Pseudomonas fluorescens*. Klein and Casida (1967) isolated BALOs on indigenous soil bacteria. Afinogenova et al. (1981) used 54 strains of bacteria of different taxonomic status to isolate BALOs from city sewage and river water. Richardson (1990) isolated BALOs from man-made water systems on lawns of strains of *Legionella pneumophila*. Jurkevitch et al. (2000) used phytopathogenic bacteria as potential prey for isolation of the predators from soil and the rhizosphere. Predators were isolated on *Pseudomonas corrugata*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *Agrobacterium tumefaciens*. Schwudke et al. (2001) isolated BALOs from feces and sewage on *Proteus mirabilis* and *Citrobacter freundii*. It is notable that all the bacteria used to isolate BALOs belong to the proteobacteria.

2.2 Enrichment Methods

Isolation of BALOs from various environments may require a preliminary enhancement of cell numbers as these predators can be present in low numbers in freshwater and terrestrial habitats. Or, one may want to isolate a BALO that is lytic toward a specific strain or species of bacterium. In these instances, populations of predators that use the prey cell of choice are first increased in number before isolation by plaque formation. Because prey cells can be pregrown, the medium for enrichment cultures does not have to be tailored to the prey cell of choice. The use of dilute nutrient media discourages the growth of heterotrophic bacteria in the sample and also minimizes bacteriophage development. Enrichment cultures in full-strength nutrient broth (NB) or Luria broth (LB) will not be particularly successful. A standard cultivation medium for BALOs is 1/10 strength NB (dilute nutrient broth, DNB). There are variations on the composition of DNB, with regard to the concentration of additional cations or the addition of yeast extract and casamino acids. All use 0.08% Difco NB. Seidler and Starr (1969) supplemented the medium with 2 mM $CaCl_2 \cdot 2H_2O$ and 3 mM $MgCl_2 \cdot 6H_2O$. Ruby's DNB (Ruby 1992) included 0.05% casamino acids, 0.01% yeast extract, 1 mM $CaCl_2 \cdot 2H_2O$, and 0.1 mM $MgCl_2 \cdot 6H_2O$. For genetic experiments with *E. coli*, Cotter and Thomashow (1992) used DNB with 1 mM $CaCl_2 \cdot 2H_2O$ and 0.1 mM $MgCl_2 \cdot 6H_2O$.

We have used the following method for the isolation of BALOs from raw sewage (Koval and Hynes 1991). A 200-ml sample of raw sewage is supplemented with 50 ml of dilute nutrient medium, incubated with shaking at 30 °C for 1 h and then centrifuged at 3000 g for 10 min. The supernatant

(a 20 ml aliquot) is added to an equal volume of pregrown prey cells (which have been washed and resuspended in the same dilute nutrient medium). This enrichment culture is incubated at 30 °C for 48 h, or until the presence of bdelloplasts (infected prey cells) or attack-phase BALOs is confirmed by phase contrast microscopy. The enrichment culture is then centrifuged at 7000 g for 10 min and the supernatant passed through a series of 0.45-μm filters. The filtrate is diluted and plated for plaques.

We have also used the method of Ruby (1992) for enrichment of BALOs from soil samples. A 50-ml overnight culture of prey cells is centrifuged and resuspended in HM buffer (Sect. 2.4) or DNB to a concentration of 10^9 or 10^{10} cells/ml. Soil (100 mg) is then added to 20–50 ml of the bacterial suspension. The enrichment cultures are incubated with rapid shaking at room temperature or at 30 °C, until the presence of bdelloplasts or attack-phase BALOs is confirmed. The slurry is centrifuged at 2000 g for 5 min and filtered through a 0.45-μm filter. The filtrate is then diluted and plated for plaque growth. It should be noted that the enrichment method may yield less diverse BALO populations than direct isolation.

For both methods, if the number of bacteriophages is higher than the number of BALOs in the enrichment cultures, the filtrate can be centrifuged at 27 000 g to concentrate the BALOs and reduce the number of bacteriophages, which remain in the supernatant (Varon and Shilo 1970).

2.3 Preparation of Pure Cultures

The double-layer agar technique is essential for the isolation of BALOs in pure culture as they are purified by repeated plaque formation. Predators from a well-separated plaque are picked up with a flamed loop and transferred to 5 ml of dilute nutrient broth (DNB) in a test tube. Prey cells are added (0.5 ml of a 24-h culture) and the coculture incubated with shaking at 30 °C until lysis of prey cells has occurred. This suspension of predator cells is serially diluted and plated for single-plaque production. This process is repeated three times, from which one obtains a pure culture of a strain of predator (Stolp and Starr 1963). If necessary, the suspension of predators from a single plaque can be passed through a 0.45-μm filter before plating for plaque formation.

2.4 Coculture Methods

Prey cells are pregrown to stationary phase in a medium suitable for their growth (as BALOs do not require actively growing cells, in contrast to bacteriophages). They are then added to DNB, usually as a 10% inoculum, along with predators. Any nutrients in the original culture medium are diluted $\sim 1/10$, and thus prey cells do not have to be centrifuged and washed before

use in cocultures. An alternative approach is to prepare cocultures in a buffer (Thomashow and Rittenberg 1979). This procedure is possible because BALOs do not replicate outside of prey cells and thus do not require exogenous nutrients. The suspending medium provides a weak buffering environment with cations required for predation. In early studies (Crothers and Robinson 1971) 25-mM HEPES buffer, pH 7.8 with 2 mM $CaCl_2 \cdot 2H_2O$ was used for cocultures of *B. bacteriovorus* strain 6-5-S growing on *E. coli* ML35. Our lab uses HM buffer with 3 mM HEPES, pH 7.6 containing 1 mM $CaCl_2$ and 0.1 mM $MgCl_2$ (Flannagan et al. 2004), but both options are valid. However, HEPES buffer is not a suitable medium for all Gram-negative prey bacteria, as might be expected. Cocultures with *Aeromonas salmonicida* had to be prepared in Tris buffer (Koval and Hynes 1991).

The requirement for cations may vary with the strain of BALO (Huang and Starr 1973). Calcium is required for efficient predation in buffer systems, as it probably functions to facilitate attachment between two negatively charged surfaces. Magnesium has also been included in some buffer compositions for cocultures, but magnesium alone (without calcium) did not support efficient growth of *B. bacteriovorus* strain 6-5-S (Crothers and Robinson 1971).

There are advantages for cocultures prepared in a buffer system, rather than DNB. The metabolic activities of the prey cell are minimal. Also, as there is no growth of prey cells in a buffer, the efficiency of predation can be measured by counts of prey cells at the beginning and end of growth. In DNB most prey cells will have an initial growth period, in which prey cells increase in number, and then predation begins. Specific compounds can be added to the buffer as probes for the process under investigation. A practical advantage is that the risk of contamination is decreased in a buffer system.

As BALOs are aerobic bacteria and most require culture conditions that provide efficient aeration, cocultures should be set up to keep a steady flow of oxygen (20–25 ml in a 125-ml flask for example, with circular shaking at $\sim$ 120 to 150 rpm). In our experience, predation of a 50-ml coculture in a 125-ml flask never really gets started, nor does it proceed to completion. Erlenmeyer flasks with baffles, or a reciprocating shaker, provide even better aeration.

How is predation monitored in cocultures? There are three independent methods that can be used for assessment.

(1) Measurement of turbidity. Typically there is a decrease in turbidity of cocultures, due to the lysis of prey cells. The number of predators increases, but due to their small size the resulting turbidity is often less than the initial turbidity of the coculture. Turbidity measurements can be conveniently done using a Klett colorimeter and side arm flasks (Koval and Hynes 1991), or a spectrophotometer. No samples need to be removed for measurement of turbidity and the risk of contamination is reduced. The culture volume remains the same, i.e., is not reduced. Cocultures can also be set up in microtiter plates (Jurkevitch et al. 2000; Lambert et al. 2003), which have the advantage of small volumes, the ability to screen large numbers of isolates,

and the availability of plate readers for initial and final turbidity readings. However, while measuring turbidity works well for "aggressive" predators that can clear a population of prey cells, not all BALOs are so efficient and unattacked prey cells can remain. Thus a large decrease in turbidity during predation may not be apparent in all cases. Or, if a large number of prey cells are used initially in the coculture, and if DNB is used (rather than HM buffer) such that the number of prey cells increases initially, the resulting number of progeny cells can also be large. Thus the initial and final turbidities of the coculture may not be too different. Therefore, one should also examine cocultures by phase contrast microscopy to assess predation.

(2) Phase contrast microscopy. This is an essential method to monitor growth of BALOs in cocultures, either for maintenance or for other experimental conditions. A turbidity reading does not reveal the stage of the life cycle of predators or the progression of predation in the batch coculture.

(3) Plaque-forming numbers. This is the definitive method to prove that the predators have multiplied in a coculture. Quantification by an increase in plaque-forming units over the time of incubation will confirm growth of the predators.

Stages in the BALO life cycle can be conveniently measured by the use of synchronous cultures (Thomashow and Rittenberg 1979). The principle of the method is simple, in that the multiplicity of infection is adjusted (a predator to prey ratio of $\sim$ 2–3 : 1) so that each prey cell is attacked and penetrated by a predator within 20 to 30 min of mixing the organisms and synchronous formation of bdelloplasts ensues. This technique has been used mostly with *B. bacteriovorus* and *E. coli* cocultures. It will be most useful for timing of cell cycle events and studies on the regulation of gene expression in predators.

2.5 Maintenance and Preservation

Starr and Huang wrote (1972) that "In the present state of knowledge, maintaining cultures of *Bdellovibrio* is a rather exasperating art." Hopefully we have progressed since then.

The reality about maintenance of BALOs is that bdellovibrio progeny, once released from the bdelloplast, are faced with starvation conditions "in the outside world." There is evidence that BALO populations are maintained in nature for relatively long periods of time (and hence our continued success in obtaining new isolates). However, under laboratory conditions, there is heterogeneity of survival potential among isolates (Varon and Shilo 1980). Varon compared the survival of different *Bdellovibrio* strains under nongrowing conditions (as cell lysates, or by resuspension and storage of predators in DNB). The viable numbers of predators (as PFUs) was determined after 3, 6, and 9 months. After 3 months there was a 10^4 to 10^6 decrease in cell numbers in the lysates, while survival was somewhat higher for all strains in DNB.

Other studies indicated a dramatic decrease in the viability of cell suspensions in the first 18 to 24 h, with the surviving populations remaining constant for the next week or so.

With these comments in mind, stock cultures of BALOs can be prepared by storage at 4 °C of cocultures prepared in DNB or HM buffer and containing mainly attack-phase cells. Screw-capped test tubes work well for storage. Cultures can be stored for 3 to 4 weeks, before subculture. Some isolates may require more frequent transfers to maintain viability. It is advisable every 6 months or so to plate out the predator for plaques to ensure that the lytic activity of the predator has been maintained. Alternatively, BALOs can be stored as plaques in agar medium.

For experiments, a coculture should be prepared from a stock culture by incubation for 24 to 48 h. This culture of fresh, new predators can then be used for the next coculture for experimental purposes. Methods for long-term preservation of BALOs are well described (Jurkevitch 2000).

3
Characterization of Isolated BALOs

3.1
Microscopy

3.1.1
Light Microscopy

Phase contrast light microscopy is the preferred mode for visualization of predators. In enrichment cultures and cocultures they can be identified as very small cells, rapidly motile with a "darting" motion. Use of the 100× oil immersion lens is essential. Depending upon the age of the culture, predators can also be seen attached to prey cells, or as multiple motile progeny cells inside the bdelloplast cell wall near the end of the life cycle. Light micrographs of cultures are best taken with preparations of cells deposited on 2% agarose coated slides (Pfennig and Wagener 1986). The cells are then all at the same depth of focus. The stages in the life cycle can be clearly identified (Fig. 1).

3.1.2
Transmission Electron Microscopy

For characterization of the morphology and life cycle of predators, negative stains and thin sections can be prepared for transmission electron microscopy. Uranyl acetate (1%, pH 4.4) is the preferred negative stain for determining cell shape and appearance of the single, polar, sheathed flagellum (Fig. 2). Neutral negative stains (e.g., ammonium molybdate, phos-

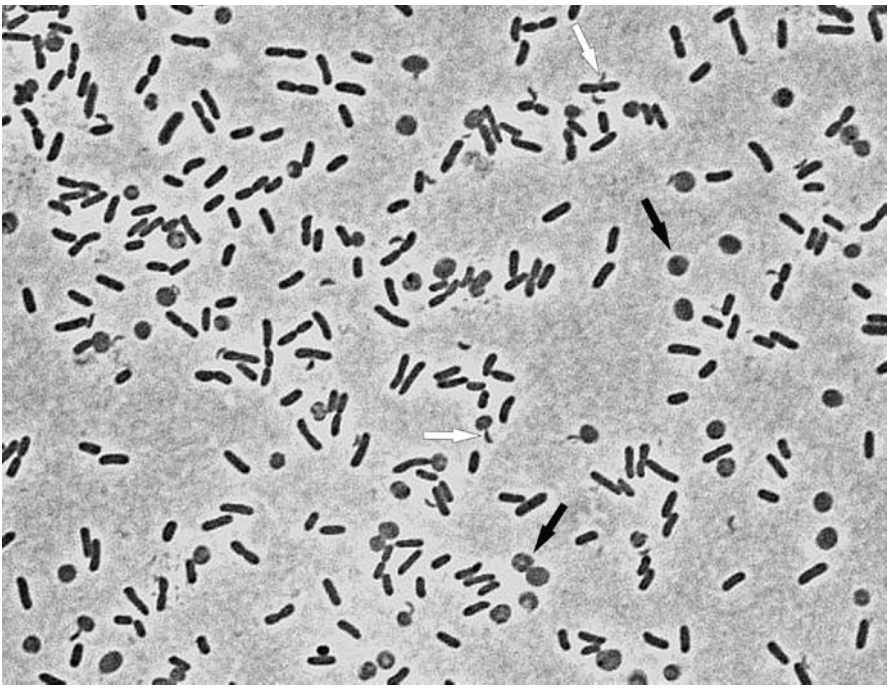


Fig. 1 Phase contrast microscopy of a co-culture of *Bdellovibrio bacteriovorus* 109J and *E. coli* ML35. *White arrows* indicate predators attached to prey cells. *Black arrows* point to a bdelloplast

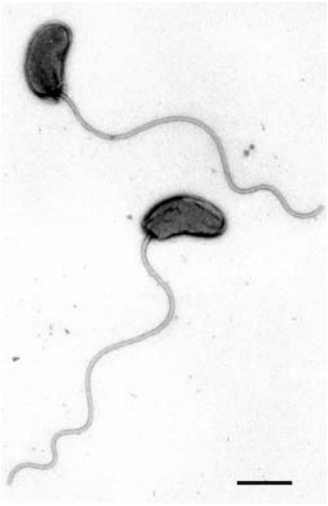


Fig. 2 Electron micrograph of negatively stained (1% uranyl acetate) BALO cells. Note the vibroid shape and the single, polar sheathed flagellum with damped waveform. The *scale bar* represents 0.5 μm

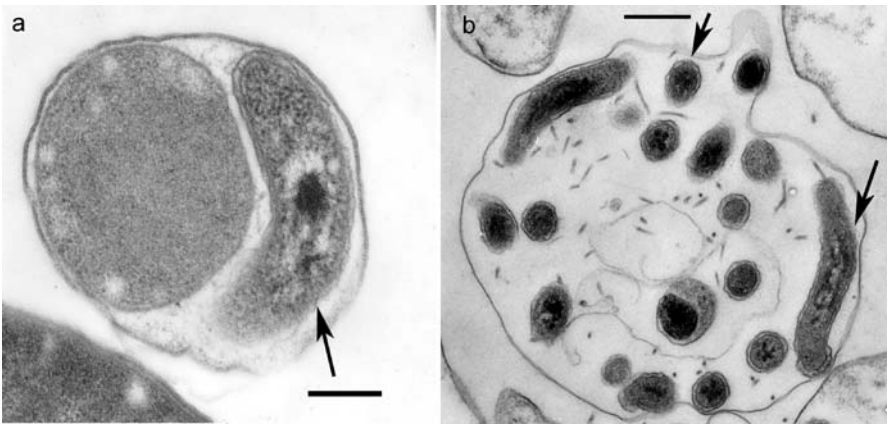


Fig. 3 Electron micrographs of thin sections of bdelloplasts. **a** Bdelloplast of *E. coli* ML35 with *Bdellovibrio bacteriovorus* 109J. One unseptated progeny cell is indicated by the *arrow*. The *scale bar* represents 0.2 μm **b** Bdelloplast of *Aquaspirillum serpens* VHL in the later stages of infection with *Bdellovibrio bacteriovorus* 6-5-S. Multiple progeny cells have been produced, and are seen in longitudinal or cross section (*arrows*). Flagella are also easily seen at the pole of the one cell cut longitudinally, and as they traverse the residual space in the bdelloplast. The *scale bar* represents 0.5 μm

photungstic acid) can cause excessive blebbing of the outer membrane and disruption of the flagellar sheath (Abram and Davis 1970; S. Koval and C. Elwood, unpubl. data). On occasion, and with luck, bdelloplasts can be seen in negative stains (Abram et al. 1974), but usually they stain too darkly and the predator in the periplasm cannot be clearly seen. Confirmation of bdelloplast formation in the life cycle of a prokaryotic predator is best done by thin sections (Fig. 3). These sections clearly show the predator inside the periplasmic space adjacent to the diminishing protoplasm of the prey cell. Depending upon the plane of the section through the bdelloplast, the helical, nonseptated filament may appear associated with the cytoplasmic membrane of the prey cell, but this association has not been addressed experimentally. The attachment of predator to the surface of prey cells can be seen in thin sections (Abram et al. 1974). *B. bacteriovorus* penetrates the outer membrane of the prey cell and produces a localized dissolution of the peptidoglycan. A "bulge" of outer membrane forms around the attack-phase pole of the predator. The integrity of the prey cell's cytoplasmic membrane, as visualized in thin sections, is not compromised. Penetration into the periplasm must occur fairly rapidly, as it is rare to see BALOs "half-way" through the cell wall of prey cells. There are, however, *Bdellovibrio* strains that remain epibiotic and do not penetrate their prey (Koval 2001).

