Eugene Rosenberg
Editor-in-Chief

Edward F. DeLong
Stephen Lory
Erko Stackebrandt
Fabiano Thompson
Editors

The Prokaryotes

Prokaryotic Communities and Ecophysiology

Fourth Edition

SpringerReference

The Prokaryotes

Eugene Rosenberg (Editor-in-Chief)
Edward F. DeLong, Stephen Lory, Erko Stackebrandt and Fabiano Thompson (Eds.)

The Prokaryotes

Prokaryotic Communities and Ecophysiology

Fourth Edition

With 179 Figures and 43 Tables

Springer Reference

Editor-in-Chief
Eugene Rosenberg
Department of Molecular Microbiology and Biotechnology
Tel Aviv University
Tel Aviv, Israel

Editors
Edward F. DeLong
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA, USA

Stephen Lory
Department of Microbiology and Immunology
Harvard Medical School
Boston, MA, USA

Erko Stackebrandt
Leibniz Institute DSMZ-German Collection of Microorganisms
and Cell Cultures
Braunschweig, Germany

Fabiano Thompson
Laboratory of Microbiology, Institute of Biology, Center for
Health Sciences
Federal University of Rio de Janeiro (UFRJ)
Ilha do Fundão, Rio de Janeiro, Brazil

ISBN 978-3-642-30122-3 ISBN 978-3-642-30123-0 (eBook)
ISBN 978-3-642-30124-7 (print and electronic bundle)
DOI 10.1007/978-3-642-30123-0
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012950985

Foreword

The purpose of this brief foreword is unchanged from the first edition; it is simply to make you, the reader, hungry for the scientific feast that follows. These 11 volumes (planned) on the prokaryotes offer an expanded scientific menu that displays the biochemical depth and remarkable physiological and morphological diversity of prokaryote life. The size of the volumes might initially discourage the unprepared mind from being attracted to the study of prokaryote life, for this landmark assemblage thoroughly documents the wealth of present knowledge. But in confronting the reader with the state of the art, the Handbook also defines where more work needs to be done on well-studied bacteria as well as on unusual or poorly studied organisms.

This edition of *The Prokaryotes* recognizes the almost unbelievable impact that the work of Carl Woese has had in defining a phylogenetic basis for the microbial world. The concept that the ribosome is a highly conserved structure in all cells and that its nucleic acid components may serve as a convenient reference point for relating all living things is now generally accepted. At last, the phylogeny of prokaryotes has a scientific basis, and this is the first serious attempt to present a comprehensive treatise on prokaryotes along recently defined phylogenetic lines. Although evidence is incomplete for many microbial groups, these volumes make a statement that clearly illuminates the path to follow.

There are basically two ways of doing research with microbes. A classical approach is first to define the phenomenon to be studied and then to select the organism accordingly. Another way is to choose a specific organism and go where it leads. The pursuit of an unusual microbe brings out the latent hunter in all of us. The intellectual challenges of the chase frequently test our ingenuity to the limit. Sometimes the quarry repeatedly escapes, but the final capture is indeed a wonderful experience. For many of us, these simple rewards are sufficiently gratifying so that we have chosen to spend our scientific lives studying these unusual creatures. In these endeavors, many of the strategies and tools as well as much of the philosophy may be traced to the Delft School, passed on to us by our teachers, Martinus Beijerinck, A. J. Kluyver, and C. B. van Niel, and in turn passed on by us to our students.

In this school, the principles of the selective, enrichment culture technique have been developed and diversified; they have been a major force in designing and applying new principles for the capture and isolation of microbes from nature. For me, the "organism approach" has provided rewarding adventures. The organism continually challenges and literally drags the investigator into new areas where unfamiliar tools may be needed. I believe that organism-oriented research is an important alternative to problem-oriented research, for new concepts of the future very likely lie in a study of the breadth of microbial life. The physiology, biochemistry, and ecology of the microbe remain the most powerful attractions. Studies based on classical methods as well as modern genetic techniques will result in new insights and concepts.

To some readers, this edition of *The Prokaryotes* may indicate that the field is now mature, that from here on it is a matter of filling in details. I suspect that this is not the case. Perhaps we have assumed prematurely that we fully understand microbial life. Van Niel pointed out to his students that—after a lifetime of study—it was a very humbling experience to view in the microscope a sample of microbes from nature and recognize only a few. Recent evidence suggests that microbes have been evolving for nearly 4 billion years. Most certainly, those microbes now domesticated and kept in captivity in culture collections represent only a minor portion of the species that have evolved in this time span. Sometimes we must remind ourselves that evolution is actively taking place at the present moment. That the eukaryote cell evolved as a chimera of certain prokaryote parts is a generally accepted concept today. Higher as well as lower eukaryotes evolved in contact with prokaryotes, and evidence surrounds us of the complex interactions between eukaryotes and prokaryotes as well as among prokaryotes. We have so far only scratched the surface of these biochemical interrelationships. Perhaps the legume nodule is a pertinent example of nature caught in the act of evolving the "nitrosome," a unique nitrogen-fixing organelle. The study of prokaryotes is proceeding at such a fast pace that major advances are occurring yearly. The increase of this edition to four volumes documents the exciting pace of discoveries.

To prepare a treatise such as *The Prokaryotes* requires dedicated editors and authors; the task has been enormous. I predict that the scientific community of microbiologists will again show its appreciation through use of these volumes—such that the pages will become "dog-eared" and worn as students seek basic information for the hunt. These volumes belong in the laboratory, not in the library. I believe that a most effective way to introduce students to microbiology is for them to isolate microbes from nature, that is, from their habitats in soil, water, clinical specimens, or plants. *The Prokaryotes* enormously simplifies this process and should encourage the construction of courses that contain a wide spectrum of diverse topics. For the student as well as the advanced investigator, these volumes should generate excitement.

Happy hunting!

Ralph S. Wolfe
Department of Microbiology
University of Illinois at Urbana-Champaign

Preface

During most of the twentieth century, microbiologists studied pure cultures under defined laboratory conditions in order to uncover the causative agents of disease and subsequently as ideal model systems to discover the fundamental principles of genetics and biochemistry. Microbiology as a discipline onto itself, e.g., microbial ecology, diversity, and evolution-based taxonomy, has only recently been the subject of general interest, partly because of the realization that microorganisms play a key role in the environment. The development and application of powerful culture-independent molecular techniques and bioinformatics tools has made this development possible. The fourth edition of *the Handbook of the Prokaryotes* has been updated and expanded in order to reflect this new era of microbiology.

The first five volumes of the fourth edition contain 34 updated and 43 entirely new chapters. Most of the new chapters are in the two new sections: Prokaryotic Communities and Bacteria in Human Health and Disease. A collection of microorganisms occupying the same physical habitat is called a "community," and several examples of bacterial communities are presented in the Prokaryotic Communities section, organized by Edward F. DeLong. Over the last decade, important advances in molecular biology and bioinformatics have led to the development of innovative culture-independent approaches for describing microbial communities. These new strategies, based on the analysis of DNA directly extracted from environmental samples, circumvent the steps of isolation and culturing of microorganisms, which are known for their selectivity leading to a nonrepresentative view of prokaryotic diversity. Describing bacterial communities is the first step in understanding the complex, interacting microbial systems in the natural world.

The section on Bacteria in Human Health and Disease, organized by Stephen Lory, contains chapters on most of the important bacterial diseases, each written by an expert in the field. In addition, there are separate general chapters on identification of pathogens by classical and non-culturing molecular techniques and virulence mechanisms, such as adhesion and bacterial toxins. In recognition of the recent important research on beneficial bacteria in human health, the section also includes chapters on gut microbiota, prebiotics, and probiotics. Together with the updated and expanded chapter on Bacterial Pharmaceutical Products, this section is a valuable resource to graduate students, teachers, and researchers interested in medical microbiology.

Volumes 6–11, organized by Erko Stackebrandt and Fabiano Thompson, contain chapters on each of the ca. 300 known prokaryotic families. Each chapter presents both the historical and current taxonomy of higher taxa, mostly above the genus level; molecular analyses (e.g., DDH, MLSA, riboprinting, and MALDI-TOF); genomic and phenetic properties of the taxa covered; genome analyses including nonchromosomal genetic elements; phenotypic analyses; methods for the enrichment, isolation, and maintenance of members of the family; ecological studies; clinical relevance; and applications.

As in the third edition, the volumes in the fourth edition are available both as hard copies and e-books, and as eReferences. The advantages of the online version include no restriction of color illustrations, the possibility of updating chapters continuously and, most importantly, libraries can place their subscribed copies on their servers, making it available to their community in offices and laboratories. The editors thank all the chapter authors and the editorial staff of Springer, especially Hanna Hensler-Fritton, Isabel Ullmann, Daniel Quiñones, Alejandra Kudo, and Audrey Wong, for making this contribution possible.