3.1.3 Scanning Electron Microscopy

This method of electron microscopy affords another view of cells in cocultures. It allows one to see whole cell interactions and associations in a way that is not possible with negative staining, due to overstaining of cells and flattening of bdelloplasts. Some excellent use of scanning electron micrographs to illustrate the life cycle of *B. bacteriovorus* has been made (Hampton 2004). In a coculture of *B. bacteriovorus* 109J and *E. coli* ML35, predators can be seen attached to prey cells (Fig. 4). The proximal part of the flagellum is seen on attached predator cells, but the distal part that lies on the support surface (the Nucleopore filter in Fig. 4b) is "lost" as it has little relief and is not readily detected by scanning electron microscopy. The same flagellar images were seen earlier (Fratamico and Whiting 1995). The round, wrinkled bdelloplasts of *E. coli* are easily distinguished from uninfected, rod-shaped

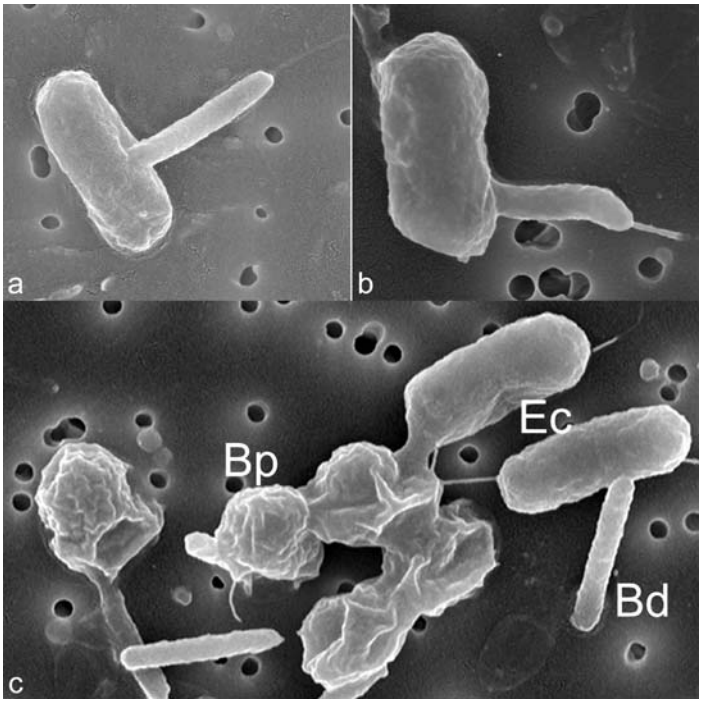


Fig. 4 Scanning electron micrographs of cells in a co-culture of *Bdellovibrio bacteriovorus* 109J and *E. coli* ML35. **a** The impact and penetration by the predator cell is seen. The flagellum on the opposite pole of the predator is barely visible. **b** This micrograph shows the proximal end of the flagellum on the predator, and the loss of relief as it flattens out on the support filter. **c** The co-culture contains prey cells (Ec), predators (Bd) and bdelloplasts (Bp)

prey cells (Fig. 4c). Scanning electron microscopy is particularly useful in the study of epibiotic predators, when the mode of cell division and multiplication needs to be assessed. Scanning electron microscopy was used in our lab to examine the life cycle of a novel strain of *Bdellovibrio*, which remains extracellular during predation. This study confirmed that no bdelloplast was formed during predation and that the empty, stalked *Caulobacter crescentus* prey cell retained its original shape (Shemesh et al. 2003).

3.1.4 Atomic Force Microscopy

The value of atomic force microscopy lies in its ability to visualize cells nearer to their "natural state" and with high spatial resolution (nanometer detail). It can be used to measure elasticity and rigidity properties of microbial surfaces and long-range forces over membranes. Thus atomic force microscopy can be considered a form of high-resolution microscopy. Although transmission and scanning electron microscopy can provide higher resolution, these microscopic techniques operate in a high vacuum environment, precluding the study of living systems. Also, electron microscopy techniques do not provide vertical resolution of images, and thus the height of surface features cannot be measured. Atomic force images can be acquired in liquid medium (underwater), which means that this technique may be used for imaging living entities. However, some technical considerations need to be addressed. Conditions must be determined for adhesion of cells to a substratum, and this requires trial and error. If the cell density is too high, individual cells and their surface properties are not clearly seen. If the cell density is too low, it may be difficult to find a cell in the sample. Although the instrumentation is smaller, simpler, and less expensive than a comparable electron microscope, the operation is not. Samples for atomic force microscopy cannot be "scanned" for detection of representative cells, as can be done with transmission or scanning electron microscopy. It takes a long time to find a suitable cell and then do the analysis. Thus atomic force microscopy is not a practical method for routine checking of bacterial cultures and will not replace electron microscopes for routine ultrastructural analyses.

With these precautions in mind, what knowledge can be gained about BALOs using this microscopy method? The stages in the life cycle are already known and have been determined by other independent methods of microscopy. We know that individual BALO cells can be visualized by atomic force microscopy (S. Koval, unpubl. observations), and bdelloplasts are nicely imaged, with good turgor pressure (Fig. 5), and not wrinkled as in scanning electron microscopy images (Fig. 4c). The progeny cells can be clearly seen inside. A good use of atomic force microscopy would be to do force measurements on cells during the life cycle to try to detect changes in turgor pressure during bdelloplast formation and utilization of nutrients.

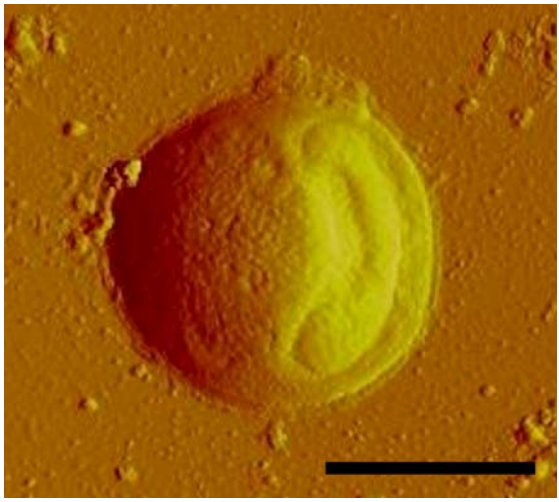


Fig. 5 Atomic force micrograph (contact mode) of a bdelloplast of *Escherichia coli* ML35 infected with *Bdellovibrio bacteriovorus* 109J. The predator inside the bdelloplast is clearly outlined in this image. The *scale bar* represents 1 μm

Nunez et al. (2005) used atomic force microscopy to study the interaction of *B. bacteriovorus* with *E. coli* cells in a biofilm. They showed that *Bdellovibrio* could invade and reduce the population of prey cells in a biofilm, but little other information was gained about this process by use of atomic force microscopy. Kadouri and O'Toole (2005) also examined the susceptibility of *E. coli* biofilms to predation by *B. bacteriovorus* by use of phase contrast and epifluorescence microscopy and environmental scanning electron microscopy. Their analyses confirmed the reduction in prey cell numbers and provided quantitative data on the impact of bdellovibrios on biofilm populations.

An experimentally unanswered question in BALO biology concerns the timing of flagellum loss during attack and penetration of prey cells. As discussed in Sect. 3.1, the sheathed flagellum is not readily seen by scanning electron microscopy. The use of atomic force microscopy was considered as a possible method to visualize this event in the life cycle. But often flagella are not seen on attack-phase cells imaged by contact mode atomic force microscopy in cocultures (S. Koval, unpubl. obs.) or in biofilms (Nunez et al. 2005).

3.2 Prey Range

The extent of the prey range of a BALO isolate is a useful practical criterion in the description of a predator, although it has no taxonomic relevance.

Nevertheless, knowledge of the prey range has ecological and biocontrol implications. The first strain of predatory prokaryotes isolated from German soils and described by Stolp and Petzold (1962) was characterized by a lytic activity limited to members of the *Pseudomonadales*. It lysed exclusively fluorescence pseudomonads and xanthomonads. Subsequent isolates of predatory prokaryotes from California soil and sewage samples by Stolp and Starr (1963) revealed differences in their prey activity spectra. Some had a restricted prey range and others were able to attack a broad spectrum of prey bacteria. Other studies on the prey range of isolates of BALOs have shown that most predators have a broad prey range. *Peredibacter starrii* A3.12 (formerly *Bdellovibrio starrii* and then *Bacteriovorax starrii*) was isolated from soil on *Pseudomonas fluorescens*, and its lytic activity on plates was restricted to *Pseudomonas* spp. (Stolp and Starr 1963). The type strain of *B. bacteriovorus* strain 100^T and the well-studied strain 109J are active against some enteric bacteria and pseudomonads. Strain 100^T was isolated on *Erwinia amylovora* and strain 109 on *E. coli* B.

The results of analyses of prey range depend on experimental conditions. Stolp and Starr (1963) analyzed the "activity spectra" of their new isolates by plaque formation and coculture analysis. These are still the two tests that should be used to test prey range. When plated with an excess of susceptible prey bacteria in top agar, BALOs are capable of producing single plaques (as PFU) or confluent lysis, depending upon the concentration of predators (Stolp and Starr 1963). A lytic effect on prey bacteria can also be simply demonstrated by placing 10 to 20 μl of a predator suspension on the top soft agar layer inoculated with prey bacteria (a drop lysis test). A large, clear lytic area is produced. This test appears to parallel individual plaque formation by a predator isolate (Stolp and Starr 1963). It was used to analyze the predation pattern of BALOs from Great Salt Lake, Utah (Pineiro et al. 2004) and our lab has used it (D. McNeely and S. Koval, unpubl. data) to test the prey range of various BALOs. In some cases, results were not concordant between the two methods. Therefore it is advisable to do liquid cocultures and follow the incubations by light microscopy, to look for bdelloplast formation and an increase in the number of progeny cells. As some new isolates may not be as "aggressive" as others, two independent methods of prey range analysis are recommended.

3.3 Phylogenetic Characterization

Phenotypic characterization as described above is important for ecological and functional studies, but it only has limited resolution and cannot replace a phylogenetic approach for the classification of BALOs. Until recently, all known BALOs belonged to the δ-proteobacteria. Recently, a class of predators from the α-proteobacteria was characterized. To differentiate between

the different classes, they can be called d-BALOs and a-BALOs, respectively (see chapter by Jurkevitch and Davidov). d-BALOs form two families, the Bdellovibrionaceae and the Bacteriovoracaceae (Baer et al. 2000; Pineiro et al. 2004; Davidov and Jurkevitch 2004) that encompass the genera *B. bacteriovorus*, and *Bacteriovorax stolpii, B. marinus, B. littoralis*, and *Peridibacter starrii*, respectively. These groups were determined using mainly the 16S rRNA gene as a phylogenetic marker. Amplified rDNA restriction analysis (ARDRA) is a convenient, simple, and quite powerful method for an initial characterization of BALO isolates. It enables a first classification based on the restriction patterns obtained from digested PCR products of the 16S rRNA gene of BALOs compared to known, characterized isolates (Davidov and Jurkevitch 2004). Naturally, a more precise analysis requires the sequencing of the gene itself, and of other marker genes if possible, followed by phylogenetic analysis (Baer et al. 2000; Pineiro et al. 2004; Davidov and Jurkevitch 2004).

4 Enumeration

The procedures for plaque formation are used throughout BALO methodology: direct isolation, enrichment cultures, purification of predators, and quantification of predator numbers. For enumeration purposes it is very important that plaques be clearly visible on the lawns of prey bacteria. As discussed in Sect. 2.1, development of plaques is favored by low-nutrient media. While DNB is often the medium of choice, other media more adapted to certain prey types have also been used. YPSC is a medium used for *Aquaspirillum* spp. (Koval and Hynes 1991) and was adapted by Huang for studies on predation by bdellovibrios on aquaspirilla. It is a low-nutrient medium for these freshwater spirilla and thus appropriate for BALO plaque formation. Our lab has compared some media (YP/10, YPSC, and DNB) for visibility and clarity of isolated plaques, using different BALOs (strains 109J, 6-5-S, and JSS) and a variety of prey cells in the lawn (*E. coli*, *Aquaspirillum serpens*, *Caulobacter crescentus*, and *Burkholderia cenocepacia*; S. Koval, unpubl. data). DNB provided the best results for all combinations. The use of an obliquely illuminated colony counter also assists in plaque visibility (Stolp 1981).

Plating is performed by preparing dilutions of a sample of predators in HM buffer and combining 100 μl of these dilutions with 200 μl of a prey cell suspension ($\sim 10^{10}$ cells ml^{-1}) in 4 ml of 0.6% DNB agar at 42 °C. This mixture is poured onto the surface of a DNB (1.5% agar) plate. Plates are incubated at 30 °C for 3 to 4 d. As a guide, an overnight prey cell culture (50 ml) can be centrifuged and resuspended in 5 ml of DNB medium. This usually provides a sufficient cell density for lawns. It is essential that the agar plates be prepared just before use, as dry agar does not promote the growth of plaques, which remain tiny and therefore difficult to find and count.

Another method for enumerating predators is to do direct microscopic counts using a fluorescence dye. With this method, attack-phase cells, bdelloplasts, and uninfected prey can be discriminated by staining with DAPI (2 μM) and cells counted by epifluorescence microscopy using a grid ocular (Shemesh et al. 2003; Shemesh and Jurkevitch 2004).

5
Culture-Independent Methods

The development of culture-independent methods for the identification of prokaryotes in natural habitats is a much needed addition to other methods currently available for BALOs. As Schwudke et al. (2001) and Shemesh et al. (2003) point out, the study of environmental BALOs is difficult because of the necessity of a dedicated isolation procedure, which is often not successful for the uninitiated researcher. Most microbial ecologists are unfamiliar with predatory prokaryotes, and their experimental design would not include the more laborious double-layer agar growth medium and prey cells. Thus BALOs are seldom included in microbial community compositions or analyses. Moreover, BALO isolates differ in prey range. As most prey may not be amenable to cultivation (and the number of prey one can use in the laboratory is anyways restricted) quantification using plaque counts certainly always yields underestimates of the actual numbers of BALOs in the environment. Two methods utilizing 16S rRNA sequence information for microbial ecology studies and their applicability to studies on BALOs are described.

5.1
Fluorescence In Situ Hybridization (FISH)

The use of fluorescencely labeled oligonucleotide probes specific for rRNA sequences of certain groups of bacteria is referred to as fluorescence in situ hybridization, or FISH. FISH can be thought of as a phylogenetic stain as it allows the phylogenetic identification of microorganisms in environmental settings. The fluorescence signal is detected by epifluorescence microscopy or confocal scanning laser microscopy. Probes can be designed to specifically target narrow to broad phylogenetic groups (from species to domain). Probe design involves identifying short regions (usually 15 to 25 nucleotides in length) in a sequence alignment unique to the group of interest. The specificity of the probe can be examined by comparative analysis of aligned 16S rDNA sequences available in online public database search programs such as Basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST/), ARB software package (http://www.arb-home.de), and the Ribosomal Database Project (RDP II; http://rdp.cme.msu.edu). Mismatches to nontarget organisms must be taken into consideration. In general, the nontarget organisms usually have

three or more mismatches that make the probe specific for the intended target organisms (Lathe 1985). However, oligonucleotide probes can distinguish between complementary and nearly complementary sequences on the basis of single mismatches if high stringency hybridization conditions are established in the FISH protocol (Amann et al. 1990). The sequence of the selected probe may also need to be modified to meet probe design criteria such as minimal melting temperature. The design and evaluation of 16S rRNA targeted probes are thoroughly discussed by Hugenholtz et al. (2002).

We have recently designed an oligonucleotide probe, designated as BDE 525, specific for the genus *Bdellovibrio* and labeled at the 5′-end with the indocarbocyanine dye CY3 (K. Mahmoud et al., unpubl. data). The specificity of the probe was first examined with newly deposited 16S rDNA sequences

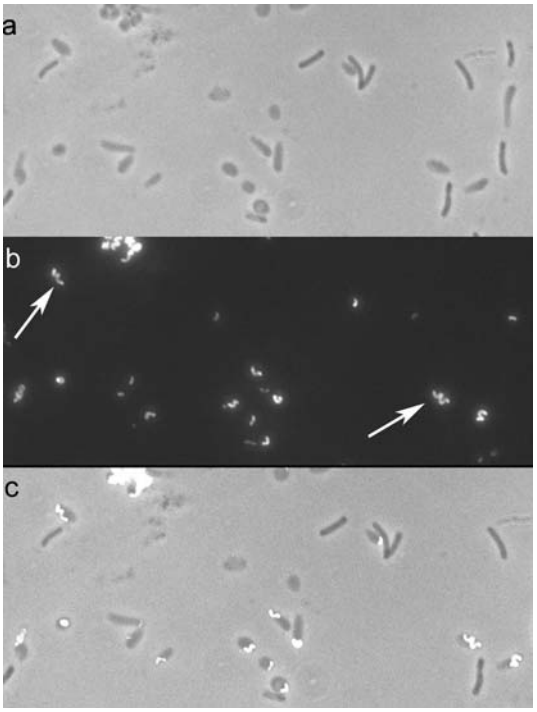


Fig. 6 Fluorescence in situ hybridization with probe BDE 525 of cells in a co-culture of *Bdellovibrio bacteriovorus* 6-5-S with *Aquaspirillum serpens* VHL. The large size of the prey cell makes this a good model system with which to demonstrate the life cycle. **a** phase contrast micrograph. **b** epifluorescence micrograph of the same field. The fluorescence signal was detected in attack phase cells and cells within the bdelloplast. A long spiral (*arrows*) can be seen inside some bdelloplasts, which is the growing, asepate filament. **c** merged images. Only the *Bdellovibrio* cells showed the fluorescence signal, not the uninfected prey cells. The fluorescence signal was more intense in cells in the bdelloplasts than the signal from released attack phase cells

from GenBank and from RDP II (Cole et al. 2005) against target and nontarget organisms. From 42 *Bdellovibrio* sequences in the databases, the sequence of the probe perfectly matched 40 *Bdellovibrio* sequences. We tested the probe in a FISH procedure with *B. bacteriovorus* strains 109J and 6-5-S, as well as the prey-independent strain 109JA. Interestingly, the fluorescence signal detected from these cells growing in bdelloplasts was more intense than the signal from released attack-phase cells (Fig. 6). This may be due to the depletion of cellular rRNA content in the attack-phase cells, which are cells that do not undergo cell division. The growth phase of bdellovibrios in the periplasm is equivalent to the exponential phase of growth of other bacteria. The sequence of the facultative predator *Bacteriovorax stolpii* UKi2, a closely related species used as a negative control in this study, had 4 base mismatches with the sequence of the probe, and thus cells did not hybridize with the BDE 525 probe. No fluorescence signal was detected under the hybridization conditions used in the FISH procedure. The probe did not hybridize with the prey cells used (*E. coli* and *Aquaspirillum serpens*). These results indicate that the FISH technique can be used to specifically detect *Bdellovibrio* cells, both in the bdelloplast and as free-swimming attack-phase cells. The next step will be to use this procedure to study the abundance and interactions of BALOs within their habitats in the environment and thus evaluate their role in reducing or modulating bacterial populations.

5.2
PCR of Community DNA (Environmental Clones)

Ribosomal DNA (rDNA) clone libraries can be made from environmental habitats and clones screened by dot blot hybridization with group-specific oligonucleonucleotide probes. This culture-independent method has been used to study many ecosystems (especially open ocean and coastal planktonic communities) to assess microbial diversity. In one study of permanently cold marine sediments in the Arctic Ocean, Ravenschlag et al. (1999) identified rDNA sequences affiliated with *Bdellovibrio* species. Many more environmental BALO sequences are now found in the databases (Davidov and Jurkevitch 2004). The culture-independent method of identification allows the distribution of predators to be considered in the context of the phylogenetic affiliation and diversity of the accompanying prokaryotic community in a particular ecosystem. BALOs have been isolated repeatedly from marine sediments and estuarine waters (see chapter by Williams), most often using *Vibrio parahemolyticus* as the prey cell for isolation and enumeration.

The increasing number of BALO 16S rDNA sequences available in the databases (Sect. 3.3) has enabled the design of taxon-specific PCR primers that target each of the hitherto described BALO groups (Jurkevitch and Ramati 2000; Herschkovitz et al. 2005; Y. Davidov and E. Jurkevitch, unpubl. data). These sets of primers can be used to directly classify isolates at the

genera level and also enable direct detection and characterization in environmental samples, through the construction of 16S rDNA libraries, analyses by denaturing gradient gel electrophoresis followed by band sequencing. It will be of great interest to use these primers in a quantitative PCR approach. This could yield the first culture-independent quantitative estimates of the occurrence of BALOs in the environment.

6 Conclusions and Perspectives

Critical studies on the life cycle and physiology of BALOs will benefit by careful attention to growth conditions for cocultures and monitoring of these cultures by the appropriate choice of microscopy technique(s). Ecological studies will benefit immensely from the application of culture-independent approaches such as the modification and optimization of in situ hybridization techniques and of PCR-based technologies for non-culture-based identification of these predatory prokaryotes in environmental samples, and direct estimation of diversity and sequence distribution. Such studies will certainly greatly contribute to the understanding of the roles BALOs play in nature, such as quantifying BALO predation in natural communities and therefore its impact on ecosystem functioning.

Acknowledgements The author wishes to thank the following individuals for their assistance in refining the methods used in BALO research: Sandra Hynes, Paul Fox, Krista Lyle, Damian McNeely, and Khaled Mahmoud. The following assistance with microscopy is also greatly appreciated: Damian McNeely (phase contrast microscopy), Judy Sholdice (TEM and SEM), and Megan Goodwin (AFM). SEM was done at the Surface Science Western Facility at the University of Western Ontario. Thanks to Chelsea Elwood for preparation of figures for electronic submission. This research was supported by an operating grant to SFK from the Natural Sciences and Engineering Research Council of Canada.

References

Abram D, Davis BK (1970) Structural properties and features of parasitic *Bdellovibrio bacteriovorus*. J Bacteriol 104:948–965

Abram D, Castro e Melo J, Chou D (1974) Penetration of *Bdellovibrio bacteriovorus* into host cells. J Bacteriol 118:663–680

Afinogenova AV, Romai Penabad S, Konovalova SM, Churkina LG, Lambina VA (1981) Comparative characteristics of the *Bdellovibrio* strains isolated from river water and sewage. Mikrobiologiia 50:378–385

Amann RI, Krumholz L, Stahl DA (1990) Fluorescence-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J Bacteriol 172:762–770

Baer ML, Ravel J, Chun J, Hill RT, Williams HN (2000) A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax* gen. nov.

as *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* comb. nov., respectively. Int J Syst Evol Microbiol 50:219–224

Cole JR et al (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res 33:D294–296

Cotter TW, Thomashow MF (1992) A conjugation procedure for *Bdellovibrio bacteriovorus* and its use to identify DNA sequences that enhance the plaque-forming ability of a spontaneous host-independent mutant. J Bacteriol 174:6011–6017

Crothers SF, Robinson J (1971) Changes in the permeability of *Escherichia coli* during parasitization by *Bdellovibrio bacteriovorus*. Can J Microbiol 17:689–697

Davidov Y, Jurkevitch E (2004) Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax-Peredibacter* clade as *Bacteriovoraceae* fam. nov. Int J Syst Evol Microbiol 54:1439–1452

Flannagan RS, Valvano MA, Koval SF (2004) Downregulation of the *motA* gene delays the escape of the obligate predator *Bdellovibrio bacteriovorus* 109J from bdelloplasts of bacterial prey cells. Microbiology 150:649–656

Fratamico PM, Whiting RC (1995) Ability of *Bdellovibrio bacteriovorus* 109J to lyse gram-negative food-borne pathogenic and spoilage bacteria. J Food Protect 58:160–164

Hampton T (2004) Researchers eye Predatory bacterium for novel antimicrobial strategies. J Am Med Assoc 291:1188–1189

Herschkovitz Y, Lerner A, Davidov Y, Okon Y, Jurkevitch E (2005) *Azospirillum brasilense* does not affect population structure of specific rhizobacterial communities of inoculated maize (*Zea mays*). Environ Microbiol 7:1847–1852

Huang JC, Starr MP (1973) Effects of calcium and magnesium ions and host viability on growth of bdellovibrios. Antonie Van Leeuwenhoek 39:151–167

Hugenholtz P, Tyson GW, Blackall LL (2002) Design and evaluation of 16S r RNA-targeted oligonucleotide probes for fluorescence in situ hybridization. Methods Mol Biol 179:29–42

Jurkevitch E (2000) The Genus *Bdellovibrio*. In: Dworkin M et al (eds) The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, 3rd edn, release 3.1, 20 January 2000. Springer, Berlin Heidelberg New York. http://link.springer-ny.com/link/service/books/10125/

Jurkevitch E (2006) In: Coico R, Kowalik T, Quarles J, Stevenson B, Taylor R (eds) Isolation and classification of *Bdellovibrio* and like organisms in Current Protocols in Microbiology. Wiley, New York, pp 7B11–17

Jurkevitch E, Ramati (2000) Design and uses of *Bdellovibrio* 16S rRNA-targeted oligonucleotides. FEMS Microbiol Lett 184:265–271

Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66:2365–2371

Kadouri D, O'Toole GA (2005) Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 71:4044–4051

Klein DA, Casida LE Jr (1967) Occurrence and enumeration of *Bdellovibrio bacteriovorus* in soil capable of parasitizing Escherichia coli and indigenous soil bacteria. Can J Microbiol 13:1235–1241

Koval SF (2001) Cover page. J Bacteriol 183:11

Koval SF, Bayer ME (1997) Bacterial capsules: no barrier against *Bdellovibrio*. Microbiology 143:749–753

Koval SF, Hynes SH (1991) Effect of paracrystalline protein surface-layers on predation by *Bdellovibrio bacteriovorus*. J Bacteriol 173:2244–2249

Lambert C, Smith MCM, Sockett RE (2003) A novel assay to monitor predator-prey interactions for *Bdellovibrio bacteriovorus* 109J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol 5:127–132

Lathe R (1985) Synthetic oligonucleotide probes deduced from amino acid sequence data. Theoretical and practical considerations. J Mol Biol 183:1–12

Nunez ME, Martin MO, Chan PH, Spain EM (2005) Predation, death, and survival in a biofilm: *Bdellovibrio* investigated by atomic force microscopy. Colloids Surf B Biointerfaces 42:263–271

Pfennig N, Wagener S (1986) An improved method of preparing wet mounts for photomicrographs of microorganisms. J Microbiol Meth 4:303–306

Pineiro SA, Sahaniuk GE, Romberg E, Williams HN (2004) Predation pattern and phylogenetic analysis of *Bdellovibrionaceae* from the Great Salt Lake, Utah. Curr Microbiol 48:113–117

Ravenschlag K, Sahm K, Pernthaler J, Amann R (1999) High bacterial diversity in permanently cold marine sediments. Appl Environ Microbiol 65:3982–3989

Rendulic S et al (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692

Richardson IR (1990) The incidence of *Bdellovibrio* spp. in man-made water systems: coexistence with legionellas. J Appl Bacteriol 69:134–140

Ruby EG (1992) The Genus *Bdellovibrio*. In: Balows A, Truper HG, Dworkin M, Harder W, Scheifer KH (eds) The Prokaryotes. Springer, Berlin Heidelberg New York, pp 3401–3415

Schwudke D, Strauch E, Krueger M, Appel B (2001) Taxonomic studies of predatory bdellovibrios based on 16S rRNA analysis, ribotyping and the *hit* locus and characterisation of isolates from the gut of animals. Syst Appl Microbiol 24:385–394

Seidler RJ, Starr MP (1969) Factors affecting the intracellular parasitic growth of *Bdellovibrio bacteriovorus* developing within Escherichia coli. J Bacteriol 97:912–923

Shemesh Y, Jurkevitch E (2004) Plastic phenotype resistance to predation by *Bdellovibrio* and like organisms in bacterial prey. Environ Microbiol 6:12–18

Shemesh Y, Davidov Y, Koval S, Jurkevitch E (2003) Small eats big: ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator-prey interactions. Agronomie 23:1–7

Starr MP, Huang JC (1972) Physiology of the bdellovibrios. In: Rose AH, Tempest DW (eds) Advances in Microbial Physiology. Academic, London, pp 215–261

Starr MP, Stolp H (1976) *Bdellovibrio* methodology. In: Norris JR (ed) Methods in Microbiology. Academic, London, pp 217–244

Stolp H (1981) The Genus *Bdellovibrio*. In: Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG (eds) The Prokaryotes. Springer, Berlin Heidelberg New York, pp 618–629

Stolp H, Petzold H (1962) Untersuchungen über einen obligat parasitischen Mikroorganismus mit lytischer Aktivität fur *Pseudomonas*-Bakterien. Phytopathologische Zeitschrift 45:364–390

Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* Gen. et Sp. N., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie Van Leeuwenhoek 29:217–248

Thomashow MF, Rittenberg SC (1979) The Intraperiplasmic Growth Cycle – The Life Style of the Bdellovibrios. In: Parish JH (ed) Developmental Biology of Prokaryotes. University of California Press, Berkeley, CA, Blackwell, London

Varon M, Shilo M (1970) Methods for separation of bdellovibrio from mixed bacterial population by filtration through Millipore filters or by gradient differential centrifugation. Rev Intern Oceanogr Med 18–19:145–152

Varon M, Shilo M (1980) Ecology of aquatic bdellovibrios. In: Droop MR, Jannasch H (eds) Advances in Aquatic Microbiology. Academic, London, pp 1–48

Microbiol Monogr (4)
E. Jurkevitch: Predatory Prokaryotes
DOI 10.1007/7171_2006_058/Published online: 9 November 2006

Ecology of the Predatory *Bdellovibrio* and Like Organisms

Henry N. Williams [1] (✉) · Silvia Piñeiro [2]

[1]Environmental Sciences Institute, Florida A&M University, Tallahassee, FL 32301, USA
henryneal.williams@famu.edu

[2]School of Medicine, University of Maryland, Baltimore, Baltimore, MD 21201, USA

Abstract This work explores what is known about the ecology of the *Bdellovibrio* and like-organisms (BALO). Recent studies of these incredibly unique predatory bacteria have revealed new information on some of their genomic features, distribution in the environment, environmental determinants that may select for the predators and their interactions with other bacteria. However, little remains known about the ecology of BALOs and their role in nature. Lack of advances in applications of newer molecular methodologies to study the predators remains a key barrier to their investigation. Nevertheless, a brighter future awaits as molecular techniques aimed at improving their detection and enumeration are being applied. On the basis of culture methods the predators are known to be ubiquitous exhibiting both geographical and seasonal distribution patterns.

Geographically, in some cases different strains of BALOs occur in different habitats. One strain has been observed only in some estuarine systems. Apparent factors that determine the distribution of the predators include salinity, food source, temperature and oxygen. The tolerance to salt has defined two groups, the marine or halophilic strains that require salt and the freshwater/terrestrial (F/T) group that is inhibited by salt and is found in freshwater and in soil. A major food source for BALOs is biofilm, a surface-associated bacterial community where large numbers of bacteria susceptible to the predators may

be found. Although results of previous studies failed to show any meaningful chemotactic attraction by BALOs, recent studies have revealed that some strains of BALOs do show preference for certain bacterial strains when a mixture of different bacteria are available.

The role of BALOs in nature has remained elusive. Although direct evidence is still lacking, the results of several studies have provided collateral information that makes it difficult to discount BALOs having a role in bacterial mortality. Supporting evidence includes their obligate requirement for prey bacteria and predatory lifestyle that leads to the killing of its bacterial prey, their ubiquity in the environment, and the susceptibility of many environmental Gram-negative bacterial species. Where BALOs have been applied to control bacterial populations to prevent disease in plants and animals and reduce bacterial contamination on environmental surfaces, the predators have shown some degree of effectiveness. BALOs continue to be one of the most fascinating yet mysterious organisms in the microbial world. Greater efforts are needed to unravel their ecology and potential as biological control agents.

1 Introduction

Bdellovibrio was the name originally assigned to the predatory, intraperiplasmic bacteria. However, as the organisms were further studied it became apparent that the diversity among isolates demanded a separation of some groups into different species and genera. Several genera have now been described and more are quite likely in the future. Since these recent developments may not be widely recognized, to minimize confusion we have elected to refer collectively to the original genus and others that have been reported as the *Bdellovibrio* and Like Organisms (BALOs). It is not intended that this designation will remain permanent as it will be important to refer to specific genera once the revised taxonomy is widely known and accepted.

The first report describing the predatory *Bdellovibrio* in 1962 (Stolp and Petzold 1962) sparked intrigue and fascination about the possible impact and role of this predator on microbial communities in the environment. There was speculation that this unique bacterium that preyed upon many Gram-negative bacteria could control populations of susceptible organisms. However, this hypothesis generated only a few investigations on the interactions of bdellovibrios with prey bacteria in nature. In the decade following their discovery, much of the research on the predators focused on unraveling the *Bdellovibrio's* life cycle and the mechanisms of predator-prey interactions in laboratory studies (Starr and Baigent 1966; Varon and Shilo 1968; Seidler and Starr 1969). The first reports of the predatory bacteria described them as ectoparasites that attached to the outer membrane of the prey and ultimately caused its lysis without entering the cell (Stolp and Starr 1963). However, thin section electron microscopy studies conducted later revealed that the *Bdellovibrio* actually penetrated the cell wall and assumed residence in the periplasmic space of its prey (Shilo and Bruff 1965). There it disrupts the

permeability of the cytoplasmic membrane, grows into a long filamentous cell at the expense of prey molecules that leach into the periplasm, multiplies by segmentation and fragmentation of the long filament and lyzes the remaining ghost of its prey releasing progeny into the environment to repeat the cycle.

BALOs are not the only predatory bacteria that have been described (see Jurkevitch and Davidov, 2006, in this volume). However, the BALOs are the only known bacterial predator that invades the periplasmic space of its prey and has a life cycle consisting of an extracellular and intraperiplasmic phase.

In the first decade, and ensuing years, of the discovery of *Bdellovibrio*, reports of isolation of the organism appeared occasionally in the literature. It soon became apparent that there were two major types of the predatory bacteria, one requiring salt that was restricted to marine or salt water ecosystems (Shilo 1966; Mitchell and Yankofsky 1967) and another that had low tolerance for salt and was found in freshwater and soil (Varon 1968). It also became obvious that the BALOs are ubiquitous in distribution having been recovered from sites around the world. The freshwater bdellovibrios have been recovered from water, soil, sewage, rhizosphere, water distribution systems and the intestinal track of animals and humans (Klein and Casida 1967; Germida 1987; Fry and Staples 1974, 1976; Keya and Alexander 1975; Richardson 1990; Edao, 2000). The saltwater predators have been isolated from oceans, seas, salt lakes, estuaries, gills of blue crabs and in one case from the intestinal track of a rabbit (Taylor et al. 1974; Marbach et al. 1976; Williams et al. 1980; Sánchez-Amat and Torrella 1989; Kelley and Williams 1992; Pineiro et al. 2004). One habitat where BALOs have been found in relatively large numbers is surface biofilm (Williams et al. 1995).