Eugene Rosenberg
Editor-in-Chief

About the Editors

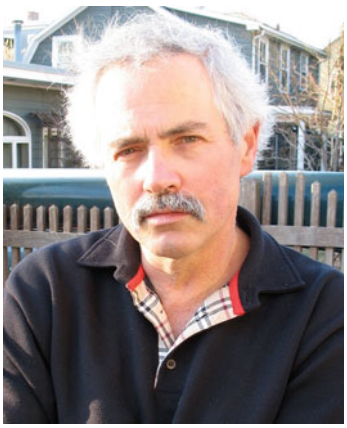
Eugene Rosenberg (Editor-in-Chief)
Department of Molecular Microbiology and Biotechnology
Tel Aviv University
Tel Aviv
Israel

Eugene Rosenberg holds a Ph.D. in biochemistry from Columbia University (1961) where he described the chemical structures of the capsules of *Hemophilus influenzae*, types B, E, and F. His postdoctoral research was performed in organic chemistry under the guidance of Lord Todd in Cambridge University. He was an assistant and associate professor of microbiology at the University of California at Los Angeles from 1962 to 1970, where he worked on the biochemistry of *Myxococcus xanthus*. Since 1970, he has been in the Department of Molecular Microbiology and Biotechnology, Tel Aviv University, as an associate professor (1970–1974), full professor (1975–2005), and professor emeritus (2006–present). He has held the Gol Chair in Applied and Environmental Microbiology since 1989. He is a member of the American Academy of Microbiology and European Academy of Microbiology. He has been awarded a Guggenheim Fellowship, a Fogarty International Scholar of the NIH, the Pan Lab Prize of the Society of Industrial Microbiology, the Proctor & Gamble Prize of the ASM, the Sakov Prize, the Landau Prize, and the Israel Prize for a "Beautiful Israel."

His research has focused on myxobacteriology; hydrocarbon microbiology; surface-active polymers from *Acinetobacter*; bioremediation; coral microbiology; and the role of symbiotic microorganisms in the adaptation, development, behavior, and evolution of animals and plants. He is the author of about 250 research papers and reviews, 9 books, and 16 patents.

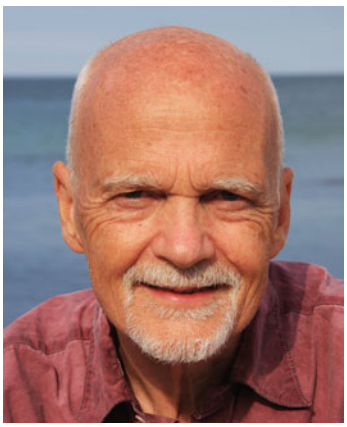
Edward F. DeLong
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA
USA

Edward DeLong received his bachelor of science in bacteriology at the University of California, Davis, and his Ph.D. in marine biology at Scripps Institute of Oceanography at the University of California, San Diego. He was a professor at the University of California, Santa Barbara, in the Department of Ecology for 7 years, before moving to the Monterey Bay Aquarium Research Institute where he was a senior scientist and chair of the science department, also for 7 years. He now serves as a professor at the Massachusetts Institute of Technology in the Department of Biological Engineering, where he holds the Morton and Claire Goulder Family Professorship in Environmental Systems. DeLong's scientific interests focus primarily on central questions in marine microbial genomics, biogeochemistry, ecology, and evolution. A large part of DeLong's efforts have been devoted to the study of microbes and microbial processes in the ocean, combining laboratory and field-based approaches. Development and application of genomic, biochemical, and metabolic approaches to study and exploit microbial communities and processes is his another area of interest. DeLong is a fellow in the American Academy of Arts and Science, the U.S. National Academy of Science, and the American Association for the Advancement of Science.



Stephen Lory
Department of Microbiology and Immunology
Harvard Medical School
Boston, MA
USA

Stephen Lory received his Ph.D. degree in Microbiology from the University of California in Los Angeles in 1980. The topic of his doctoral thesis was the structure-activity relationships of bacterial exotoxins. He carried out his postdoctoral research on the basic mechanism of protein secretion by Gram-negative bacteria in the Bacterial Physiology Unit at Harvard Medical School. In 1984, he was appointed assistant professor in the Department of Microbiology at the University of Washington in Seattle, becoming full professor in 1995. While at the University of Washington, he developed an active research program in host-pathogen interactions including the role of bacterial adhesion to mammalian cells in virulence and regulation of gene expression by bacterial pathogens. In 2000, he returned to Harvard Medical School where he is currently a professor of microbiology and immunobiology. He is a regular reviewer of research projects on various scientific panels of governmental and private funding agencies and served for four years on the Scientific Council of Institute Pasteur in Paris. His current research interests include evolution of bacterial virulence, studies on post-translational regulation of gene expression in *Pseudomonas*, and the development of novel antibiotics targeting multi-drug-resistant opportunistic pathogens.



Erko Stackebrandt
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures
Braunschweig
Germany

Erko Stackebrandt holds a Ph.D. in microbiology from the Ludwig-Maximilians University Munich (1974). During his postdoctoral research, he worked at the German Culture Collection in Munich (1972–1977), 1978 with Carl Woese at the University of Illinois, Urbana Champaign, and from 1979 to 1983 he was a member of Karl Schleifer's research group at the Technical University, Munich. He habilitated in 1983 and was appointed head of the Departments of Microbiology at the University of Kiel (1984–1990), at the University of Queensland, Brisbane, Australia (1990–1993), and at the Technical University Braunschweig, where he also was the director of the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (1993–2009). He is involved in systematics, and molecular phylogeny and ecology of Archaea and Bacteria for more than 40 years. He has been involved in many research projects funded by the German Science Foundation, German Ministry for Science and Technology, and the European Union, working on pure cultures and microbial communities. His projects include work in soil and peat, Mediterranean coastal waters, North Sea and Baltic Sea, Antarctic Lakes, Australian soil and artesian wells, formation of stromatolites, as well as on giant ants, holothurians, rumen of cows, and the digestive tract of koalas. He has been involved in the description and taxonomic revision of more than 650 bacteria taxa of various ranks. He received a Heisenberg stipend (1982–1983) and his work has been awarded by the Academy of Science at Göttingen, Bergey's Trust (Bergey's Award and Bergey's Medal), the Technical University Munich, the Australian Society for Microbiology, and the American Society for Microbiology. He held teaching positions in Kunming, China; Budapest, Hungary; and Florence, Italy. He has published more than 600 papers in refereed journals and has written more than 80 book chapters. He is the editor of two Springer journals and served as an associate editor of several international journals and books as well as on national and international scientific and review panels of the German Research Council, European Science Foundation, European Space Agency, and the Organisation for Economic Co-Operation and Development.

Fabiano Thompson
Laboratory of Microbiology
Institute of Biology
Center for Health Sciences
Federal University of Rio de Janeiro (UFRJ)
Ilha do Fundão
Rio de Janeiro
Brazil

Fabiano Thompson became the director of research at the Institute of Biology, Federal University of Rio de Janeiro (UFRJ), in 2012. He was an oceanographer at the Federal University of Rio Grande (Brazil) in 1997. He received his Ph.D. in biochemistry from Ghent University (Belgium) in 2003, with emphasis on marine microbial taxonomy and biodiversity. Thompson was an associate researcher in the BCCM/LMG Bacteria Collection (Ghent University) in 2004; professor of genetics in 2006 at the Institute of Biology, UFRJ; and professor of marine biology in 2011 at the same university. He has been a representative of UFRJ in the National Institute of Metrology (INMETRO) since 2009. Thompson is the president of the subcommittee on the Systematics of Vibrionaceae–IUMS and an associate editor of *BMC Genomics* and *Microbial Ecology*. The Thompson Lab in Rio currently performs research on marine microbiology in the Blue Amazon, the realm in the southwestern Atlantic that encompasses a variety of systems, including deep sea, Cabo Frio upwelling area, Amazonia river-plume continuum, mesophotic reefs, Abrolhos coral reef bank, and Oceanic Islands (Fernando de Noronha, Saint Peter and Saint Paul, and Trindade).