This work will review what is currently known about the ecology of the BALOs. The distribution and abundance of the organisms in nature will first be reviewed, followed by other topics including diversity and taxonomic changes and how these alter the perspective of the ecology of BALOs, prey range, the role of the bacterial community in selecting for BALOs and their role in nature.

Being about 1/5th the size of a typical bacterium, the BALOs have been called "the world's smallest hunters". The physical aspects of the BALOs life cycle have been well documented (Starr and Baigent 1966; Varon 1968) and are described elsewhere in this volume. Several reports have documented the biochemical and physiological properties of the predators' growth cycle within the prey and the source of nutrients supplied by the prey (Varon et al. 1969; Starr and Baigent 1966; Rittenberg and Langley 1975; Diedrich 1988). However, many gaps in knowledge remain, especially in regard to the genetic controls at play in the various stages of the life cycle. Such investigations are now being addressed, facilitated by the first description of a whole genome sequence of a BALO, *Bdellovibrio bacteriovorus* 100 (Rendulic et al. 2004). Work on sequencing other strains is currently underway in several laboratories.

Much of the work on the *Bdellovibrio* life cycle was done on just a few strains at a time when BALOs were treated as a homogenous population, a single genus and specie, with little known diversity. Recently, several reports have documented that the BALOs are not a homogenous group but rather are very diverse potentially consisting of several genera and multiple species (Baer et al. 2000, 2004; Davidov and Jurkevitch 2004; Williams et al. 2004). Therefore, it can not be assumed, that all BALOs have identical features as the few strains described in early studies. For example, differences in specific stages in the life cycle may occur among diverse groups. One recent finding revealed that in at least one strain the long held belief that the flagella of *Bdellovibrio* was shed during its penetration into the prey cell appears not to be the case (Lambert et al. 2006). Whether this is universal for all BALOs or is strain specific will require further investigation. This applies also to other features of the BALO life cycle, prey interactions, metabolic properties, ecology, etc.

Up to now it has been assumed that the BALOs are obligate predators. Clearly all evidence to date suggests that they require prey to complete their life cycle and propagate. This perception drives much of our perspectives of the BALOs, especially their dependence on other bacteria as the sole source of nutrients, and their ecology. However, considering that there has been little effort to determine if prey-independent BALOs exist in nature, this possibility should not be dismissed outright and studies should be encouraged to address the issue.

2
Methods for Ecological Studies of BALOs

Few advances in ecological methods to study the BALOs have been made in the past several decades and only now is there beginning to be application of recently developed molecular techniques. Unraveling the ecology of the BALOs requires suitable methods, especially with their unique growth requirement for prey, which has negated their cultivation in pure culture directly from environmental samples. Although this is one of the greatest barriers for investigators, there has not been sufficient study aimed at developing media and methodologies for recovery of BALOs independent of a prey bacterium. Since traditional methods for characterizing bacteria require utilization of pure cultures. They are not suitable for the study of the BALOs. Characterization and diversity assays of BALO isolates have typically been limited to a few phenotypic properties as prey-susceptibility patterns and salinity and temperature tolerances. Since traditional cultural methods are limited, molecular genetic methods may be better for characterizing BALOs.

Until the early 1990s, few studies characterized genetic properties of BALOs and these were typically limited to %G+C ratios and DNA/DNA hy-

bridization (Seidler et al. 1972). The subsequent use of the 16S rRNA gene sequence by Donze et al. (1991) and later by Jurkevitch and Ramati (2000) and Snyder et al. (2002) have revealed much greater diversity among the BALOs than was previously known (see Jurkevitch and Davidov, 2006, in this volume; Snyder 2002; Davidov and Jurkevitch 2004). The use of molecular-based non-cultural methods for detecting the predators has been slow in developing and only in the last several years have there appeared reports of 16S rRNA-derived oligonucleotides for in situ and dot-blot hybridization (Jurkevitch and Ramati 2000; see Koval, 2006, in this volume) and primers for PCR-based analysis of environmental samples for *Bdellovibrio* and *Bacteriovorax*, *Peridibacter* and *Micavibrio* (Snyder et al. 2002; Jurkevitch and Ramati 2000; Pineiro et al. 2004; Herschkovitz et al. 2005; Davidov et al. 2006). At least two laboratories have used specific primers to detect or confirm BALO isolates (see Jurkevitch, 2006, in this volume; Mahamoud et al. 2005).

Utilization of quantitative methods such as fluorescence in situ hybridization (FISH), flow cytometry or real time PCR to enumerate BALOs is just being reported or considered. This lag in the use of molecular and microscopic techniques to study the BALOs can be attributed in large part to the fact that so few investigators have consistently undertaken the challenges to investigate these organisms in the past 20 years due largely to the lack of suitable methods. This has contributed to the meager knowledge that exists on the ecology of these predatory bacteria. As genome sequences continue to be described as discussed elsewhere in this volume (Tudor and McCann), further study and development of molecular tools can be expected. As of now however, there remains a dearth of investigations devoted to ecological studies of the BALOs.

The methods that have been and continue to be used routinely for the growth and isolation of BALOs have substantial limitations. As with other bacteria, the culture method for BALOs is limited by the medium used. The double agar overlay method used to study bacteriophages has been adapted to isolate and propagate the BALOs since their discovery. In fact, the BALOs were discovered while attempting to isolate bacteriophage from soil (Stolp and Petzold 1962). Upon examination of the double agar plates for phage plaques, the investigators observed late-developing plaques (colonies) that increased in size with continued incubation, which is atypical for phage. Microscopic examination of these plaques revealed small comma shaped, highly motile, predatory bacteria later named the *Bdellovibrio* (more details described in the introductory part of this volume). Another method also used to recover viruses, the broth enrichment culture technique, has been applied for the recovery of BALOs as well (technical details can be found in Koval, 2006, in this volume). Ideally in culture methods, the formulation of the media should foster the growth of the great majority of cells in a population. However, it has been well documented that only a small percent of the bacteria present in environmental samples can be recovered on culture media (Daley 1979).

Nevertheless, at this time there remain practical reasons for using the culture method including to obtain livecells that can be tested to characterize and differentiate new isolates.

A key component of media to grow BALOs is the prey. Although many BALO isolates prey on a number of bacterial species, some prey only on a few (Taylor et al. 1974; Schoeffield and Williams 1990; Pineiro et al. 2004) (Table 1). In cases where BALOs prey on multiple bacterial strains, the efficiency of predation and predator growth may vary between the prey strains. Thus, the choice of prey becomes an important decision for investigators attempting the recovery of BALOs from environmental samples. A primary concern is whether the prey is capable of supporting the growth of all subpopulations of BALOs or even the specific strains of interest to the investigator.

Several studies have evaluated different bacterial species for recovery and enumeration of BALOs in saltwater environments. In one of the first comprehensive studies of susceptibility of various bacteria to the predators, Taylor et al. (1974) investigated the predation pattern of 13 marine BALO isolates recovered from waters off the coast of Oahu, Hawaii against a battery of 42 bacterial strains including marine and terrestrial species. Two different media were used, basal medium agar and yeast extract agar prepared with half-strength artificial seawater. Incubations were at 25 °C. Many bacterial species were susceptible to predation by all of the BALO isolates tested. However, *Vibrio* species were among the most susceptible. In contrast, *Pseudomonas* species were resistant to attack by the BALO isolates. Marine BALOs typically showed greater predation efficiency on marine than non-marine bacteria, although there were exceptions.

Likewise, it has been reported by others that *Vibrio* sp., including *V. parahaemolyticus*, were typically most susceptible to saltwater BALOs and most efficient at recovering the predators (Taylor et al. 1974; Schoeffield and Williams 1990; Sutton and Besant 1994; Rice et al. 1998). Sanchez-Amat and Torrella (1989) reported that when enrichment cultures established by adding yeast extract to seawater samples were plated on a strain of *Pseudomonas*, *V. alginolyticus* and *V. parahaemolyticus*, in every case of four experiments the greatest number of BALO PFUs were recovered on *V. parahaemolyticus*.

In another comprehensive study, the author and co-workers compared 44 bacterial species for efficiencies at recovering BALOs from two different environments, a tidal pond at the University of Maryland Horn Point Environmental Laboratory and a tank at the National Aquarium in Baltimore, both in Maryland, USA (Schoeffield and Williams 1990) (Fig. 1). *Vibrio parahaemolyticus* strain P-5 yielded significantly more BALO plaques than the other test prey bacteria. Further, when the material from plaques formed on each of the test bacteria were subcultured onto top agar lawns of *V. parahaemolyticus*, plaques were produced 97% of the time. This suggests that the plaques appearing on the test bacteria were enumerated also on *V. para-*

Table 1 Prey susceptibility assay. Results were considered: positive + = 2(1, 1), negative – = 0(0, 0) and variable *V* = 1(1, 0). (From Pineiro et al. 2004, by permission)

Source of bacterial strains used in prey-susceptibility test	Test bacteria strains	BALO Isolates								
		GSL2[a]	*GSL3*[a]	*GSL4*[a]	*GSL4A*[a]	*GSL4B*[a]	*GSL4E*[a]	*SJ*[b]	*OC7*[c]	*JS10*[d]
Crabs, courtesy of Dr. D. Johnson, Veterans Administration Medical Center (VAMC), Baltimore	P5	+	+	+	+	+	+	+	+	+
Great Salt Lake, UT	GSLP1	+	+	+	+	V	+	+	+	+
Great Salt Lake, UT	GSLP2	+	+	+	+	+	+	+	+	+
Great Salt Lake, UT	GSLP3	+	–	+	+	+	+	+	–	–
Great Salt Lake, UT	GSLP4	+	+	+	+	+	+	+	+	+
Virginia Beach, VA	VP1	+	+	–	–	–	–	+	+	–
Virginia Beach, VA	VP2	+	+	+	V	V	+	+	V	–
Virginia Beach, VA	VP3	+	–	V	V	V	–	+	+	+
Virginia Beach, VA	VP4	+	–	V	V	+	V	V	V	–
Virginia Beach, VA	VP5	V	–	+	V	+	+	–	+	V
Mediterranean Sea, Romano Beach, Spain	MedP1	+	+	+	+	+	–	+	+	V
Mediterranean Sea, Romano Beach, Spain	MedP3	–	–	–	–	–	–	–	+	+
Mediterranean Sea, Romano Beach, Spain	MedP4	–	–	–	–	–	–	–	–	–
Dr. Judith Johnson, VAMC Baltimore	*V. vulnificus*	–	–	–	–	–	–	+	+	–
Dr. Judith Johnson, VAMC Baltimore	*V. cholerae* 01	–	+	+	+	+	+	+	+	–

[a] Great Salt Lake, UT
[b] St. John Island, Virgin Islands
[c] Ocean City, MD
[d] Chesapeak Bay, MD

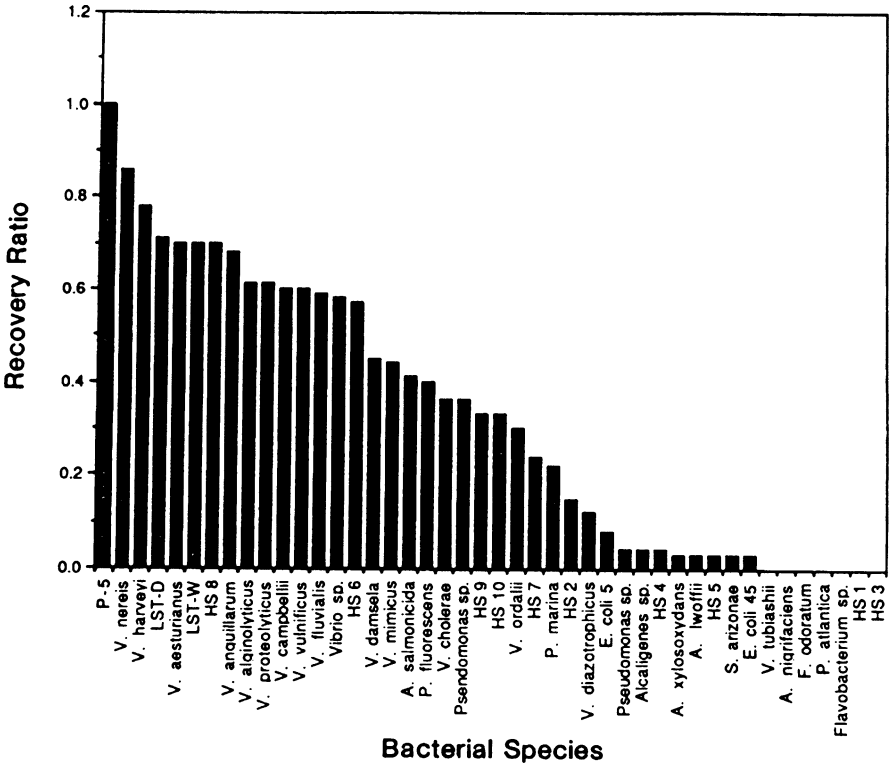


Fig. 1 Quantitation efficiencies of various bacterial prey compared with that of *V. parahaemolyticus* P-5 (Horn Point trials 1 to 9). (From Schoeffield et al. 1990 by permission)

haemolyticus. The results also revealed that as a group, the *Vibrio* species were two to three times more efficient at recovering BALOs than non-*Vibrio* species (Fig. 2).

Sutton and Besant (1994) tested 36 BALO isolates, 12 from each of three habitats, a sandy beach, mangrove and fringing coral reef, in the Great Barrier Reef for predation against 39 bacteria including marine and some non-marine strains. A different test medium (basal medium) and incubation conditions (27 °C) than used in other studies were applied. The results of the prey susceptibility studies concluded, as did the previous study by Taylor et al. (1974), that *Vibrio* species were among the most susceptible bacteria to BALOs.

Rice et al. (1998) tested the ability of BALOs from Chesapeake Bay waters, sediment and biofilm to prey upon autochthonous bacterial isolates. Again, *Vibrio* species were typically among the most susceptible and efficient at recovering BALOs.

Although there may not be a universal prey that can be used for the recovery of all BALOs, the data supports the use of *V. parahaemolyticus* to recover

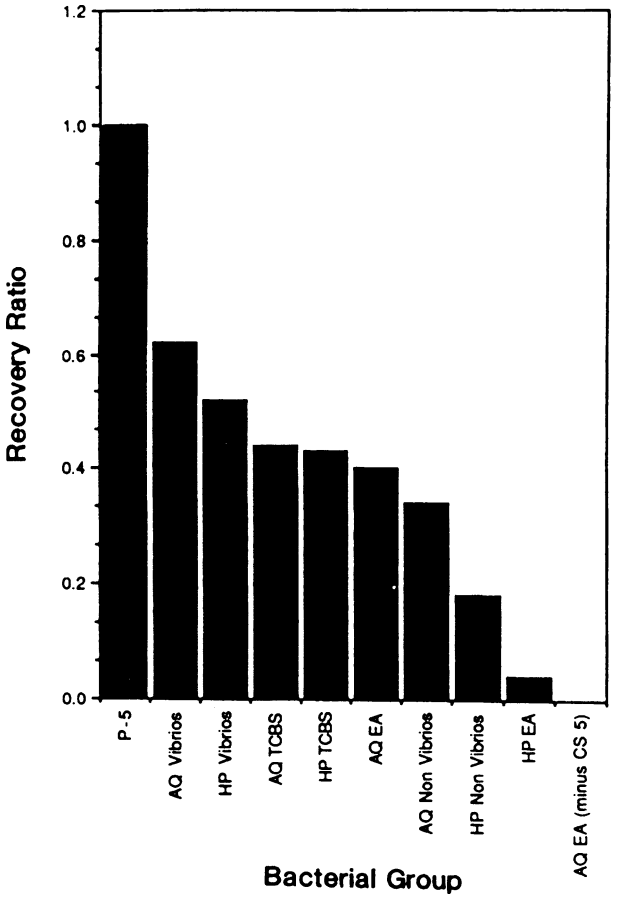


Fig. 2 Comparison of the mean quantitation efficiencies of *Vibrio* species, non-vibrio species, TCBS isolates, and EA isolates recovered from pond (HP) and aquarium (AQ) water samples. *V. parahaemolyticus* P-5 was used as the standard reference organism. (From Schoeffield et al. 1990 by permission)

saltwater BALOs by the culture technique. Nevertheless, continued testing of other bacteria is encouraged.

Quantitative studies comparing the efficiency of different bacteria in recovering the freshwater-terrestrial (F/T) BALOs have also been reported. Among the most commonly used prey are strains of *E. coli* (Dias and Bhat 1965; Klein and Casida 1967; Staples and Fry 1973). However, variations in the bacterial species most efficient at recovering the predators have been observed, depending sometimes on the source of the samples. From sewage and activated sludge samples, Dias and Bhat (1965) observed *E. coli* to yield more plaques than eight other species. Among the bacteria yielding the lowest number of plaques and, hence least efficient at recovery of BALOs, were *Pseu-*

domonas aeruginosa, *P. chlororaphis* (previously *P. aureofaciens*) and *Serratia marcescens*. Klein and Casida (1967) reported that *P. aeruginosa*, *P. fluorescens* and *P. putida* were lyzed by BALO strain 167-1 but not OX9-1, both of which lyzed *E. coli*.