Table of Contents

List of Contributors

Judith P. Armitage
OCISB, Department of Biochemistry
University of Oxford
Oxford
UK

Bonnie L. Bassler
Department of Molecular Biology
Princeton University
Princeton, NJ
USA

Lucy Belmar
Departamento de Oceanografía and Programa de Postgrados en Oceanografía
Universidad de Concepción
Concepción
Chile

David G. Bourne
Australian Institute of Marine Science
Townsville
Australia

John P. Bowman
Tasmanian Institute of Agriculture
University of Tasmania
Hobart, TAS
Australia

Rosalie R. R. Coelho
Instituto de Microbiologia Paulo de Góes
Universidade Federal do
Rio de Janeiro
Brazil

Rolf Daniel
Department of Genomic and Applied Microbiology
Georg-August-Universität Göttingen
Göttingen
Germany

J. L. Darcy
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Seana Davidson
Department of Civil and Environmental Engineering
University of Washington
Seattle, WA
USA

Uwe Deppenmeier
Department of Biological Sciences
University of Wisconsin-Milwaukee
Milwaukee, WI
USA

Jason J. Flowers
Department of Civil and Environmental Engineering
University of Washington
Seattle, WA
USA

Thilo M. Fuchs
Lehrstuhl für Mikrobielle Ökologie, Department für Biowissenschaftliche Grundlagen, Wissenschaftszentrum Weihenstephan
Technische Universität München
Freising
Germany
and
Zentralinstitut für Ernährungs– und Lebensmittelforschung (ZIEL), Abteilung Mikrobiologie
Technische Universität München
Freising
Germany

Ferran Garcia-Pichel
School of Life Sciences
Arizona State University
Tempe, AZ
USA

Gerhard Gottschalk
Institut für Mikrobiologie und Genetik
Georg-August-Universität Göttingen
Göttingen
Germany

Yitzhak Hadar
Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food & Environment
The Hebrew University of Jerusalem
Rehovot
Israel

Steven J. Hallam
Department of Microbiology and Immunology
University of British Columbia, Life Sciences Institute
Vancouver, BC
Canada
and
Graduate Program in Bioinformatics
University of British Columbia, Life Sciences Institute
Vancouver, BC
Canada

William P. Hanage
Department of Epidemiology
Harvard School of Public Health
Boston, MA
USA

Meredith Hullar
Public Health Sciences
Fred Hutchinson Cancer Research Center
Seattle, WA
USA

Masahiro Ito
Graduate School of Life Sciences
Toyo University
Gunma
Japan
and
Japan and Bio–Nano Electronics Research Centre
Toyo University
Kawagoe
Saitama
Japan

Rainer Jaenicke
Institut für Biophysik und physikalische Biochemie
Universität Regensburg
Regensburg
Germany

Dale Kaiser
Department of Biochemistry
Stanford University School of Medicine
Stanford, CA
USA

Donovan P. Kelly
School of Life Sciences
The University of Warwick
Coventry
UK

J. Knelman
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Terry Ann Krulwich
Department of Pharmacology and Systems Therapeutics
Mount Sinai School of Medicine
New York, NY
USA

R. C. Lynch
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Andrew Macrae
Instituto de Microbiologia Paulo de Góes
Universidade Federal do
Rio de Janeiro
Brazil

Kevin C. Marshall*

Melissa B. Miller
Department of Pathology and Laboratory Medicine
University of North Carolina at Chapel Hill
Chapel Hill, NC
USA

Dror Minz
Institute for Soil, Water and Environmental Sciences
ARO, Volcani Research Center
Bet-Dagan
Israel

D. R. Nemergut
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Klaus Neuhaus
Lehrstuhl für Mikrobielle Ökologie, Department für
Biowissenschaftliche Grundlagen, Wissenschaftszentrum
Weihenstephan
Technische Universität München
Freising
Germany
and

*Deceased

Zentralinstitut für Ernährungs– und Lebensmittelforschung (ZIEL), Abteilung Mikrobiologie
Technische Universität München
Freising
Germany

Maya Ofek
Institute for Soil, Water and Environmental Sciences
ARO, Volcani Research Center
Bet-Dagan
Israel

Aharon Oren
Division of Microbial and Molecular Ecology, The Institute of Life Sciences, and The Moshe Shilo Minerva Center for Marine Biogeochemistry
The Hebrew University of Jerusalem
Jerusalem
Israel

Jörg Overmann
Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
Braunschweig
Germany

Raquel Peixoto
Instituto de Microbiologia Paulo de Góes
Universidade Federal do
Rio de Janeiro
Brazil

Jakob Pernthaler
Limnological Station, Institute of Plant Biology
University of Zurich
Kilchberg
Switzerland

Martin F. Polz
Parsons Laboratory, Department of Civil and Environmental Engineering
Massachusetts Institute of Technology
Cambridge, MA
USA

M. Rhodes
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Alexandre S. Rosado
Instituto de Microbiologia Paulo de Góes
Universidade Federal do
Rio de Janeiro
Brazil

Siegfried Scherer
Lehrstuhl für Mikrobielle Ökologie, Department für Biowissenschaftliche Grundlagen, Wissenschaftszentrum Weihenstephan
Technische Universität München
Freising
Germany
and
Zentralinstitut für Ernährungs– und Lebensmittelforschung (ZIEL), Abteilung Mikrobiologie
Technische Universität München
Freising
Germany

Bernhard Schink
Department of Biology
University of Konstanz
Constance
Germany

S. K. Schmidt
Department of Ecology and Evolutionary Biology
University of Colorado
Boulder, CO
USA

Ruth A. Schmitz
Institut für Allgemeine Mikrobiologie
Christian-Albrechts-Universität Kiel
Kiel
Germany

Kathryn A. Scott
OCISB, Department of Biochemistry
University of Oxford
Oxford
UK

Lawrence J. Shimkets
Department of Microbiology
The University of Georgia
Athens, GA
USA

David A. Stahl
Department of Microbiology and Civil and Environmental Engineering
University of Washington
Seattle, WA
USA

Alfons J. M. Stams
Laboratory of Microbiology
Wageningen University
Wageningen
The Netherlands

Reinhard Sterner
Institut für Biophysik und physikalische Biochemie
Universität Regensburg
Regensburg
Germany

Andreas Teske
Department of Marine Sciences
University of North Carolina at Chapel Hill
Chapel Hill, NC
USA

Osvaldo Ulloa
Departamento de Oceanografía
Universidad de Concepción
Concepción
Chile

Nicole S. Webster
Australian Institute of Marine Science
Townsville
Australia

Ann P. Wood
School of Biomedical Sciences
Department of Biochemistry
King's College London
London
UK

Jody J. Wright
Department of Microbiology and Immunology
University of British Columbia, Life Sciences Institute
Vancouver, BC
Canada

Prokaryotic Communities

1 Structure and Function of Microbial Communities

David A. Stahl[1] · *Jason J. Flowers*[2] · *Meredith Hullar*[3] · *Seana Davidson*[2]
[1]Department of Microbiology and Civil and Environmental Engineering, University of Washington, Seattle, WA, USA
[2]Department of Civil and Environmental Engineering, University of Washington, Seattle, WA, USA
[3]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Introduction

For the greater part of its history, microbiology has been a science of the single cell. The cell has dominated thought and experiment. So much so that discussion of other forms of organization, if addressed at all, was most often the subject of hallway speculation rather than a serious question of investigation. It is heartening that within a couple of decades or so the science of microbiology has begun to address the dimensions of organization that transcend the single cell. For example, the study of biofilms as organized systems is now more acceptable, and several high-profile publications have emerged that examine the physiological basis of structure and function at a multicellular level of monospecific biofilm communities, revealing mechanisms that specifically regulate collective behavior in microorganisms (Ng and Bassler 2009; Fuqua et al. 1994, 1996; Hastings and Greenberg 1999; Whiteley et al. 2001). Although there remains an essential reductionism to these studies, they have demonstrated the importance of intrapopulation, and possibly interpopulation, communication systems in controlling the structure and activity of multispecies microbial systems.

Two decades ago, one of the authors contributed a short article to the American Society for Microbiology (ASM) News (Stahl 1993), noting a fundamental variance between microbiology and general biological sciences. Microbiology was not built upon a foundation of natural history, for lack of tools to observe and categorize microbes in nature. Morphology was of little utility—the simple shapes of microorganisms conceal their remarkable diversity. Culture-based studies provided only a sketchy census of natural diversity, and conventional biochemical tests did not serve a phylogenetically based system of classification. It was only through comparative sequencing, first of proteins and later of nucleic acids, that inferences of phylogenetic relationship among microorganisms could be made (Zuckerkandl and Pauling 1965; Jukes and Cantor 1969). Today, we see a field transformed by comparative analyses of genes, complete genomes, and metagenomics. The metric provided by a molecular clock introduced an evolutionary perspective and phylogenetic dimension most forcefully represented by a universal tree of life (Woese 1987; Woese et al. 1990). This single phylogeny revealed the vast diversity of microbial life, reducing the plants and animals to a peripheral branch (*Fig. 1.1*). This has fueled more general recognition that the primary biology of our planet is microbial and provided an essential framework to conduct a census of diversity (Pace 2009; Huse et al. 2008; Rappe and Giovannoni 2003; Sogin et al. 2006; Stahl et al. 1984; Olsen et al. 1986; Ward et al. 1990; DeLong et al. 1994; Amann et al. 1995). Within that framework, both cultured and uncultured organisms could be related through a common metric based on the sequence divergence of common biopolymers (e.g., DNA-encoded RNA and protein components of the cellular translational and transcriptional machinery). As environmental metagenomic surveys have revealed, the natural diversity vastly exceeds that represented in pure culture, revealing major lines of descent (kingdom and phylum levels) that were previously unrecognized (Tringe et al. 2005; Venter et al. 2004). The astounding implication is that we are only now beginning to explore significantly the biological diversity of our planet.

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_34,

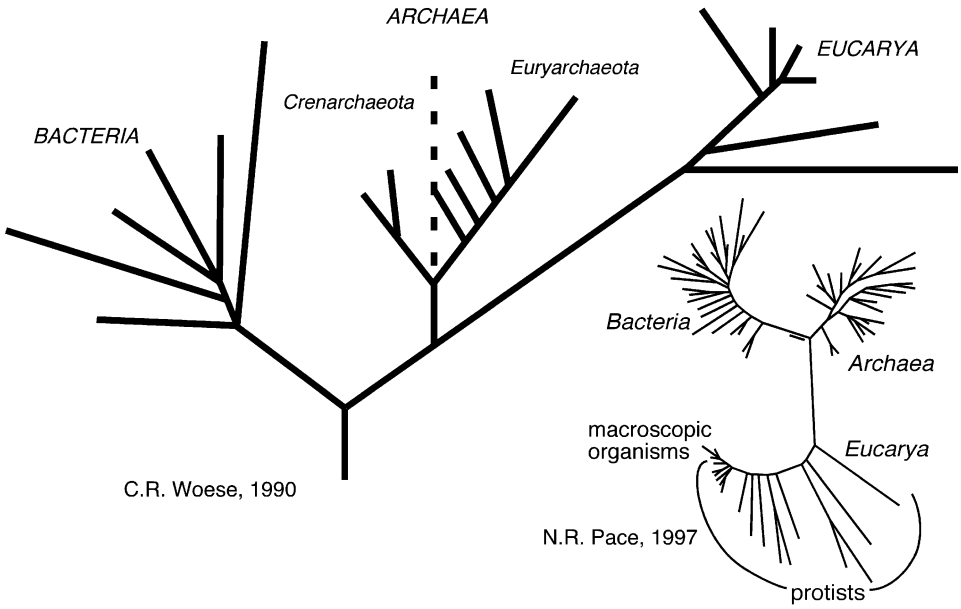


Fig. 1.1

Universal phylogenetic tree in rooted form, showing the three domains. Branching order and branch lengths are based upon rRNA sequence comparisons (Woese et al. 1990). *Inset* tree illustrates elaborations of existing branches (Pace 1997), but maintenance of the fundamental framework determined in the earlier phylogeny

This introduction must include a disclaimer. Although our subject is microbial community structure, the accepted unit of community structure, the species, remains poorly conceptualized. Complete genome sequences and expansive molecular diversity surveys have not provided much illumination on the "species problem." For example, comparative analyses of different strains of *Escherichia coli*, *Shewanella* species, and *Prochlorococcus* isolates have revealed a remarkable plasticity in genome sequence among organisms coherent by traditional phenotypic criteria (Wren 2000; Hayashi et al. 2001; Perna et al. 2001; Pennisi 2001; Dobrindt et al. 2003; Coleman et al. 2006; Rodrigues et al. 2011; Rocap et al. 2003). Additionally, metagenomic sequencing of a simplified bacterial community in the environment, an acid-mine drainage, revealed a significant intrapopulation variation (Tyson et al. 2004). Microbial geneticists were startled by the large fraction of genes of unknown function in the genome sequence of *E. coli*. Each newly sequenced genome contains a similarly high fraction of unknowns (20–30%). Nonetheless, clinical labs would assign *E. coli* strains differing by as much as 30% to the same species without difficulty. In contrast, strains of other well-described genera and species are much more uniform in genome sequence and organization. For example, the genome sequence of *Mycobacterium bovis* is greater than 99.95% identical to *M. tuberculosis*, having no genes that uniquely distinguish it from *M. tuberculosis* (Garnier et al. 2003). This apparent incongruity illustrates one of the limitations of characterizing microorganisms in populations of clonally derived cells—the pure culture—and of emphasizing selected features (such as host range or pathogenicity) in formal descriptions. Here, we simply emphasize that laboratory culture is not an appropriate context for fully appreciating any organism—divorced from a native habitat (niche) of which we have little understanding. The high proportion of genes of unknown function in every completed genome is another reflection of the pure culture paradigm. The environment is the context in which genomes evolved, function, and continue to evolve. It is the only context in which they can be fully understood.

The habitat in which microbial communities reside includes the interplay between biotic and abiotic factors. Microbiologists, again largely because of the pure culture paradigm, have tended to emphasize the abiotic features of habitat (electron donor, electron acceptor, salinity, temperature, pressure, etc.). These are the standards by which organisms have traditionally been classified. However, these factors are in most instances only a thin slice of the parameter space defining their ecology and evolution. As was long ago expressed by Darwin, "...the most important of all causes of organic change is one which is almost independent of altered physical conditions, namely, the mutual relation of organisms to organisms. If some of these many species become modified and improved, others will have to be improved in a corresponding degree or they will be exterminated" (Darwin 1969). Also, a system-wide analysis of archaeal, bacterial, and protist populations in the ocean suggested that correlations between microbial members provided greater influence on the observed population structure than abiotic environmental parameters (Steele et al. 2011). Thus, more complete understanding of any organism must encompass the features of higher order biological organization that have shaped the organism's evolution and contemporary "niche."

A retrospective by Moselio Schaechter (2003) suggests that we have so far experienced two golden ages in microbiology. The first age followed recognition of microorganisms in general

terms—as examples of the unity of biochemical processes, as agents of disease and spoilage, and as primary engines of biogeochemical transformations. The technological development that heralded the beginning of this first golden age was the pure culture method. Advances in molecular biology and associated methods of genetic analysis introduced the second golden age. We are now poised at the beginning of a third golden age which is beginning to develop a more complete understanding of systems of organization (ecology), their origins, and mechanisms of change (evolution) through next-generation sequencing technologies, improved instrumentation speed and sensitivity for protein and metabolite identification, and increased computational power which is causing the coalescing of multiple disciplines needed to address the complexity of systems-level organization. An empirical observation has been that predictions of higher levels of organization in biological systems are not readily derived from understanding gained at lower levels (Mayr 1982). Thus, advances in technology in concert with disciplinary coalescence will foster investigations that move far beyond the study of clonal lines in laboratory culture to studies of natural systems comprised of diverse interacting populations (Heidelberg et al. 2010).

Structure and Function of Microbiological Systems

Microbiological systems can be characterized in three general ways: (1) the historical (considering system origins and evolution), (2) the proximate (characterizing the immediate structure and function of a system), and (3) the temporal (addressing the interplay of biotic and abiotic elements that shape a system over diel, annual, and decadal temporal periods). No one way of characterization provides complete understanding—all three must be considered. In turn, each characterization must incorporate the different dimensions of structure and function. These dimensions encompass both the biotic (phylogenetic, evolutionary, and metabolic) and the abiotic (chemical and physical). We have organized this chapter by considering both the different ways microbiological systems are characterized and the multiple dimensions of structure and function that serve characterization.

The Historical System

The Phylogenetic Dimension

The evolution of our planet is intertwined with the evolutionary history of its microorganisms. Neither the planet nor any one organism is fully intelligible apart from that ancestry. Today, a reasonable representation of the phylogenetic relationships among all life is available (Pace 2009; Woese et al. 1990; Woese 2000). The "universal tree," inferred by comparative sequencing of the small ribosomal subunit rRNA, remains the canonical structure (*Fig. 1.1*). Although recognition that horizontal gene transfer (HGT) may have eroded some of the fine detail of structure (Doolittle 1999), there is an emerging consensus that microorganisms do display an organismal genealogy and that many genes are phylogenetically informative (Pace et al. 2012; Ochman et al. 2000; Daubin et al. 2003). Within-group divergence in gene content (in part attributable to HGT) appears to more reflect acquisition of cosmopolitan genes adaptive to a specific environment (Woese 2004).