Staples and Fry (1973) tested 10 bacterial strains for the recovery of BALOs from river water and sewage. *Achromobacter* sp. yielded more plaques in more samples than the other bacteria tested on NB 500 medium. *E. coli* yielded more samples positive for BALOs than *P. aeruginosa*, but the difference was not significant. *E. coli* strains have been used in other studies either as the single prey or in combination with *Achromobacter* sp. (Fry and Staples 1976). Studies have also used *E. coli*, *Pectobacterium carotovorum* (previously *Erwinia carotovora*), *Alcaligenes faecalis* and *Pseudomonas fluorescens* as reported by Afinogenova et al. 1981. This group also reported that of 10 laboratory stock cultures of BALOs and 12 freshly isolated strains from Russia out of the river Oka and sewage from the City of Puschino, none preyed upon *Pseudomonsas aeruginosa*, *Stenotrophomonas* (previously *Pseudomonas*) *maltophila* and *Brevundimonas* (previously *Pseudomonas*) *vesicularis*.

Jurkevitch et al. (2000) reported testing five soil BALO isolates for predation on 22 bacteria, most associated with plants either as pathogens or having growth-enhancing activity. None of the test prey bacteria were lyzed by all five of the BALO isolates tested. The most susceptible prey organisms were lyzed by four of the five BALO isolates. *Pseudomonas syringae* pv *tomato* and *P. corrugata* were lyzed by the same four predator isolates yielding an identical lysis pattern. *Enterobactor agglomerans* and *Chromobacterium violaceum* each were lyzed by four BALO isolates, but some were different from those that preyed on the two *Pseudomonas* sp. thus yielding a different lysis pattern. *S. maltophila*, *P. putida*, *Serratia marcescens*, *P. carotovorum* subspecies *carotovorum* 2 and *Vibrio fluvialis* were only preyed upon by one or two of the predator isolates tested. When soil and rhizosphere samples were cultured for the recovery of the predators using various bacteria as prey, the most efficient at recovering BALOs from soil samples was *P. carotovorum* subsp. *carotovorum* 24 and *P. corrugata* PC.

From the results of the several studies referenced above on the efficiency of recovery of F/T BALOs, no one genus or specie emerged in prominence as was the case with *Vibrio* sp. for the saltwater BALOs. Perhaps habitats such as soil, rhizosphere, water and sewage, may have different bacterial communities that may select for different strains of the predators that prey more efficiently on the autochthonous bacteria. Likewise, the predators may require specific prey unique to the community for maximum recovery. Nonetheless, *E. coli* and *Achromobacter* are likely good choices for isolating the predators from sewage and river water. Soils and rhizosphere may require the use of multiple preys including indigenous species. Perhaps it is prudent to use more

than a single bacterium species when attempting to isolate F/T BALO from any source.

In many of the reports cited above and others describing testing of bacteria for susceptibility to BALOs, there has been a lack of uniformity in how the tests have been conducted. In most cases there was variation in culture media and battery of test bacteria and used incubation times (Afinogenova et al. 1981; Sánchez-Amat and Torrella 1989; Schoeffield and Williams 1990; Sutton and Besant 1994; Taylor et al. 1974). Methods to achieve uniformity in medium thickness, freshness of the medium and other important controls in the conduct of the test are frequently not described and are assumed to not have been taken into consideration. This variation in test methods has made it impossible to compare and apply broadly the results from different reports. This is a serious flaw in the study of BALOs and one which should be corrected in future studies to maximize the utility and value of the test and advance understanding of the interactions of the predators with various bacteria. However, in spite of the deficiencies, when the data from the different studies are analyzed a few general trends are evident. The prey organisms that have been identified as the most efficient for recovery of F/T BALOs differ from those most efficient for recovering the salt water BALOs, although there may be some overlap. For example, *E. coli* is preyed upon by both saltwater and F/T. *Vibrio* sp. is the clear choice for saltwater BALOs whereas *Achromobacter* sp. *P. corrugata* and *E. coli* appear best for the F/T predators.

In addition to the bacterium selected to incorporate into a medium for recovery of BALOs, the chemical formulation of the culture medium may also influence the efficiency of recovery of the predators. Williams (1979) tested media having various chemical formulations and found differences in plaquing efficiency for the BALOs (Fig. 3). Even different dilutions of the same medium can yield varying results (Fig. 3). Typically, BALOs can be better detected when grown in a dilute medium as opposed to an undiluted enriched medium. For example, significantly higher numbers of F/T BALO plaques were recovered on nutrient agar (top agar concentration 0.7%) diluted 500 times than on the medium diluted 10 times (Staples and Fry 1973). The plaques also developed more rapidly and were larger in diameter. Similar observations have been made for the saltwater BALOs (Williams 1979). In the case of the saltwater strains, polypeptone 20 medium (Pp20 agar) (Williams 1979; Williams et al. 1980) was found to be as, or more, efficient than the other media tested. It must be considered always that the saltwater BALO strains require sodium chloride at a minimum concentration of 0.5% for sustained growth (Taylor et al. 1974; Marbach et al. 1976). Other salts are also required for increased growth efficiency (Marbach and Shilo 1978). To the contrary, the freshwater BALOs do not tolerate sodium chloride at concentrations above 0.5%, but recovery is increased with other salt ions such as magnesium and calcium (Varon and Shilo 1968).

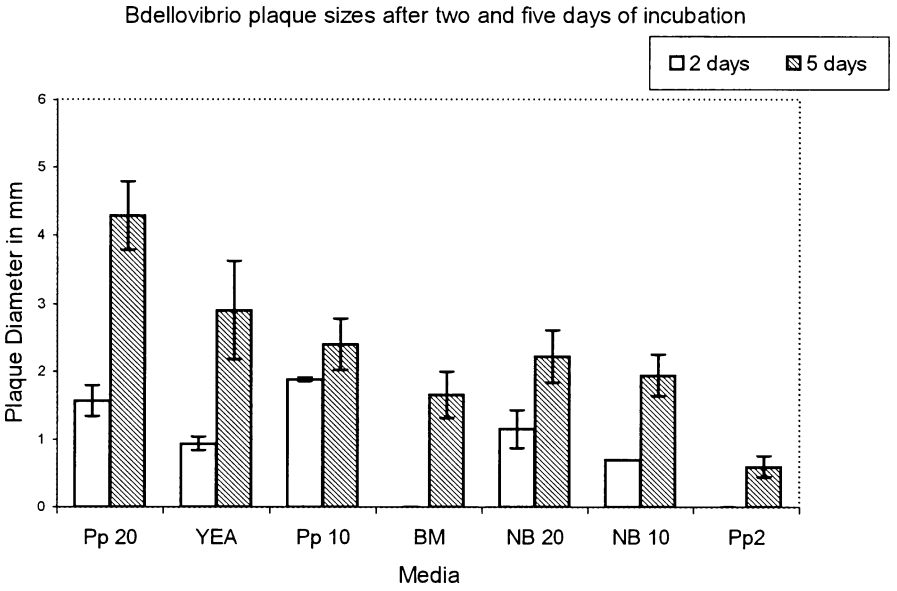


Fig. 3 Mean diameter of plaques produced by *Bacteriovorax* OC1, a marine strain, when grown on *V. parahaemolyticus* on various media after two (*empty columns*) and five (*stripes*) days incubation. (From Williams 1979, by permission)

Other important factors in the efficiency of the recovery of BALOs is incubation temperature and time. The optimum conditions may vary according to species. For saltwater BALOs, plaque formation on plates is optimal at incubation temperatures between 22 and 25 °C. Temperatures above 30 °C significantly decreased plaque formation (Marbach et al. 1976; Williams 1979). To the contrary, approximately 30 °C is optimal for growth of the F/T BALOs (Uematsu et al. 1971). Under optimal incubation conditions, plaques typically appear between 48 and 96 h for both the saltwater and F/T BALOs. In some cases the authors have observed more rapid or delayed plaque formation for some isolates.

Although the culture method for detection of bacteria is considered by some as being antiquated, BALOs represent an example where, to date, culture remains the only method that can be applied to the quantitative detection of these predatory bacteria. Fortunately, this situation is expected to change in the near future with several laboratories examining molecular-based techniques. This should lead to more accurate estimates of the number of the predators in nature. Culture will remain an important tool for determining prey-susceptibility assays, growth conditions and interactions of BALOs with other bacteria.

3
The Distribution of the BALOs

BALOs have been recovered from widely dispersed geographical areas and many different types of ecosystems (Dias and Bhat 1965; Fry and Staples 1976; Jurkevitch et al., 2000; Klein and Casida 1967; Marbach et al. 1976; Pineiro et al. 2004; Richardson 1990; Sanchez-Amat and Torrella 1989; Sutton and Besant 1994; Taylor et al.1974; Williams et al.1980). The evidence is quite convincing that these predatory bacteria are ubiquitous in distribution. This is also evident as more and more BALO clones are detected in environmental samples in molecular surveys (see Jurkevitch and Davidov, 2006, in this volume).

However, not all strains of the bacteria are found in every type of ecosystem. The nature of the physical, chemical and biological properties of some ecosystems may exclude certain types of BALOs. The F/T group found in soils and freshwater has a low tolerance for salt and is limited to ecosystems with low salt concentrations. In the Gunpowder River in Maryland, samples collected by my laboratory group from a site where the water salinity fluctuates between fresh (< 0.3%) and brackish (> 0.5%) were cultured for both F/T and saltwater BALOs. The former were recovered when the salinity was below 0.5% and the latter when the salinity was greater. We were not able to recover both types in the same sample at any time. Sutton and Besant (1994) also reported unsuccessful attempts to recovered freshwater BALOs at sites in marine habitats that were near freshwater inputs.

3.1
Distribution in Saltwater ECO Systems

The marine or halophilic BALOs are ubiquitous in saltwater systems. Most of the early studies of BALOs in saltwater environments were of samples taken from oceans and seas (Taylor et al. 1974; Marbach et al. 1976; Torrella et al. 1978). In many cases, the samples were from a single or limited number of sites in the same body of water. One of the first and most comprehensive studies of BALOs in the marine environment was in the Pacific Ocean (Taylor et al. 1974). The numbers of BALOs recovered were low (< 1 plaque-forming unit (PFU)/ml of water sample). Quantitative studies reported by Shilo (1966) indicated a higher number of these bacterial predators in the Mediterranean Sea (40–50 PFU/ml). In both these studies the water samples were treated by filtration to reduce microbial contaminants prior to culturing. Such methods typically have the undesirable feature of also reducing the number of BALOs in the sample (Shilo 1966; Staples and Fry 1973) (Table 2).

Results of studies in our laboratory, in which water samples were cultured without methods that reduce the number of BALOs, revealed that typically the predators are more prevalent in estuaries than in ocean coastal waters (Williams 1979) (Figs. 4 and 5).

Table 2 Effect of filtering river water samples on the numbers of *Bdellovibrios* recovered using NB-10 medium and 2 different hosts. (From Staples and Fry 1973, by permission)

Filter (μm)	N° cells/ml on *Escherichia coli* B	*Aerobacter aerogenes*
None	44	40
0.8	10*	10
0.6	34*	3*
0.45	8*	0*

* significantly different at $P = 0.05$

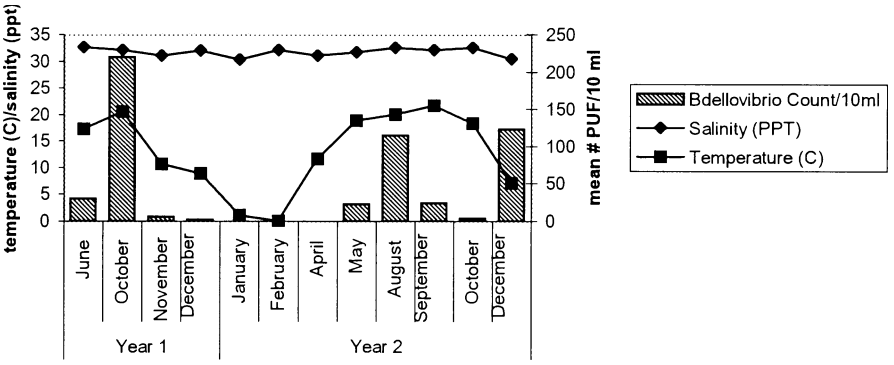


Fig. 4 Quantitation of marine bdellovibrios from ocean water collected from the beach at Ocean City, Maryland over an annual cycle. (Adapted from Williams 1979, by permission)

Hence, detection and ecological monitoring studies of these organisms may be facilitated in an estuarine environment and yield more information than the open ocean where their numbers are much lower. On the basis of this premise, in the late 1970s, the author initiated studies on the distribution of BALOs in the Chesapeake Bay, one of the world's largest and most productive estuarine systems. This was the first extensive geographic distribution study of saltwater BALOs and involved both spatial and temporal distributions in the estuary (Fig. 5) (Williams et al. 1980, 1982; Williams and Falkler 1984; Williams 1988). The results revealed greater numbers of BALOs accured in the mid-region of the estuary where the salinity was in the moderate range.

Variations in the abundance of BALOs in the water column have been described. Williams (1987) reported that the air-water surface microlayer yielded the greatest concentration of BALOs in the water column, up to 10^6 plaque-forming units per ml. Below the surface microlayer, the BALOs appeared to be rather evenly distributed in the bulk water column. An assessment of the vertical distribution of BALOs over a 24 h cycle at a site in the Miles River, a tributary of the Chesapeake Bay, found no significant difference

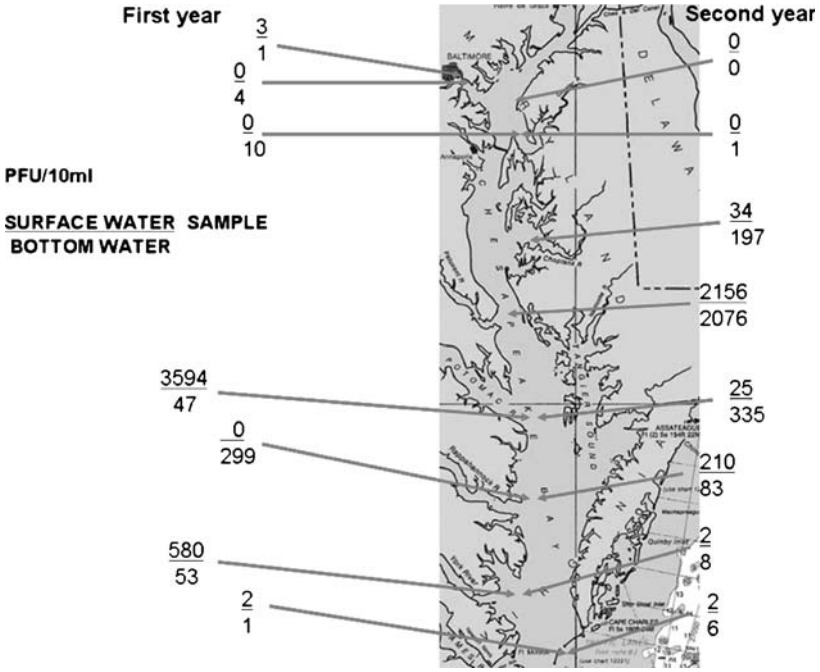


Fig. 5 Distribution of salt water bdellovibrios in the Chesapeake Bay in two consecutive years, 1978 and 1979. (Adapted from Williams et al. 1980, by permission)

in the numbers recovered at several depths in the water column (Williams and Falkler 1984). Conflicting results were reported by Sutton and Besant (1994) who did observe differences in the vertical distribution of BALOs in 1 m of water at three environmentally distinct tropical marine habitats in the Great Barrier Reef in Australia. In the summer, greater numbers were recovered in subsurface waters collected at 40 cm than in bottom water, but the reverse was reported for the winter months. Midwater samples were reported to have the least number of predators. Perhaps the differences in the vertical distribution of BALOs between the Miles River and the Australia coastal samples is due to differences in the properties of the BALOs at the two sites and/or in the environmental properties at the two sites.

The three habitats investigated in the Australian waters were a mainland sandy beach, a mangrove area and a fringing coral reef. The number of BALOs recovered from the sites varied with the greatest and most consistent number being from the mangrove area. BALOs were recovered from all mangrove samples, but not from all reef or beach samples. The lowest numbers were reported in the reef area. This investigation represented the first study on the distribution of specific strains of BALOs in the environment. Strain specificity was based primarily on differences in the predation pattern of the BALO isolates on a battery of selected bacterial species (prey susceptibility pattern).

The results revealed variations in the predator strains recovered from the different habitats. The BALOs recovered from the mangrove site yielded a different predation pattern than those from the beach or coral reef areas.

Prior to 2000, most distribution studies reported make no distinction between the different species or strains recovered, as there were no practical, reliable, genetic or biochemical methods to distinguish specific types. Among the saltwater BALOs there had not been recognition of specific genotypes or strains. The prey-susceptibility pattern phenotype used by Besant and Sutton (1990) and others has been utilized inconsistently and, consequently, is not reliable to compare results from different studies. Therefore, little or no information existed on the distribution of specific strains of BALOs in the environment.

Efforts to better define specific strains and their distribution were initiated several years ago by Snyder et al. (2002), Baer et al. (2004) and recently by Pineiro et al. (2004). These investigators analyzed the 16S rRNA gene sequence to assess the diversity and phylogenetic relationships among saltwater BALO isolates. It became apparent that the saltwater BALOs were highly diverse, consisting of a number of genotypes and/or strains and species. Differences in the sequences of the small subunit ribosomal gene among the BALOs made it possible for the first time to monitor the distribution of different BALO genotypes in the environment. The value of this tool is illustrated by the author's studies in the Chesapeake Bay. Prior to the 1990s, it was assumed that the isolates recovered in the estuary were typical marine BALOs, the same as those in the Atlantic Ocean as this is the source of saltwater into the bay. Subsequently, the author and his colleagues have analyzed and compared numerous isolates from the Chesapeake Bay and various oceans and seas and a salt lake (Pineiro et al. 2004). Several genotypes were identified in the Chesapeake Bay, including a distinct type not found thus far in ocean waters. It has subsequently been recovered from the Pamlico Sound/Neuse River estuarine system in North Carolina raising the possibility that this genotype may be restricted to estuarine environments and may represent a new specie or genus. Since estuaries vary in form and structure, freshwater input, salinity gradient, etc., further study involving different types of estuaries are needed to show if the estuarine type BALOs occurs universally in these bodies. A few other isolates recovered only from a single habitat were also found at other sites including the Great Salt Lake.