Recognition that a microbial genealogy could be inferred from comparative analysis of appropriate gene homologs (orthologs) had significant impact on our perspective and understanding of the structure and function of microbiological systems. The most immediate impact was derived from recognition that our census of biological diversity was incomplete; the several thousand named species of microorganisms scattered among thinly populated parts of the universal tree was not an adequate representation of diversification, spanning the greater part of the evolutionary history of this planet. Subsequent application of molecular methods to directly describe environmental microbial diversity has confirmed that the greater part of biological diversity is microbial and that the greater part of microbial diversity has yet to be described (Pace 2009; Amann and Ludwig 2000; DeLong and Pace 2001; Torsvik and Ovreas 2002).

The phylogenetic dimension embodies the connection between the diversification of life represented in the canonical tree and the emergence of functional groups (e.g., methanogens, acetogens, phototrophs, sulfate reducers, and nitrifiers) in both early and contemporary biospheres. If microorganisms have diversified to fill both general and specific niches, the record of this diversification will be captured by their genealogy. However, there is considerable confusion concerning the ability to infer physiology from phylogenetic affiliation. There are many examples of close phylogenetic relationship between organisms that have remarkably different physiological attributes, for example, close relationships between phototrophs and autotrophs, and between autotrophs and heterotrophs (for examples, see Lane et al. 1992). Conversely, there are also examples of phylogenetically defined groups that are remarkably coherent in physiological characteristics. The *Desulfovibrio* (Devereux et al. 1989, 1990), methanogenic groups (Raskin et al. 1994), and nitrifiers (Head et al. 1993; Teske et al. 1994) are notable examples of phylogenetic and physiological coherence. Part of this apparent discordance almost certainly derives from failure to recognize ecologically significant features. We suspect that this is in large part a consequence of using the pure culture phenotype to infer environmental activity outside of the context of the complex environment and interactions between organisms of a microbial community. As methods are further developed for direct observations of populations within an environmental setting, we anticipate that many additional phylogenetically cohesive characters will be revealed.

A general relationship between habitat and taxonomic groups has long been recognized. However, molecular tools are refining the characterization of that relationship. An early observation of direct correspondence was made in a microbial mat community in which the depth distribution of different sulfate-reducing populations corresponded with members affiliated with discrete phylogenetic clades (Risatti et al. 1994). A similar correspondence

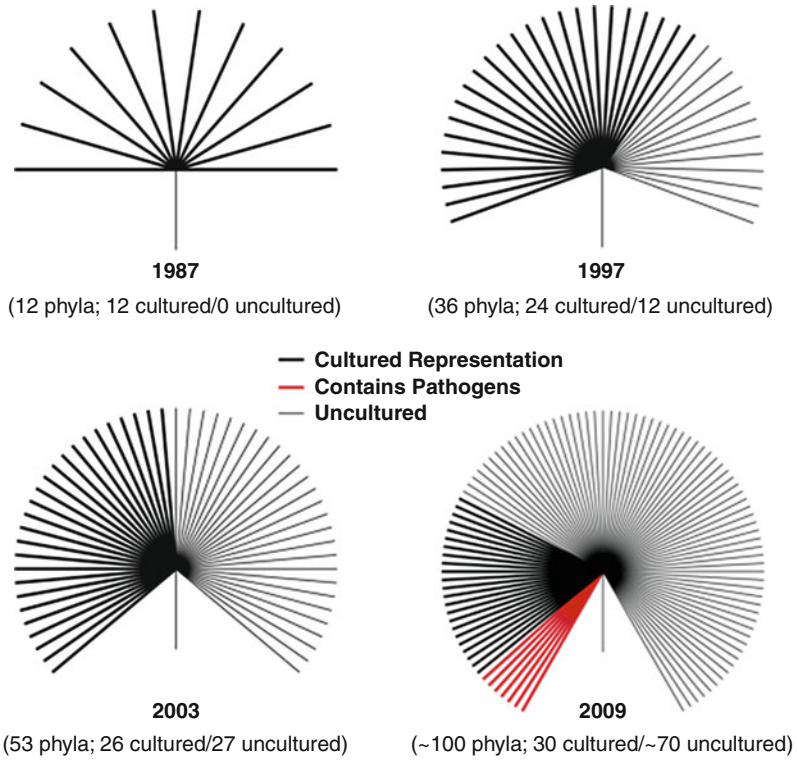


Fig. 1.2
Evolutionary distance tree of the bacterial domain showing the increase in recognized (“cultured”) divisions and putative (candidate/“uncultured”) divisions from 1987 to 2009 (Figure courtesy of Norman Pace at University of Colorado, Boulder, USA)

between phylogenetic affiliation and aquatic habitat has been noted. The ubiquitous SAR11 group (*Pelagibacter*), first identified by Giovannoni and associates (1990), appears to be exclusively marine (Morris et al. 2002). However, the greater assemblage of organisms to which the marine SAR11 belongs includes more distantly related freshwater representatives (Bahr et al. 1996; Field et al. 1997; Morris et al. 2002; Carlson et al. 2009; Glockner et al. 1999; Zwart et al. 1998). *Prochlorococcus* appears to be almost exclusively marine assemblage of unicellular cyanobacteria. An early suggestion that genetic variation among *Prochlorococcus* isolates corresponded to different light-adapted populations was subsequently confirmed (Moore et al. 1998). Recent genomic sequencing of 12 strains of *Prochlorococcus* provides genetic evidence for these ecotypes including genes that explained the high-light and low-light adapted clades (Kettler et al. 2007). The population structure and thus phylogeny of planktonic bacterial communities is associated with general features of habitat (marine vs. freshwater and depth-associated changes in physical and chemical variables).

Although a correlation between closely related populations and habitat is not unexpected, we also ask the more general question—how are the most ancient of evolutionary divergences (domain and division level; Hugenholtz et al. 1998) related to the structure and activities of contemporary systems? If the emergence and diversification of a new lineage were primarily explained by a key evolutionary innovation, then retention of that innovation among contemporary representatives would both serve to characterize the lineage and the defining evolutionary innovation. A few notable examples support this, suggesting a centrality of the phylogenetic dimension (*Fig. 1.2*). The first example is one of remarkable biochemical innovation, the invention of oxygenic photosynthesis. There is good support that this innovation was a consequence in part of horizontal gene transfer between early anoxygenic phototrophs, each having distinct photosystems. The melding of those photosystems into a two-photosystem apparatus defined the emergence of the cyanobacteria (Buttner et al. 1992; Mulkidjanian et al. 2006). This ancestral metabolic innovation defines all contemporary representatives of the group. In contrast, the spirochetes provide an example of structural innovation. The spiral body form combined with axial flagella offered advantage for moving in viscous environments, and today, they are abundant residents in many such habitats, including microbial biofilms and mats. A final example of both structural and physiological innovation is the endospore of named species of *Bacillus* and *Clostridium*. The bacterial endospore was apparently invented only once and likely in large part defines both the origin and successful radiation of major Gram-positive lineages.

The Evolutionary Dimension

The reflections of past innovations in microbial phylogeny raise immediate questions about mechanism and context of innovation. Complete genome sequencing has begun to more fully reveal the importance of horizontal gene transfer (HGT) as

a mechanism of biological innovation. A recent study (Smillie et al. 2011) has discovered significant horizontal gene transfers occurring across distantly related organisms. Interestingly, environment/ecology was suggested to be a greater selector for gene transfer occurrence than geography or phylogeny. Koonin et al. (2009) also found evidence for extensive amounts of HGT in prokaryotic genomes. Although extensive HGT has been suggested to have eroded a clear microbial genealogy, it appears that frequent gene transfer is more reflective of ecology than genealogy; the genealogical record is preserved in the sequence divergence of genes participating in information processing (e.g., the genes coding for the ribosomal RNAs)(Pace et al. 2012; Smillie et al. 2011). Thus, it is increasingly apparent that mechanisms of adaptive evolution (or innovation) cannot be separated from environmental context. That is, the fodder of innovation is determined by both the diversity of organisms (and genes) and the opportunities for interaction within a habitat (Smillie et al. 2011). Microbial mat communities provide a specific example of this point. Microbial mats are highly active, highly compact, and highly diverse microbial communities providing ample opportunity for intimate interactions among genetically diverse populations. If some early mat communities were based on anoxygenic photosynthesis, this community would be a plausible context for the development of oxygenic photosynthesis via horizontal gene exchange among intimately associated phototrophs. If early mats provided viscous habitats rich in organic substrates produced by phototrophs, this would also have been an ideal context for the emergence of spirochetes. These primarily heterotrophic organisms are adapted to move rapidly in highly viscous environments like microbial mats and biofilms.