BALOs have been recovered from two extreme saltwater environments, saltern ponds and the Great Salt Lake, USA. Sánchez-Amat and Torrella (1989) studied BALOs recovered from high salinity (4.2 to 20.0%) solar evaporation pond waters and adjacent coastal seawater from the southeastern Spanish Mediterranean coast. Samples were collected and processed to recover BALOs by both the enrichment and direct plating methods. BALOs were recovered from all seawater and salt pond samples except for one from the sea. The isolates were characterized by prey range, salinity growth range

and cytochrome spectra. No definitive differences between the seawater and salt pond isolates could be detected by prey range or cytochrome spectra. All isolates except one seawater strain grew at 15% total salts in liquid culture. Differences were observed between the salt pond and seawater isolates in growth at lower salinities. None of the salt pond isolates grew at 1% salinity whereas all except one of the seawater strains were observed to grow.

BALOs were isolated from several samples taken from the Great Salt Lake in winter and spring. Most of the samples yielded isolates of the predators. Isolates were purified and the 16S rRNA gene sequence analyzed and compared to others in GenBank. The results revealed several different genotypes. Most isolates had the same genotype as strains recovered from oceans and seas. One isolate was of a genotype not found in any of the other 30 saltwater locations sampled and may represent a distinct Great Salt Lake or extreme halophilic strain. Other characterizations such as salinity growth range of the various genotypes have not been done. Pineiro et al. (2004) reported that in testing bacteria susceptibility to BALO isolates from the Great Salt Lake the predators preferentially preyed upon the lake bacteria when compared to bacteria from the Atlantic Ocean and other bodies of water.

3.2 Distribution in Freshwater, Sewage and Soil

Freshwater/terrestrial BALOs (*Bdellovibrio*, F/T *Bacteriovorax*, *Peridibacter*) are defined by their intolerance to salt concentrations above about 0.5%, %G+C ratios above 40 and presence in freshwater, soil and sewage systems, but not in saltwater. The distribution of F/T BALOs in freshwater has been addressed in several studies conducted in South Wales in the River Ely. The river is considered polluted with sewage effluents from a large sewage works facility and several small sewage facilities (Staples and Fry 1973; Fry and Staples 1974, 1976). In the 1973 study, the average number of BALO plaque-forming units (PFU) per ml reported was 112 on *Achromobacter* sp., the bacterium yielding the highest counts of several different bacteria tested (Staples and Fry 1973). A year later it was reported that average numbers of BALOs ranged from 10 to 100 PFU per ml over an 18-month period. The mean water temperature during this period was below 20 °C. In a survey involving 19 rivers in South Wales and elsewhere, the average numbers of BALOs ranged up to 51 PFU per ml from 91 samples. The numbers of BALOs were reported to be influenced by river water quality. BALO counts in unpolluted waters ranged from 0 to 3 PFU per ml whereas in grossly polluted rivers the numbers ranged from 18 to 51 PFU per ml. The major source of pollution in the rivers studied was sewage effluent. BALO numbers were observed to increase significantly at the point of entry of the sewage effluent. It was suggested that the increase was due to BALOs in the effluents and not from their multiplication in the river water.

Not many studies of freshwater systems have reported the distribution of BALOs in river sediments. Fry and Staples (1976) reported a range of BALO counts from 5.5×10^1 to 2.9×10^4 PFU from sediments from seven South Wales rivers using *Achromobacter* sp. as prey. As reported for river water, unpolluted river sediments had fewer of the predators than polluted sediments. BALOs were recovered in the upper aerobic regions of sediment in the top 5 cm. Their potential prey, heterotrophic Gram-negative bacteria, were found down to 12 cm suggesting that a lack of prey was not the reason for the absence of the BALOs in the deeper sediments. The aerobic nature of the predators likely accounted for their restriction to the upper sediments.

Most, if not all, of the reports of BALOs in soils have been of the F/T predators. As is the case with aquatic systems, F/T BALOs are ubiquitous in soils. Recovery of the predators has been reported from soils in Brazil and Canada (Germida 1987), Uganda (Keya and Alexander 1975), Australia (Parker and Grove 1970) and several states in the Unites States (Klein and Casida 1967). A range of bacteria have been successfully used as prey to recover BALOs from soils including *E. coli* strains (Klein and Casida 1967), *Azospirillum brasilense* (Germida 1987) and *Rhizobium* (Keya and Alexander 1975).

Klein and Casida (1967) reported recovering BALOs from all of 23 soil samples collected from sites located in the eastern and central United States. Two samples were taken from agricultural fields being sprayed with effluent waters from a municipal sewage treatment plant and one was obtained from the bank of a stream receiving sewage treatment effluent waters. The counts from these sites ranged between 5 to 7×10^4 PFU per g soil. A non-treated control sample yielded 1×10^4 PFU per g. The numbers of BALOs recovered from the other sites ranged from $< 1 \times 10^3$ PFU per g in soils from a lake edge grass and pine area, respectively, to 9×10^4 per g from turf. Two soil BALO isolates were tested for predation range against a battery of bacteria including 25 *E. coli* serogroups and bacteria derived from soil and other sources. The isolates were observed to prey upon and lyze all 25 *E. coli* tested. For the other bacteria, the two BALO isolates yielded the same predation pattern except that one strain preyed upon three *Pseudomonas* species tested and the other did not.

Germida (1987) reported that of several bacteria tested for susceptibility to a BALO isolate recovered from soil using *A. brasilense*, this organism was the preferred prey (Germida 1987). *P. fluorescens* and *E. adhaerens* were resistant. Using an enrichment technique, BALOs were recovered from Laptosol and Podzolic soil samples from Brazil after being stored and air dried for two years. As is the case with studies reported in the aquatic environment, most reports on isolations of the predators from soil reported bulk numbers of the predators, treating all plaque-forming units the same without any character or taxonomic distinctions.

Jurkevitch et al. (2000) reported that several BALO isolates recovered from soil and rhizosphere samples using different prey bacteria produced identical restriction patterns but yielded quite different prey susceptibility patterns.

The number of PFUs recovered from the same soil sample varied depending upon the bacteria strain used as prey, but ranged from $0.3 \pm 0.09 \times 10^3$ to $5.2 \pm 0.85 \times 10^3$ per gram of soil on *A. tumefaciens* and *P. carotovorum* subsp. *carotovorum*, respectively. The number reported from the rhizosphere was $23 \pm 1 \times 10^3$ PFU per gram using *P. corrugata* as prey. Using molecular- and culture-based methods, the investigators reported three distinct populations of BALOs among isolates from soil. This represents the first comprehensive report of diversity among soil BALOs.

The presence of salt-water BALOs in soils has not been addressed. Recently, the isolation of a *Bacteriovorax* strain from a salt-laden soil that clusters with marine BALOs was reported (Davidov et al. 2006).

3.3 BALO in Man-Made Water and Sewer Systems

It is not surprising that BALOs, like many other ubiquitous microorganisms would find their way into man-made systems. In sewage, F/T BALOs are reported to be abundant, reaching 900 cells/ml (Staples and Fry 1973). BALOs have also been recovered from man-made water distribution systems by Richardson (1990). One hundred and thirty-five water samples were collected from 81 sources including shower units, cooling towers, domestic and industrial water systems, condenser/compressors, hospital calorifiers and other systems. BALOs were recovered from 57.8% of the samples. The recovery rate would likely have been higher had the samples been cultured shortly after collection rather than stored for five months before culturing. Also *Legionella pneumophila* was the only prey used and there is no evidence as to how this organism compares to other bacteria used to recover BALOs.

The calorifiers from which BALOs were isolated in this study were designed to maintain water temperature at greater than 70 °C. Sample temperatures at the time of collection were approximately 55 °C. This was surprising as it far exceeds the upper growth range of 30 to 35 °C reported for the F/T BALOs, although BALO clones have been found in thermophilic environments (Fouke et al. 2003). The authors acknowledge that distal points on the calorifiers may not have reached the higher temperature.

Singh et al. (2003) reported detecting DNA clones matching those of BALOs in the water distribution system of dental units. Most likely the direct source of BALOs in these systems was either the water reservoir which supplies the incoming water or biofilm adhering to the inner walls of the tubing.

BALOs have also been found to persist in aquarium tanks. Williams et al. (1987) reported the recovery of BALOs from three different tanks at the National Aquarium in Baltimore. The salinity of two of the tanks was similar to that of ocean waters. In tank 13A, with American cold water lobsters and octopus from northern parts of the US, the salinity ranged from 30 to 33 ppt with a mean temperature of 15.5 °C. The salinity in the coral reef tank with

Caribbean fish and sharks was 30 to 31 ppt and the mean temperature was 24.9 °C. The mean number of BALOs from tank 13A and the coral reef tank was 3.1 ± 5.2 PFU and 1.3 ± 1.8 PFU per ml, respectively. In tank 20 the average mean number of BALOs recovered was 3.7 ± 7.1 PFU per ml. This tank also had the lowest mean salinity, 17.6 ppt, which has greater similarity to brackish waters in some estuarine systems than ocean or sea waters (the mean temperature was 20.3 °C). All of the aquarium tanks were supplied with artificial sea water which would probably not contain BALOs. The likely source of the BALO is probably the marine life introduced into the tanks.

Dias and Bhat (1965) examined BALO populations in raw sewage, activated sludge and sludge effluent. BALOs lytic against a range of bacterial species including *Pseudomonas*, *Salmonella*, *Serratia*, *Proteus* and *Aerobacter* were recovered from each of the sources. One likely origin of BALOs in sewage is the human or animal fecal material entering the system. The predators may also be introduced into the sewer system pipes and tanks from surrounding waters and soil and are able to rapidly multiply on the rich abundance of diverse bacterial strains present in sewage.

3.4
Biofilm—An Econiche for BALOs

Most recovery and distribution studies of BALOs in aquatic systems have targeted the water column. However, more than three decades ago, Shilo (1969) suggested that the optimal ecosystem for the activity and proliferation of BALOs may be in aquatic habitats other than the water column. The author's laboratory has devoted considerable efforts to testing this hypothesis and biofilm is one of several habitats examined. The recovery of BALOs from biofilms was first reported by Kelley et al. (1997). Using *V. parahaemolyticus* as prey, the predators were recovered by direct plating of suspensions of biofilm prepared from well-developed epifauna on the surfaces of oyster shells retrieved from a basket suspended in the Patuxent River (Maryland, USA). The numbers of predator PFUs recovered were three-fold greater than that found in equivalent volumes of samples from the water column and sediments (Table 3) (unpublished data).

Later, biofilm adherent to the soft outer surface of sea squirts was examined and found to also harbor large numbers of BALOs. In a subsequent study (Kelley et al. 1997), BALOs were detected on previously sterilized shell and glass surfaces within 30 minutes following their submersion in natural river water. These data suggest that BALOs like many bacteria in aquatic systems prefer to be associated with surfaces than be free-floating. The results of many studies have revealed that samples of biofilms on surfaces of aquatic animals and submerged objects (Table 4) and the surface water microlayer yield the highest numbers of BALOs found in aquatic systems (Williams 1987; Kelley and Williams 1992).

Table 3 The number ($\log_{10}$) of *Bdellovibrio* and like organisms (BALOs) PFU recovered from the supernatant fluid of oyster shell epifaunae and the water column are shown

Sample number	Month of collection	BALO PFU	
		Oyster epifaunae/5 ml supernant fluid	Water column/5 ml sample
1	9-Jun	2.50×10^4	2
2	15-Jun	2.50×10^3	6
3	July	2.50×10^4	6
4	21-Sep	3.00×10^4	1.70×10^1
5	21-Sep	1.00×10^5	1.70×10^1
6	Nov	6.30×10^2	1.50×10^1
7	Jan	1.30×10^3	≤ 1
8	Feb	2.20×10^2	≤ 1
9	Mar	4.50×10^2	≤ 1
10	Apr	2.60×10^3	0

Biofilms are one of the richest sources of bacteria and food for the BALOs and may offer other benefits for the predators such as enhancement of their survival under harsh environmental conditions. A report by Kelley et al. (1997) described the recovery of BALOs from biofilm during the coldest period in the winter months when the organisms were rarely detected in the water column. The lead author of this review has made similar observations (Table 3) (Unpublished data).

Kadouri and O'Toole (2005) investigated *Bdellovibrio bacteriovorus* in single specie biofilms generated in wells of microtiter plates. *B. bacteriovorus* was observed to immediately become established and persisted quite well when introduced into respective *E. coli* and *Pseudomonas* biofilms. Similar results were reported by Nunez et al. (2003). Using atomic force microscopy, the investigators were able to observe predation by *Bdellovibrio* in live preparations of bacterial biofilms without prior fixation and staining that may cause artifacts in specimens.

3.5 BALO in Animals

An early study by Westergaard and Kramer (1977) described a failed attempt to establish colonization of BALOs in the intestinal tract of frogs. This result cast some doubt as to the presence of BALOs in animals. Kelley and Williams (1992) were able to recover only small numbers of BALOs from the intestinal tract of Maryland blue crabs, *Callinectus sapidus*. However, large numbers of the predatory bacteria were recovered from the gills of the animals. An electron micrograph of the crab gills revealed a dense biofilm of bacteria that apparently was favorable for the proliferation of BALOs. The predators were

Table 4 Mean numbers of PFU and CFU recovered from various surfaces and water following submersion times up to 120 h [a]. (From Kelley and Williams 1992, by permission)

Time (h)	No. of organisms (mean ± SD) recovered from Oyster shell N [b]	PFU [c]	CFU [d]	Glass N [b]	PFU [c]	CFU [d]	Polystyrene N [b]	PFU [c]	CFU [d]	Water N [b]	PFU [e]	CFU [f]
24	15	8.8 ± 11.0	$1.1 \times 10^5 \pm 7 \times 10^4$	11	2.2 ± 4.5	$1.7 \times 10^3 \pm 3 \times 10^3$	10	0.9 ± 1.2	$5.4 \times 10^3 \pm 1 \times 10^4$	14	$3.0 \times 10^1 \pm 7 \times 10^1$	$7.8 \times 10^4 \pm 8 \times 10^4$
48	15	$2.8 \times 10^2 \pm 8 \times 10^2$	$1.1 \times 10^5 \pm 2 \times 10^5$	11	4.5 ± 5.8	$5.8 \times 10^3 \pm 7 \times 10^3$	9	3.8 ± 7.1	$4.4 \times 10^3 \pm 6 \times 10^3$	14	$2.6 \times 10^1 \pm 4 \times 10^1$	$7.6 \times 10^4 \pm 8 \times 10^4$
72	14	$4.4 \times 10^2 \pm 8 \times 10^2$	$1.1 \times 10^5 \pm 1 \times 10^5$	11	9.2 ± 20.7	$9.1 \times 10^3 \pm 1 \times 10^4$	9	4.2 ± 6.3	$7.1 \times 10^3 \pm 9 \times 10^3$	11	$2.7 \times 10^1 \pm 5 \times 10^1$	$5.0 \times 10^4 \pm 5 \times 10^4$
120	14	$9.4 \times 10^2 \pm 2 \times 10^2$	$1.4 \times 10^5 \pm 2 \times 10^5$	11	17.2 ± 24.3	$1.8 \times 10^3 \pm 2 \times 10^4$	9	15.6 ± 36.5	$1.0 \times 10^4 \pm 2 \times 10^4$	14	$2.8 \times 10^1 \pm 5 \times 10^1$	$7.3 \times 10^4 \pm 8 \times 10^4$

[a] The mean temperature was $14.6 \pm 6.3°$ (SD), with a range of 4 to 26.7° (N-60), the mean salinity was $10.4 \pm 5.3‰$ (SD), with a range of 0 to 16.5‰ (N-60), Puerto Rico salinity data (35‰) were not included

[b] Sample size

[c] *Bdellovibrio* plaque per square centimeter on the test surface

[d] Heterotrophic bacterium CFU per square centimeter on the test surface

[e] *Bdellovibrio* PFU per millimeter

[f] Heterotrophic bacterium CFU per millimeter

found not only in fresh crabs (14.8 ± 24.2 PFU per g of wet tissue) collected from the environment, but also from those purchased from seafood markets (19.2 ± 13.1 PFU per g of wet tissue). More recently Schwudke et al. (2001) reported the characterization of BALO isolates recovered from the gut of animals. Further study is needed on BALOs in humans and other animals.

3.6 Seasonal Distribution

In addition to geographical distribution, BALOs exhibit seasonal distribution patterns. The first report of a seasonal distribution was from studies conducted in the Patuxent River, a subestuary of the Chesapeake Bay (Williams et al. 1982). The greatest recovery of BALOs was observed in the warmer months. Both the frequency of recovery and number of predators recovered were significantly higher in late summer and early fall months, in both water and sediment samples. In the colder months, the numbers decreased and frequently BALOs could not be recovered. In all seasons, the numbers recovered and the frequency of recovery were higher in sediment than in the water column. Low, but persistent numbers of the predators recovered from the benthos in the colder seasons led the study authors to suggest that "seeds" of BALOs may survive the winter in sediment. As the water temperature increases in the spring and summer months, conditions become favorable for the proliferation of the organisms and they repopulate the water column. This hypothesis was supported in a subsequent study that showed that as the water warmed in the spring and early summer months, the number of BALOs was observed to first increase in the sediment and later in the water column (Williams 1988). Also other studies in the author's laboratory have supported the observation that BALOs do not grow or survive well below 10 °C. An indirect effect that temperature may have is on the prey of BALOs. *V. parahaemolyticus* and other *Vibrio* species, a favored prey for BALOs (Rice et al. 1998), also have been shown to exhibit a seasonal distribution in the Chesapeake Bay (Colwell et al. 1977). This could mean fewer prey available to BALOs at colder temperatures.