Novelty can also arise through isolation, limiting genetic exchange and contributing to genetic drift and local adaptation. The degree to which this phenomenon occurs in bacteria is contentious. The prevailing opinion has been that bacterial species are cosmopolitan and exhibit a worldwide distribution. Indeed, surveys using the 16S rRNA suggest a global distribution of many species that can be poles apart (Fuhrman et al. 1992, 1993; Giovannoni and Cary 1993; Staley and Gosink 1999). This is further suggested from a metagenomic survey of two lab-scale bioreactors in the USA and Australia, respectively, that were enriched in *Candidatus* Accumulibacter phosphatis, a bacterium involved in phosphorus removal in wastewater treatment that revealed that the dominant strains were >95% similar over 79% of the genome (Martin et al. 2006). The adage "everything is everywhere and nature selects" (Beijerinck 1913) suggests that geographic barriers do not restrict bacterial dispersal. In this view, bacterial distribution is solely determined by the global dispersal of preadapted populations. This view is primarily based on the 16S rRNA gene divergence, which may not be representative of changes in other genes that define specific adaptive traits. The alternative, a biogeography in part determined by evolutionary adaptation to the local environment, is now receiving some support. Several studies have shown that geographically separated populations sharing identical or very similar16S rRNA sequences differ at other genetic loci (Moore et al. 1998; Casamayor et al. 2002; Rocap et al. 2002). A study by Whitaker et al. (2003) found that strains of the extremophile *Sulfolobus* was clustered geographically rather than by environmental variables that characterized different hot springs. Their multilocus analysis revealed that the genetic distances between populations increased proportionally with geographic distance, suggesting that dispersal of populations and exchange of genetic material between geographically distant groups were limited. Comparative genomic analysis of seven *Sulfolobus islandicus* genomes from three locations confirmed these trends (Reno et al. 2009). Using the 16S rRNA gene as a marker, Papke et al. (2003) also observed genetic differences among thermophilic cyanobacteria from different geographic regions. Their distribution patterns were also ascribed to biogeographic isolation. These and other cumulative data suggest that global distribution patterns reflect both endemic and cosmopolitan groups. This is also supported by recent studies of pure cultures and cocultures maintained over many generations in the laboratory, demonstrating the capacity for continued and rapid adaptive change from a single clonal line of descent (Elena and Lenski 2003; Hillesland and Stahl 2010).

While the exploration of phage diversity is still in its infancy with large populations being uncharacterized (Cantalupo et al. 2011), phage populations clearly have an impact on microbial evolution. Phage population abundance has been shown to be greater than their microbial hosts (Bergh et al. 1989) which suggests that an ongoing phage-host "arms race" continually acts to shape the evolution of the bacterial and viral populations (Stern and Sorek 2011). A study of 77 substrains of *Prochlorococcus* revealed unique mutations in both core and noncore genes implicated in phage resistance, possibly at the cost of reduced fitness as indicated by an associated decrease in growth rate. However, several phage sensitive substrains were also identified in this study that did not have growth deficits under the conditions tested (Avrani et al. 2011). Ongoing studies of phage-bacteria dynamics will almost certainly lead to a greater understanding of how these dynamics shape population structure, possibly contributing to the emergence of new ecotypes.

Although specific examples are limited, available data point clearly to the importance of contingency in metabolic innovation and adaptive radiation of microorganisms. For example, widespread dispersal may follow major metabolic innovations that derive from interspecies horizontal gene transfer within a complex community, whereas adaptive radiation reflected by biogeographic patterns may arise from more restrictive mechanisms of genetic change. A less speculative discussion of the evolutionary dimension of microbial community structure and function must await more comprehensive descriptions of natural communities.

The Proximate System

Chemical and Physical Dimensions

A central consideration in discussing the structure of microbial communities concerns the interplay between physical and

biological controls of organization. Microorganisms are small and experience low Reynolds numbers—viscosity and diffusion dominate their world rather than the mixing and turbulence more familiar to us. One of the fastest and largest bacterium known, *Thiovulum majus*, is able to significantly increase substrate availability by generating advective flow through the entire colony via coordinated communal organization (slime veil formation) and motility (Fenchel and Glud 1998; Schulz and Jorgensen 2001; Thar and Fenchel 2001). More generally, diffusive delivery of nutrient solutes is more important than advective transport (Purcell 1977; Blackburn and Fenchel 1999), and diffusion determines structure at both microscopic and macroscopic scales. At microscopic dimensions, a diffusive "sphere" surrounds every metabolically active prokaryotic cell such that substrate concentrations only approach that of the bulk solution several cell diameters away from the microbe's surface. Microorganisms have developed a variety of strategies to enhance nutrient recovery including the production of siderophores, exoenzymes, smallness, and motility (Button 1994; Blackburn et al. 1998; Blackburn and Fenchel 1999). Directed movement along a concentration gradient gives the prokaryotic cell a mechanism to move toward regions of higher nutrient concentration and by doing so increases the flux of nutrient through the cell's diffusive sphere. Fenchel et al. (2002) suggest that even in a turbulent water column, "nutrient micropatches" derived from cell lysis and excretion by protozoa have life spans sufficiently long to increase nutrient availability to bacteria not to mention the occurrence of particles or marine snow. The implications for global processes are significant; motile bacteria converging transiently on microscale nutrient patches act to accelerate nutrient uptake and secondary production on a global scale.

Layered Microbial Communities

In environments experiencing limited mixing, diffusion and light attenuation contribute to stable architecture at the macroscopic dimensions of millimeters to meters. These systems are layered both chemically and biologically. We briefly discuss several types of layered communities common to aquatic habitats, although we recognize there are layered terrestrial communities as well.

Sediments

Within a sediment, in the absence of significant advection or bioturbation, mass transport occurs primarily through molecular diffusion. Sediments vary in coarseness and porosity, but in general, the sediment matrix limits or prevents water to advect through it. Gradients are formed whenever the production or consumption of a product or nutrient (reactant) exceeds the diffusion of that product or reactant. Substrate concentration reaches a minimum (a boundary condition) at a depth at which the rate of diffusion from the bulk phase matches the consumption rate needed to sustain the minimum free energy required for maintenance. Under these conditions, substrate-concentration gradients reach steady state, yielding characteristic profiles (Nealson and Stahl 1997; Brusseau et al. 1998; *Fig. 1.3*). A key variable is the amount of organic substrate received via sedimentation of organic matter derived from primary production in the water column, from terrestrial input, or from sediment surface photosynthesis in shallow systems that allow light penetration to the bottom. The observed depth-related changes in chemical composition correspond to a progression of thermodynamically predictable redox changes. Given a variety of possible respiratory modes, those that yield the greatest free energy prevail at each depth under steady-state conditions. This begins near the surface with oxygen depletion and typically ends with the reduction of sulfate (in marine environments) to produce sulfide, or the reduction of CO_2 (in freshwater environments) to produce methane.

The Water Column

The water column of marine and freshwater systems may also exhibit similar depth-related chemical structure, most comparably when oxygen depletion extends into the water column. This is observed in small eutrophic lakes and in the permanently stratified Black Sea (Nealson and Stahl 1997; Taillefert et al. 2002). In addition to diffusion-controlled structure characteristic of sediments, the water column is divided by changes in density, temperature, and light. The attenuation of light in the world's oceans provides the most easily resolvable of gradients along which different bacterial populations distribute. For example, as previously noted, different "ecotypes" of *Prochlorococcus* are adapted to different light intensities (Moore et al. 1998; West and Scanlan 1999; Rocap et al. 2002; Kettler et al. 2007). Physiological analyses have shown that one ecotype is adapted to the higher light intensities of the upper water column, having a low chlorophyll (Chl) b/a_2 ratio, and the other (higher Chl b/a_2) to life deeper in the euphotic zone. Genome sequence comparisons have also pointed to specific adaptive differences (Rocap et al. 2003). For example, the ecotype adapted to the lower euphotic zone has lost the photolyase gene involved in the light-driven enzymatic catalysis of DNA damage repair, presumably because there is little selective advantage to maintain this function under low-light conditions.

Variables other than light also contribute to depth-related structure of oceanic provinces. Unlike the relationship to thermodynamic preference observed in chemically stratified lakes and sediments, the controlling variables in the marine water column have yet to be fully resolved (DeLong et al. 2006). For example, the archaeal phylotypes common in the open ocean vary in depth-related abundance patterns (Massana et al. 2000). Studies of the Antarctic Peninsula showed that planktonic euryarchaeotes were most abundant in surface waters (Massana et al. 1998). Increasing archaeal abundance with depth (ca. 25% of total rRNA) was associated with a shift in dominance from euryarchaeal to crenarchaeal rRNA

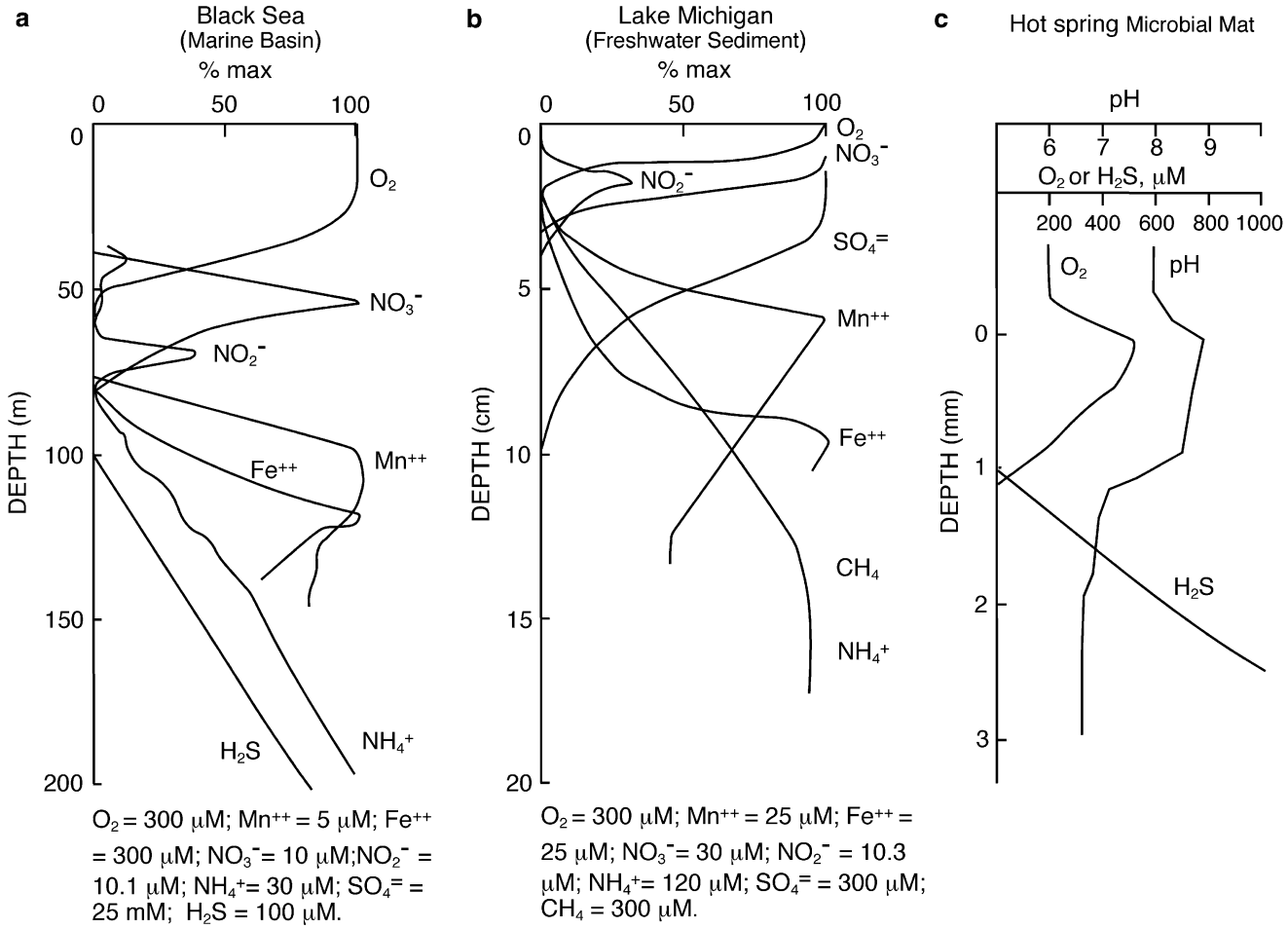


Fig. 1.3

Chemically stratified freshwater sediment from Lake Michigan and chemically stratified water column of the Black Sea. Although the distribution of the chemicals is similar, the scales over which the chemicals are distributed vary from centimeters in lake sediment to meters in the Black Sea water column (After Nealson and Stahl 1997)

(Massana et al. 1998). Another study at the Hawaii Ocean Time-series (HOT) station examining the depth-related abundance of these archaeal groups (pelagic euryarchaeota vs. pelagic crenarchaea) revealed a similar depth-related pattern of abundance (Karner et al. 2001). Pelagic archaea initially assigned to the Crenarchaeota (now assigned to the Thaumarchaeota) comprised a large fraction of total picoplankton below the euphotic zone (>150 m), approaching 39% of total DNA-containing picoplankton detected. These groups are surely physiologically distinct, as suggested by a time-series study in the Santa Barbara Channel that showed the abundance patterns of these two archaeal groups correlated with general environmental variables (Massana et al. 1997). Isolation of members of the Thaumarchaeota has revealed that many are chemotrophic ammonia oxidizers, with the potential for autotrophic growth (Brochier-Armanet et al. 2012; Konneke et al. 2005), while metagenomic sequencing suggests that at least some members of the marine Euryarchaeota are photoheterotrophs (Iverson et al. 2012). Assignment of physiological traits to populations previously identified by 16S rRNA sequence alone is contributing to the framework essential for relating distribution patterns to specific niches. Other abundant marine picoplankton *Pelagibacter ubique* (SAR11) and SAR211 and SAR203 clusters show characteristic depth-related abundance patterns (Giovannoni et al. 1996; Field et al. 1997; Wright et al. 1997; Morris et al. 2002). Success in culturing representatives of the SAR 11 cluster suggests that temperature, not light (Rappe et al. 2002; Zengler et al. 2002), may be an important factor in the growth and distribution of members of this assemblage. Comparison of the genomic content of a SAR11 isolate from the Oregon coast, *Pelagibacter ubique HTCC1062*, to a Sargasso Sea metagenome revealed that while the nearly the entire genome was represented, there were an additional four hypervariable regions which might confer unique ecological differences (Wilhelm et al. 2007). Further investigation into the distribution of SAR11 clades Ia, Ib, and II near Bermuda revealed depth and seasonal pattern differences, pointing to multiple environmental variables controlling population distribution (Carlson et al. 2009).

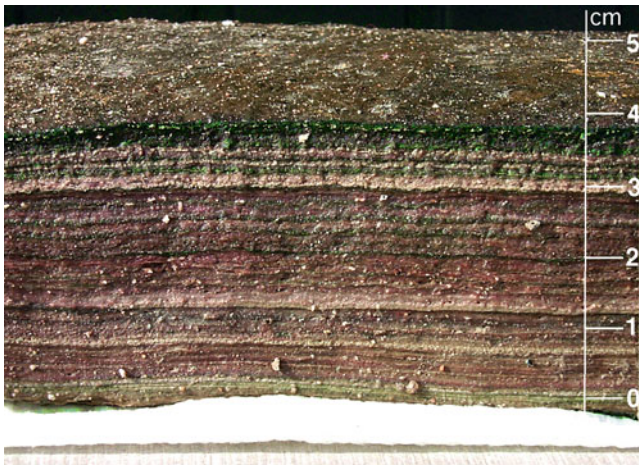


Fig. 1.4
An example of a layered community from Guerrero Negro, Baja California Sur. The colors denote pigments from different microbial groups (Photograph courtesy of Jesse Dillon at California State University, Long Beach)

The biotic and abiotic controls of population structure should be more clearly resolved as genomic and metagenomic sequencing further explore the marine environment.

Microbial Mats

Microbial mats are among the most visibly conspicuous of layered communities (*Fig. 1.4*). Built by photosynthetic or chemolithotrophic bacteria, they share features of structure similar to sediment and water column communities, in that population distribution is governed by light availability and diffusive flux of substrates and metabolites. However, mats provide for much closer physical and metabolic interactions among contributing populations. The most abundant and versatile photosynthetic mat builders today are the oxygen-producing cyanobacteria (Cohen and Rosenberg 1989). The most common types of chemolithotrophic mats are comprised of filamentous sulfur-oxidizing bacteria, generally occurring on sediment surfaces at the interface between gradients of reduced sulfur species and the oxidants oxygen or nitrate (Jorgensen and Revsbech 1983; Jannasch et al. 1989; Sassen et al. 1993).

The cyanobacterial mats are complete microbial ecosystems, comprised of primary producers (cyanobacteria) and populations of consumers that together mediate all key biogeochemical cycles (*Fig. 1.5*). Remarkably, this ecosystem can be represented by a fragment of microbial mat only several centimeters on a side. Although this general type of microbial community is thought to have existed for over 3.5 billion years (Cohen and Rosenberg 1989), the evolution of metazoan grazers, competition with macrophytes, and changing oceanic carbonate chemistry triggered the decline of the extensive mat communities (represented by stromatolitic fossils) at the end of the Proterozoic (Grotzinger and Knoll 1999). Today's mats develop conspicuously only in aquatic environments where environmental stress limits or excludes grazing, most commonly in hypersaline or thermal habitats. These are among the best studied of microbial communities and have provided a superb context for studies relating structure and function (Cohen and Rosenberg 1989).