Seasonal influence on BALO populations was also reported in warmer tropical waters off Australia by Sutton and Besant (1994). The approximate mean water temperature at the study sites ranged from a low of 23 °C in the winter to a high of 29 °C in summer. This is substantially higher than the temperature range in the Patuxent River and Chesapeake Bay which is typically from < 2 °C to 30 °C. BALO numbers in the Chesapeake Bay were observed to increase between 15 to 23 °C (Fig. 6) (Williams et al. 1982), whereas in the tropical waters the numbers were significantly decreased at 23 °C (Fig. 7) (Sutton and Besant 1994). Another difference between the isolates from the tropical waters and those from the Chesapeake Bay is the temperature growth range. All tropical isolates grew at 35 °C whereas those in our laboratory

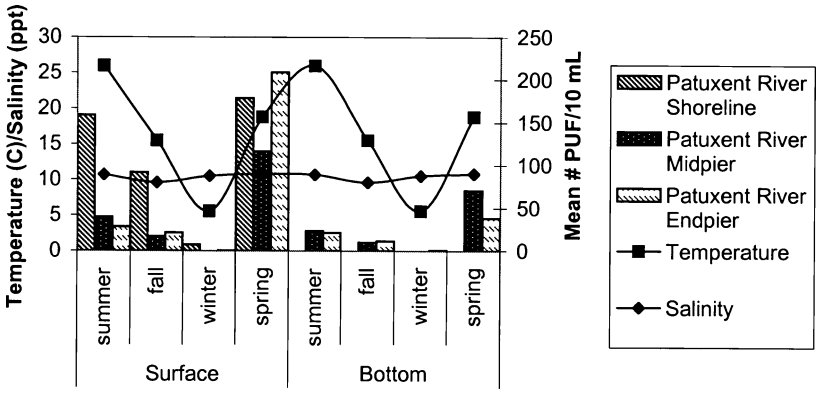


Fig. 6 Seasonal distribution of BALOs recovered from the Patuxent River, Solomons, Maryland, USA. (Williams HN et al. 1982, by permission)

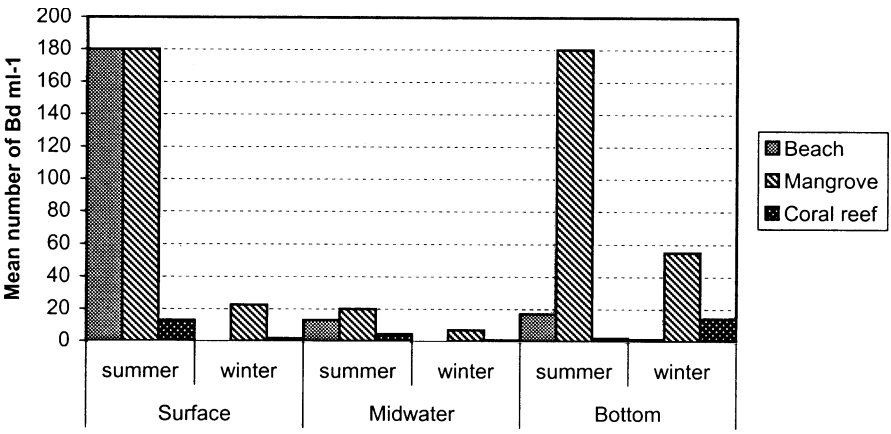


Fig. 7 Season distribution of BALOs at a beach, mangrove and coral reef sites in the tropical coastal waters of Australia. (Adapted Sutton DC and Besant PJ 1994, by permission)

recovered from temperate climates typically did not grow at 35 °C. These data suggest that the tropical isolates may have developed an adaptation for warmer temperatures in contrast to the isolates from temperate climates. These differences may be manifested as well in the genome of the isolates from the two regions.

3.7 Selective Determinants of BALO Distribution

Although BALOs are widely dispersed in the environment, not all ecosystems, habitats or environmental niches equally support the predators as there are

wide variations in the numbers and strains recovered from various sites. One of the most striking examples of this is the distinct population of BALOs we first recovered in the Chesapeake Bay and thus far has only been found in estuarine ecosystems (Williams et al. 2005). This observation suggests that factors in estuarine ecosystems, that may be absent in ocean and sea environments, select for, or favor, the unique predator strain. Or perhaps the estuarine genotype can not survive the ocean environment or compete with the genotypes found in the oceans, seas and salt lake. The factors that may influence the number and distribution of BALOs are likely multiple and complex. At best, we can only consider those that are most obvious until more environmental data is collected and correlated with the number of BALOs. Among the most likely determinants of BALO occurrence and distribution are food source and temperature and salinity ranges. These are considered in the following paragraphs.

As with all biota, one of the most apparent and important factors influencing the distribution of BALOs is availability of a food source. Typically, the predators can be expected to be most abundant where its food supply is greatest. Varon and Zeigler (1978) observed that the probability of a *Bdellovibrio* meeting the prey becomes smaller as prey density decreases. It then follows that greater numbers of BALOs will be found in areas with high prey populations. As far as is known, the sole food source of wild-type BALOs is prey bacteria. Since most BALO strains studied have a wide prey range and are capable of preying upon many different species of Gram-negative bacteria their food sources are likely available in most econiches. However, the abundance of the food supply will vary in different ecosystems. For example, the numbers of bacteria in smaller estuaries, rivers and lakes with more coastal land mass is typically several orders of magnitude greater than in open ocean waters. In some cases, up to a 1000-fold higher number of BALOs were reported from Chesapeake Bay waters than in ocean waters (Figs. 4 and 5). Presumably, the Chesapeake Bay and other more eutrophic environments support more abundant bacterial growth which results in higher numbers of bacteria and prey for the BALOs.

Sutton and Besant (1994) reported correlations of numbers of BALOs with numbers of total colony-forming units (CFU) from three distinct sites and correlation with total numbers of bacteria (as determined by direct microscopic counts) at two of the sites. In the Chesapeake Bay, Williams et al. (1980) found no correlation between numbers of BALOs and total CFU. The contrasting results in the two studies may reflect differences in the bacterial populations in the Chesapeake Bay and the tropical waters around Australia, especially the proportion that are susceptible to the BALOs. Correlations may not be found in every case study for various reasons including the presence of high populations of non-prey bacteria that do not support growth of BALOs. Further study is needed to better define correlations between numbers of total bacteria and BALOs.

As discussed above, biofilm represents another high density bacterial community to which large numbers of BALOs have been associated. Another source that yields high numbers of BALOs is raw sewage which also has high bacteria abundance (Table 5) (Fry and Staples 1976). Klein and Casida (1967) reported that soils sprayed by effluent water from sewage treatment plants had greater numbers of BALOs than regular soils.

Although the association of high abundances of bacteria with high numbers of BALOs can be observed in many different types of habitats, there are exceptions such as the intestinal tract of some animals. Westergaard and Kramer (1977) failed to recover BALOs from the intestinal tracts of frogs. Kelley and Williams (1992) recovered low numbers of BALOs from the intestinal track of *C. sapidus* (blue crabs), but high numbers in the gills of the animals. Apparently, there were present in the animal intestine tracts some inhibitory factors not favorable for BALO growth that negated the high numbers of bacteria. Such factors may include temperature, inhibitory chemicals or toxins, etc.

Although the influence that large communities of bacteria may have in selecting for high numbers of BALOs is apparent, another important factor is the composition of the bacterial communities. Since not all bacteria are susceptible to all strains of BALOs, and the predators may show preference in its predation even among susceptible bacteria, the composition of organisms in bacterial communities influences both the qualitative and quantitative properties of the BALO population.

Preferential predation by BALO strains has been documented in both laboratory and field studies. Rogosky et al. (2006) reported preferential predation when BALO strains were presented with several different prey bacteria simultaneously in a laboratory mesocosm. The basis for this selection is not known, however, such preferential behavior may select for certain strains of the predators. The dynamics of such systems is treated in Wilkinson, 2006, in this volume. Preferential predation has also been observed in environmental

Table 5 Relative abundance of *Bdellovibrio* and like organisms (BALOs) in Miskin sewage works, estimated using NB-500 medium with *Achromobacter* sp. (Fry and Staples 1973, by permission)

Site sewage works	No. of samples	Mean no. of *Bdellovibrio* per ml	per g (dry wt)
Raw inflow	7	222	
Filter inflow	7	135	
Filter effluent	7	226	
Filter film	9		2.7×10^4
Final settlement sludge	12		ND [a]

[a] ND, Not detectable

isolates. Pineiro et al. (2004) reported that the BALOs in the Great Salt Lake preferentially preyed upon bacteria isolated from the lake rather than bacterial isolates from ocean waters. Evidence of preferential predation was also reported in soil and adjacent rhizosphere, which have different bacterial community structures. Using both molecular and culture methods, Jurkevitch et al. (2000) found that the BALO populations also differed in the two habitats. Restriction analysis revealed that the soil BALOs belonged to two ribotypes representative of the *B. bacteriovorus* group and *B. stolpii* UKi2, respectively, whereas the predators recovered from the rhizosphere were identical to the ribotype of *B. bacteriovorus* W.

The BALOs from the soil, rhizosphere and total root extract also showed marked differences in their prey susceptibility patterns against the same battery of bacteria with a single exception. In this case the total root extract isolate (BEP2) and the rhizosphere isolate (BRP4) yielded identical prey susceptibility patterns. These two isolates were also identical by 16S rRNA gene sequence and restriction patterns. One BALO isolate, TRA2, from total root extract, was the only isolate among five tested that preyed upon several *Rhizobium* and *Sinorhizobium* species and the bacterium on which it was isolated, *Agrobacterium tumefaciens* C58. This isolate was recovered only from root extract and not from soil. Differences in the bacterial populations in soil, rhizosphere and root extract may be responsible for selecting the distinct BALO populations observed in the respective sites. Recent studies (Herschkovitz et al. 2005) showed that the dominant rhizobacterial populations change with plant growth and that *Bdellovibrio* spp. rhizosphere populations change as well. The results of these studies clearly point to a role for the structure of bacterial communities in selecting for populations of BALOs in defined niches in an ecosystem and may be a factor in selecting for separate sets of genes and separate phenotypes for the predators. This appears to be the case in soil and rhizosphere (Jurkevitch et al. 2000) as well as in marine habitats (Sutton and Besant 1994).

As described above salinity is a major determinant governing the distribution of freshwater and saltwater BALOs. A recent discovery in our laboratory noted in a previous section of this review revealed subpopulations among the saltwater BALOs based on apparent adaptation to low and moderate salinities or extreme salinities. The distribution of the subpopulations also appears to be governed by salinity as suggested by studies in the Chesapeake Bay. Although BALOs have been recovered from all regions of the Chesapeake Bay (Fig. 5), the distinct estuarine BALO genotype appears to be restricted to the mid and northern upper regions of the estuary where the salinity ranges from < 5 ppt to 15 ppt. They have not been recovered from the southern lower bay and mouth region where the salinity is highest (20 to 30 ppt) and the ocean water influence is greatest. In this region were found the BALO genotypes typically found in oceans and seas. We have observed that many of the BALOs isolated from Chesapeake Bay waters were able to tolerate and grow at lower

salinities than ocean isolates. Previously, Sanchez-Amat and Torrella (1989) observed that BALO strains recovered from high salinity saltern ponds could not tolerate growth at lower salinities as did isolates recovered from nearby ocean waters. These observations confirm the important role of salinity in the distribution of subpopulations of saltwater BALO.

Although salinity may be the important factor in selecting for the distinct Chesapeake Bay genotype, the influence of the composition of the bacterial community in the bay (Bouvier and del Giorgio 2003) and other estuaries which differs in species and abundance from that of ocean environments can not be discounted.

The distribution of BALOs is also likely subject to the availability of oxygen. Schoeffield et al. (1996) reported that BALOs would not grow when inoculated with prey cells under anaerobic conditions. Fry and Staples (1976) reported recovering BALOs only in the top 5 cm of river sediment. The investigators attributed the restriction of BALOs to the upper regions of sediment to their requirement for oxygen rather than to insufficient prey since coliforms and other Gram negative heterotrophic bacteria were recovered at depths down to 12 cm with little decrease in numbers below 2 cm. Williams (1988) reported similar distribution in sediment samples obtained from the Patuxent River in Maryland. There, BALOs were recovered only in the top 7.5 cm of sediment.

4 Interactions of BALOs with Other Bacteria

As predatory bacteria, BALOs interact with other bacteria in a manner that is uniquely different from that of any other known prokaryote. The physical interaction of a BALO with its prey is initiated by an attack that almost immediately paralyzes the metabolic machinery of the prey and rapidly leads to its death. Although BALOs were first described as ectoparasites, and some reports have described strains of predators lyzing prey without penetrating the cell wall (Yair et al. 2003), most BALOs appear to be of the intraperiplasmic nature (Starr and Baigent 1966; Nunez et al. 2003; Rendulic et al. 2004). Predation is a vital part of the life cycle and multiplication of BALOs, as it is the only known mechanism for the predators to derive food for energy and anabolism. Gram negative bacteria are the only known food source for these predators. Some BALO strains interact with a broad spectrum of Gram-negative bacteria while other strains appear to prey only on a few select organisms. However, not all Gram negative organisms are susceptible to BALO predation as described in previous sections above. Thus far, attempts to grow BALOs in the laboratory on non-cellular, artificial media or cell types other than Gram negative organisms, including eukaryotic cells, have not met with success (Lenz and Hespell 1978).

It has been established that Gram positive bacteria are not subject to predation by BALOs (Afinogenova et al. 1981). What makes a bacterium susceptible to BALOs or what enables the predators to attack some bacteria but not others remains a mystery.

Apparently, bacteria that are susceptible to the predators have some specific, requisite cellular features that allow the attackers access through the cell wall and into the periplasmic space, although no such features have been described. To distinguish prey from non-prey cells, it would appear that the BALOs possess some specific prey recognition mechanism, but none has been elucidated.

4.1 BALO Interactions in Mixed Bacterial Populations

One of the interesting aspects of BALO interactions with other bacteria is the response of the predators when there are available many prey strains and species as occurs in nature. Some reports have concluded that the collision and interaction between BALOs and other bacteria occur randomly, suggesting that the predators do not show any preferences when in the presence of mixed populations of prey cells (Straley and Conti 1977). These authors described a chemotaxis assay system to measure directed movement of BALOs toward several bacterial species. The data suggested that the *Bdellovibrio* did not use chemotaxis to locate prey cells. Varon (1981) suggested that mixed bacterial populations affected the predator-prey interaction in different ways: some bacteria competed with the original prey for the predator, others enhanced the activity of the predator and others inhibited it. A recent report by Lambert et al. (2003) suggested that chemotaxis may play at least a minor role in attracting the predators to their prey. Rogosky et al. (2006) reported that when cells of BALOs were inoculated into a suspension of washed cells of *E. coli* and *Pantoea agglomerans* (previously *Erwinia herbicola*) in equal numbers, the predator preferentially preyed upon the *Pantoea*. When the mixed suspension of cells included *E. coli* and *Serratia marcescens* and *E. coli* and *Enterobacter* respectively, the BALOs preferentially lyzed the *Serratia* and *Enterobacter*. The attachment of the predators to mixed bacterial species was also studied: it was observed that attachment to the different prey varied widely with the predator attaching most rapidly to *Pantoea agglomerans* with 91% of the predator cells attached after 4 min. *Salmonella enterica* exhibited the lowest attachment efficiency with only 12% of the predators attached after 20 min. The data revealed that the attachment efficiency was consistent with the predation efficiency with more rapid attachment occurring with the more preferred prey. The results of these studies clearly suggest that the interaction of BALOs with prey is not initiated by a random collision event, but rather by a non-random mechanism, and that the predators have some means of prey-recognition. This represents one of the most interest-

ing areas of study of the interactions between BALO and prey and should be vigorously pursued.

5 The Role of BALOs in Nature

Since the discovery of the unique bacterial predators more than four decades ago, the role of BALOs in nature has been a matter of interest and controversy. Following the first report of these predatory bacteria, there was speculation that the organisms could play a role in the control of susceptible bacterial populations in nature and could perhaps be exploited as an agent of biological control of undesirable bacteria. Although viruses have since captured much of the attention regarding factors responsible for bacterial mortality, they are not responsible for all microbial lysis. BALOs are also likely important contributors. The results from many studies conducted over the last four decades have revealed that in the environment BALOs are active, dynamic members of the microbial community (Sutton and Besant 1994; Rice et al. 1998; Pineiro et al. 2004).

BALOs' ubiquitous distribution can only be explained by their attack and multiplication which kills their prey bacteria providing potential for the predators to exert some control on the bacterial population in nature. BALOs predatory behavior, requirement for prey bacteria and the susceptibility of many environmental microbes to the predators support a role for these unique bacteria in bacterial mortality. Results from studies conducted in the Chesapeake Bay revealed that nearly 70% of bacteria isolated were susceptible to the autochthonous BALO population (Rice et al. 1998). *Vibrio* species are a preferred prey for the halophilic BALOs and therefore may be the genus most controlled by their predation in nature (Schoeffield and Williams 1990; Rice et al. 1998; Sutton and Besant 1994).

The significance of BALOs' contribution to bacterial mortality will depend upon many factors, a most critical of which is their abundance. This is analogous to the uncovering of the role of viruses in aquatic systems, which was realized only following the discovery of their great abundances in the oceans (Fuhrman 1999). Another important indicator of the role of BALOs in bacterial mortality is the proportion and type of susceptible bacteria in any habitat.

In exploring BALOs' role in bacterial mortality, food web dynamics and the shaping of community structure within particular environments, the diversity in the predator population, as manifested by different strains or species must be taken into account. This is illustrated by the example of a specific genotype that has been associated with a specific habitat, the Chesapeake Bay and some other estuaries (Williams et al. 2005). This is the first observation of a phylogenetically distinctive BALO strain associated with a specific aquatic ecosystem.

The role of BALOs in nature has eluded investigators due in part to a lack of properly designed studies, which is also related to a lack of investigative tools to accurately detect, monitor, quantitate and characterize these predatory bacteria in environmental samples. Methodologies to accomplish such tasks would advance greatly the study of BALOs in the environment and aid the pursuit of many important unanswered questions such as: how much of bacterial mortality is attributed to BALOs? Which functional groups do they most affect? What is their impact on environmental processes associated with bacteria including the cycling of nutrients and the energetics of ecosystems and ecosystem function? There is now sufficient data upon which to base some reasonable assumptions helpful in generating hypotheses on the activities of BALOs in the environment.

6
BALO as Bacterial Control Agents in Biological and Environmental Systems

Since attack by BALOs is a lethal event for its prey, the predators have attracted interest as biological control agents (Nakamura 1972; Fratamico and Cooke 1996). In animals, the most dramatic example to date of the effectiveness of BALOs in reducing infections was reported by Nakamura in 1972. Inoculations of *B. bacteriovorus* suspensions in the eyes of rabbits experimentally infected with *Shigella flexneri* eliminated or substantially reduced infection. The infection rate was reduced to 0 of 4 eyes when followed at 12 h with an inoculum of *B. bacteriovorus* and 2 of 10 when *Shigella* and the predator were inoculated simultaneously. The *B. bacteriovorus* also reduced the pathogenic symptomatology of *Shigella*-related fluid accumulation in the intestinal tract in experiments using ligated rabbit ileal loops (Nakamura 1972).

Recently, Edao (2000) reported the recovery of BALOs in fecal samples from the gastrointestinal tracts of animals and humans. A correlation was found between the presence of the predators and the state of health of several domestic animals. The detection rate of BALOs was significantly lower in animal populations with enteritic and pneumonic diseases. This suggested that the presence or absence of the predators might influence the manifestation of eubiotic conditions in the intestinal tract because of their ability to control pathogenic enterobacteria, e.g. *Pseudomonas*, *Pasteurella* and *Campylobacter* (Schwudke et al. 2001). However, this is apparently not the case in all animal systems. BALOs did not become an integral component of the intestinal microflora of fish and frogs after being force-fed into the animals via an intragastric tube (Westergaard and Kramer 1977). The authors concluded that it was not feasible to lyse pathogenic, Gram negative bacteria in the alimentary tract with BALO.

BALOs have also been used to reduce numbers of bacteria in the environment. Practical examples include food preparation machinery and in agricul-

ture (Fratamico and Whiting 1995; Fratamico and Cooke 1996). Lambina et al. (1981) reported that the predators dramatically decreased the number of viable Gram-negative bacteria in polluted wastewater of a communal sewage plant. The implications and significance of BALO's potential in addressing environmental and water quality concerns in confined bodies of water such as polluted ponds, fish farms, and aquaria is worthy of consideration.

The few animal, plant and environmental studies on the use of BALOs to control bacterial populations are encouraging, but many more properly designed studies and data are needed including the influence of environmental factors such as temperature, salt concentrations and oxygen, among others.

References

Afinogenova AV, Penabad SR, Konovalova SM, Churkina LG, Lambina VA (1981) Comparative characteristics of strains of *Bdellovibrio* isolated from river water and sewage. Russian Original 50:135–274

Baer ML, Ravel J, Chun J, Hill RT, Williams HN (2000) A proposal for the reclassification of *Bdellovibrio stolpii* and *Bdellovibrio starrii* into a new genus, *Bacteriovorax* gen. nov. as *Bacteriovorax stolpii* comb. nov. and *Bacteriovorax starrii* comb. nov., respectively. Int J Syst Evol Microbiol 50:219–224

Baer ML, Ravel J, Piñeiro SA, Guether-Borg D, Williams HN (2004) Reclassification of saltwater *Bdellovibrio* sp. as *Bacteriovorax marinus* sp. nov. and *Bacteriovorax litoralis* sp. nov. Int J Syst Evol Microbiol 54:1011–1016

Bouvier T, del Giorgio PA (2003) Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): A quantitative review of published reports. FEMS Microbiol Ecol 44:3–15

Colwell RR, Kaper J, Joseph SW (1977) *Vibrio cholerae*, *Vibrio parahaemolyticus*, and other vibrios: occurrence and distribution in Chesapeake Bay. Science 198:394–396

Daley RJ (1979) Direct epifluorescence enumeration of native aquatic bacteria: uses, limitations and comparative accuracy. American Society for Testing and Materials, Special Technical Publication 29–45

Davidov Y, Jurkevitch E (2004) Diversity and evolution of *Bdellovibrio*-and-like organisms (BALOs), reclassification of *Bacteriovorax starrii* as *Peredibacter starrii* gen. nov., comb. nov., and description of the *Bacteriovorax Peredibacter* clade as *Bacteriovoracaceae* fam. nov. Int J Syst Evol Microbiol 54:1439:1452

Davidov Y, Friedjung A, Jurkevitch E (2006). High diversity of *Bdellovibrio*-and-like organisms (BALOs) in soil revealed by culture-dependent and culture-independent methods. Environ Microbiol 8:1667–1673

Dias FF, Bhat JV (1965) Microbial ecology of activated sludge II. Bacteriophages, bdellovibrio, coliforms, and other organisms. Appl Microbiol 13:257–261

Diedrich D (1988) Bdellovibrios: recycling, remodelling and relocalizing components from their prey. Microbiol Sci 5:100–103

Donze D, Mayo JA, Diedrich DL (1991) Relationships among the bdellovibrios revealed by partial sequences of 16S ribosomal RNA. Curr Microbiol 23:115–119

Edao A (2000) Research on the occurrence and importance of *Bdellovibrio bacteriovorus* in the gastrointestinal tract of humans, animals as well as in the natural environment. Dissertation, University of Leipzig, Germany

Fouke BW, Bonheyo GT, Sanzenbacher B, Frias-Lopez J (2003) Partitioning of bacterial communities between travertine depositional facies at Mammoth Hot Springs, Yellowstone National Park, USA. Can J Earth Sci 40:1531–1548

Fratamico PM, Cooke PH (1996) Isolation of *Bdellovibrio* that prey on *Escherichia coli* 0157:H7 and application for removal of prey from stainless steel surfaces. J Food Safety 16:161–173

Fratamico PM, Whiting RS (1995) Ability of *Bdellovibrio bacteriovorus* 109J to lyse Gram-negative food-bornepathogenic and spoilage bacteria. J Food Protect 58:160–164

Fry CJ, Staples DG (1974) The occurrence and role of *Bdellovibrio bacteriovorus* in a polluted river. Water Res 8:1029–1035

Fry JC, Staples DG (1976) Distribution of *Bdellovibrio bacteriovorus* in sewage works, river water, and sediments. Appl Environ Microbiol 31:469–474

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Germida JJ (1987) Isolation of *Bdellovibrio* spp. that prey on *Azospirillum brasilense* in soil. Can J Microbiol 33:459–461

Herschkovitz Y, Lerner A, Davidov Y, Okon Y, Jurkevitch E (2005) *Azospirillum brasilense* does not affect population structure of specific rhizobacterial communities of inoculated maize (*Zea mays*). Environ Microbiol 7:1847–1852

Jurkevitch E, Minz D, Ramati B, Barel G (2000) Prey range characterization, ribotyping, and diversity of soil and rhizosphere *Bdellovibrio* spp. isolated on phytopathogenic bacteria. Appl Environ Microbiol 66:2365–2371

Jurkevitch E, Ramati B (2000) Design and uses of *Bdellovibrio 16S* rRNA-targeted oligonucleotides. FEMS Microbiol Lett 184:265–271

Kadouri D, O'Toole GA (2005) Susceptibility of biofilms to *Bdellovibrio bacteriovorus* attack. Appl Environ Microbiol 71:4044–4051

Kelley J, Turng B, Williams H, Baer M (1997) Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic *bdellovibrios*. Appl Environ Microbiol 63:84–90

Kelley JI, Williams HN (1992) Bdellovibrios in *Callinectus sapidus*, the blue crab. Appl Environ Microbiol 58:1408–1410

Keya SO, Alexander M (1975) Factors affecting growth of *Bdellovibrio* on *Rhizobium*. Arch Microbiol 103:37–43

Klein DA, Casida LEJ (1967) Occurrence and enumeration of *Bdellovibrio bacteriovorus* in soil capable of parasitizing *Escherichia coli* and indigenous soil bacteria. Can J Microbiol 13:1235–1241

Lambert C, Evans KJ, Till R, Hobley L, Capeness M, Rendulic S, Schuster SC, Aizawa S-I, Sockett RE (2006) Characterizing the flagella filament and the role of motility in bacterial prey-penetration by *Bdellovibrio bacteriovorus*. Mol Microbiol 60:274–286

Lambert C, Smith MCM, Sockett RE (2003) A novel assay to monitor predator-prey interactions for *Bdellovibrio bacteriovorus* 109 J reveals a role for methyl-accepting chemotaxis proteins in predation. Environ Microbiol 5:127–132

Lambina VA, Ledova LA, Situkhina NS (1981) Participation of *Bdellovibrio* in sewage self-purification processes. Translated from Mikrobiologiya 50:140–146

Lenz RW, Hespell RB (1978) Attempts to grow bdellovibrios microsurgically-injected into animal cells. Arch Microbiol 119:245–248

Mahamoud KK, McNeely D, Koval SF (2005) Design and performance of a 16S rRNA oligonucleotide probe for the genus *Bdellovibrio* using fluorescent in situ hybridization (FISH). Abstr of 2005 Ann Conf Can Soc Microbiologists

Marbach A, Shilo M (1978) Dependence of marine bdellovibrios on potassium, calcium, and magnesium ions. Appl Environ Microbiol 36:169–177

Marbach A, Varon M, Shilo M (1976) Properties of marine *Bdellovibrios*. Microb Ecol 2:284–295

Mitchell R, Yankofsky S (1967) Lysis of *Escherichia coli* by marine micro-organisms. Nature 215:891–893

Nakamura MPD (1972) Alteration of *Shigella* pathogenicity by other bacteria. Am J Cli Nutr 25:1441–1451

Nunez ME, Martin MO, Duong LK, Ly E, Spain EM (2003) Investigations into the life cycle of the bacterial predator *Bdellovibrio bacteriovorus* 109J at an interface by atomic force microscopy. Biophys J 84:3379–3388

Parker CA, Grove PL (1970) *Bdellovibrio bacteriovorus* parasitizing rhizobium in Western Australia. J Appl Bacteriol 33:253–255

Pineiro SA, Sahaniuk GE, Romberg E, Williams HN (2004) Predation pattern and phylogenetic analysis of Bdellovibrionaceae from the Great Salt Lake, Utah. Curr Microbiol 48:113–117

Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans KJ, Goesmann A, Meyer F, Sockett RE, Schuster SC (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692

Rice TD, Williams HN, Turng BF (1998) Susceptibility of bacteria in estuarine environments to autochthonous bdellovibrios. Microb Ecol 35:256–261

Richardson IR (1990) The incidence of *Bdellovibrio* spp. in man-made water systems: coexistence with legionellas. J Appl Bacteriol 69:134–140

Rittenberg SC, Langley D (1975) Utilization of nucleoside monophosphates per se for intraperiplasmic growth of *Bdellovibrio bacteriovorus*. J Bacteriol 121:1137–1144

Sanchez-Amat A, Torrella F (1990) Formation of stable bdelloplasts as a starvation-survival strategy of marine bdellovibrios. Appl Environ Microbiol 56:2717–2725

Sánchez-Amat A, Torrella F (1989) Isolation and characterization of marine and salt pond halophylic bdellovibrios. Can J Microbiol 35:71–778

Scherff RH, DeVay JE, Carroll TW (1966) Ultrastructure of most parasite relationships involving reproductinon of *Bdellovibrio bacteriovorus* in host bacteria. Phytopathol 56:627–633

Schoeffield AJ, Williams HN (1990) Efficiencies of recovery of bdellovibrios from brackish-water environments by using various bacterial species as prey. Appl Environ Microbiol 56:230–236

Schoeffield AJ, Williams HN, Turng B, Falkler WAJ (1996) A comparison of the survival of intraperiplasmic and attack phase bdellovibrios to reduced oxygen. Microb Ecol 32:35–46

Schwudke D, Strauch E, Krueger M, Appel B (2001) Taxonomic studies of predatory bdellovibrios based on 16S rRNA analysis, ribotyping and the *hit* locus and characterization of isolates from the gut of animals. System Appl Microbiol 24:385–394

Seidler RJ, Mandel M, Baptist JN (1972) Molecular heterogeneity of the bdellovibrios: evidence of two new species. J Bacteriol 109:209–217

Seidler RJ, Starr MP (1969) Isolation and characterization of host-independent bdellovibrios. J Bacteriol 100:769–785

Shilo M (1966) Predatory bacteria. Science J 2:33–37

Shilo M (1969) Morphological and physiological aspects of the interaction of *Bdellovibrio* with host bacteria. Curr Topics Microbiol Immunol 50:174–204

Shilo M, Bruff B (1965) Lysis of gram-negative bacteria by host-independent ectoparasitic *Bdellovibrio bacteriovorus* isolates. J Gen Microbiol 40:312–328
Singh RO, Stine C, Smith DL, Spitznagel JK, Labib M, Williams H (2003) Microb diversity of biofilms in dental unit water systems. Appl Environ Microbiol 69:3412–3420
Snyder AR, Williams HN, Baer ML, Walker KE, Stine OC (2002) 16S rDNA sequence analysis of environmental *Bdellovibrio*-and-like organisms (BALO) reveals extensive diversity. Int J Syst Evol Microbiol 2089–2094
Staples DG, Fry JC (1973) Factors which influence the enumeration of *bdellovibrio bacteriovorus* and sewage and river water. J Appl Bacteriol 1–11
Starr MP, Baigent NL (1966) Parasitic interaction of *Bdellovibrio bacteriovorus* with other bacteria. J Bacteriol 91:2006–2017
Stolp H, Petzold H (1962) Untersuchungen über einen obligat parasitischen Mikroorganismus mit lytischer Aktivitat für *Pseudomonas*-Bakterien. Phytopath Z 45:364–390
Stolp H, Starr MP (1963) *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek 29:217–248
Sutton DC, Besant PJ (1994) Ecology and characteristics of bdellovibrios from three tropical marine habitats. Marine Biol 119:313–320
Taylor VI, Baumann P, Reichelt JL, Allen RD (1974) Isolation, enumeration, and host range of marine bdellovibrios. Arch Microbiol 98:101–114
Torrella F, Guerrero R, Seidler R (1978) Further taxonomic characterization of the genus *Bdellovibrio*. Can J Microbiol 24:1387–1394
Uematsu T, Shiomi T, Wakimoto S (1971) Biological and ecological studies on Bdellovibrio 2. Some biological properties of Japanese isolates of *B. bacteriovorus*. Ann Phyto Soc Jap 52–57
Varon M, Drucker I, Shilo M (1969) Early effects of bdellovibrio infection on the syntheses of protein and RNA of host bacteria. Biochem Biophy Res Comm 37:518–525
Varon M, Shilo M (1968) Interaction of *Bdellovibrio bacteriovorus* and host bacteria I. Kinetic studies of attachment and invasion of *Escherichia coli* B by *Bdellovibrio bacteriovorus*. J Bacteriol 95:744–753
Varon M, Zeigler BP (1978) Bacterial predator-prey interaction at low prey density. Appl Environ Microbiol 36:11–17
Westergaard JM, Kramer TT (1977) *Bdellovibrio* and the intestinal flora of vertebrates. Appl Environ Microbiol 34:506–511
Williams HN, Stine O, Sahaniuk GE, Pineiro SA (2005) Diverse genotypes of halophilic Bdellovibrionaceae in oceans, seas, estuaries and a salt lake. Abstr of 2005 Meeting of Amer Soc Limnol Ocean The Oceanogr Soc
Williams HN (1979) Cultural, immunologic and ecologic studies of marine bdellovibrios isolated from the atlantic ocean and the chesapeake bay. Dissertation, Univeristy of Maryland Health Science Library, Baltimore
Williams HN (1987) The recovery of high numbers of bdellovibrios from the surface water microlayer. Can J Microbiol 33:572–575
Williams HN (1988) A study of the occurrence and distribution of bdellovibrios in estuarine sediment over an annual cycle. Microb Ecol 15:9–20
Williams HN, Fakler WAJ, Shay DE (1980) Incidence of marine *Bdellovibrios* lytic against *Vibrio parahaemolyticus* in Chesapeake Bay. Appl Environ Microbiol 40:970–972
Williams HN, Falkler WAJ (1984) Distribution of bdellovibrios in the water column of an estuary. Can J Microbiol 30:971–974
Williams HN, Falkler WJ, Shay DE (1982) Seasonal distribution of bdellovibrios at the mouth of the Patuxent River in the Chesapeake Bay. Can J Microbiol 28:111–116

Williams HN, Scheoffield AJ, Guether D, Kelley J, Shah D, Falker WAJ (1995) Recovery of bdellovibrios from submerged surfaces and other aquatic habitats. Micob Ecol 29:39–48

Williams HN, Stine OC (2004) Distribution of diverse genotypes of halophilic Bdellovibrionaceae in oceans, seas, estuaries and a salt lake. Abst of 2004 meeting of Amer Soc Limnol Ocean and The Oceanogr Soc

Williams HN, Toon S, Faulk E, Falkler WAJ (1987) The incidence of bdellovibrios in an artificial environment: The National Aquarium in Baltimore. Can J Microbiol 33:483–488

Yair S, Yaacov D, Susan K, Jurkevitch E (2003) Small eats big: ecology and diversity of *Bdellovibrio* and like organisms, and their dynamics in predator-prey interactions. Agronomic 23:433–439

Subject Index