Hypersaline cyanobacterial mats are characterized by intense oxygen production during the day in the photic surface layer and by highly active sulfate reduction throughout the mat. These gradients virtually disappear at night when the entire mat turns anoxic and sulfidic (Revsbech and Jorgensen 1983; Canfield and Marais 1993). Both molecular and cultivation studies of the oxic surface layer of cyanobacterial mats have revealed high numbers of sulfate-reducing bacteria (SRB) (Ley et al. 2006; Risatti et al. 1994; Teske et al. 1998; Minz et al. 1999a, b). Although SRBs are classical anaerobes, oxygen supersaturation apparently does not interfere with the activity of near-surface populations in these mats (Jorgensen and Cohen 1977; Canfield and DeMarais 1991, 1993; Jorgensen 1994; Teske et al. 1998). An SRB isolate from a cyanobacterial mat, *Desulfovibrio oxyclinae*, showed continued sulfate reduction in presence of oxygen (Sigalevich et al. 2000). Resistance to oxygen stress was attributed in part to a tendency to form flocs, promoting low-oxygen conditions in the floc interiors, but further exploration of mechanism is required. Another SRB was found to handle oxygen stress by coupling its reduction with NADH oxidization to limit the production of reactive oxygen species (Chen et al. 1993; Kettler et al. 2007). As further discussed below, the rapid migration of some mat populations, tracking the changing position of the oxic-anoxic interface during a diel cycle, is another strategy used to cope with rapidly fluctuating environmental conditions (Dillon et al. 2009).

The chemolithotrophic *Thioploca* mats on the Chilean and Peruvian continental shelf are the most extensive microbial mats on earth (Gallardo 1977; Fossing et al. 1995). In 2002, similar mats were also found in eastern Lake Ontario as well (Dermott and Legner 2002). Abundant *Thioploca* populations residing in the upper centimeter of these mats participate in an intense sulfur cycle. The high rate of sulfate reduction (up to 1,500 nmol cm^{-3} day^{-1}) is balanced by the oxidation of sulfide by *Thioploca* such that sulfate is not appreciably depleted (Thamdrup and Canfield 1996; Ferdelman et al. 1997). This highly efficient sulfur cycle has been attributed to a close physical association between sulfate-reducing and sulfur-oxidizing bacteria. Filamentous sulfate reducers of the genus *Desulfonema* were observed growing within the *Thioploca* sheaths, suggesting a complete cycle of sulfate reduction and reoxidation within individual *Thioploca* bundles, representing an example of syntrophy (Fukui et al. 1999).

Some mat populations rely on motility to follow shifting chemical gradients. Microbes such as the filamentous microaerophilic sulfide-oxidizing bacterium *Beggiatoa* monitor local chemical and physical dimensions of habitat, using that sensory input to relocate to environments better suited to their physiology. *Beggiatoa* and several other bacterial members in the cyanobacterial mats of Guerrero Negro follow the diel

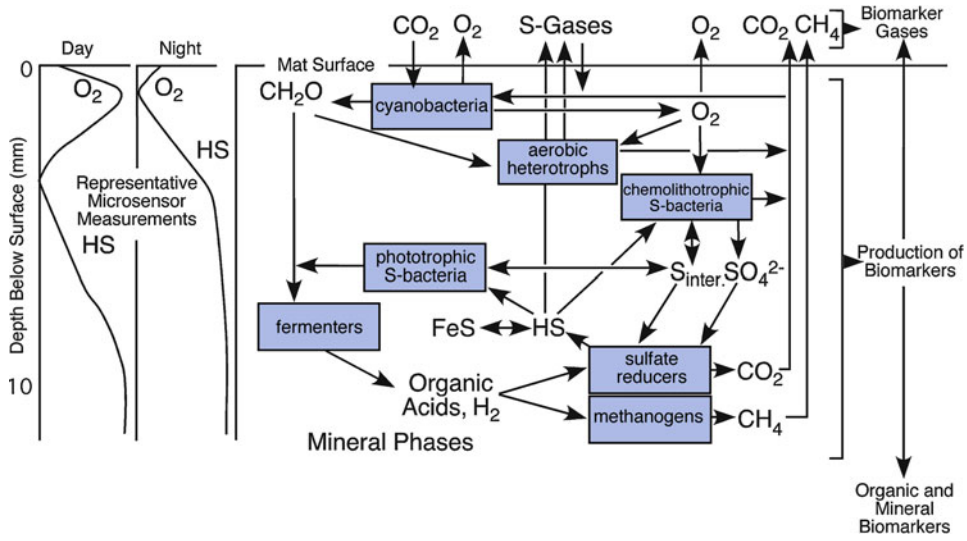


Fig. 1.5
The distribution and conversions of oxygen, sulfur, hydrogen, and carbon dioxide within a layered cyanobacterial mat community (After Fenchel and Finlay 1995)

up-and-down movement of the oxygen-sulfide interface closely (Garcia-Pichel et al. 1994; Dillon et al. 2009). Other organisms have developed a strategy to bridge spatially separated resources. This is exemplified by the discovery that *Thioploca* species use large internal vacuoles to store high concentrations of nitrate for anaerobic respiration. Much like a scuba diver fills diving tanks with oxygen to dive in an oxygen-limited water environment, *Thioploca* migrate to the sediment surface, partially emerge from their sediment-embedded sheaths to enter the water column, and charge internal vacuoles with high concentrations of nitrate (Huettel et al. 1996). They then return ("dive") to the anoxic depths of the sediment (gliding at a speed of 3–5 mm h^{-1}) to use their stored nitrate for sulfide oxidation (Maier et al. 1990; Fossing et al. 1995). There are undoubtedly many undescribed metabolic and behavioral strategies used to cope with the rapidly changing environmental conditions in microbial mat communities.

Biofilms

The term "biofilm" is the generally accepted term for microorganisms attached to a solid surface in a relatively thin film. Biofilms pervade virtually all environments and surfaces, often dominating the microbial activity distributed between the individual planktonic and aggregated habitats (van Loosdrecht et al. 1990). Characklis and Marshall (1990) have generally defined biofilms as systems displaying the following four features: (1) cells immobilized at a substratum and frequently embedded in an organic polymer matrix of microbial origin; (2) a surface accumulation, which is not necessarily uniform in time or space; (3) a significant fraction of inorganic or abiotic substances held together by the biotic matrix; and (4) transport and transfer processes are rate limiting and play a much more important role than in the suspended growth microbial systems. The fourth characteristic again highlights the importance of diffusion and reaction in controlling population structure and associated metabolic processes. As for the previously described layered communities, gradients form in response to the balance between microbial synthesis and consumption of diffusible substances, creating niches distinct from the proximal bulk water. Similar to mat communities, natural and experimental biofilm systems provide for a spectrum of stable habitat types (Monds and O'Toole 2009). Thus, a biofilm community of thickness less than 1 mm can have a diverse and stable microbial ecology amenable to study. For example, the biofilms produced by oral microbiota colonizing tooth surfaces provide a model system for experimental study and are receiving increasing attention as part of the human microbiome study (Kolenbrander 2000; Zaura et al. 2009).

The microbial populations colonizing suspended particles demonstrate many attributes of biofilms. Suspended organic particles, marine and lake "snow," conspicuous in aquatic habitats provide hot spots of nutrients and carbon sources for bacterial growth (Alldredge and Silver 1988; Cho and Azam 1988; reviewed in Turner 2002; Cho and Azam 1988). High densities of multiple microbial populations are embedded in a mucoid extracellular polysaccharide (EPS) matrix, providing opportunity for interaction. Reduced diffusion and high activity serve to create localized concentration gradients. Depletion of oxygen toward the center of larger aggregates supports the coexistence of aerobes and anaerobes (Alldredge and Cohen 1987), as revealed by the presence of sulfate-reducing bacteria (DeLong et al. 1993) and possibly nitrogen fixation (Paerl and Prufert 1987; (Riemann et al. 2010). Because microorganisms are the only biota having the capacity to utilize the dilute carbon and energy in many aquatic habitats, the colonization and transformation of these particles by microorganisms represents an

important portion of secondary production and may play an important role in food web energetics, atmospheric CO_2 exchange, and flux of nutrients to the deep-sea ecosystem through sedimentation of colonized particles (Decho and Herndl 1995; Turley and Mackie 1995). Microbial aggregates provide a link between the dilute dissolved carbon and higher trophic levels as food for larval species and protozoa (Pomeroy 1974; Karl 1994; Kiorboe et al. 2003) and serve to transfer surface water nutrients to the benthos via sedimentation (Passow 2002; Turner 2002).

Interactions among microbial populations in aggregated communities are also modulated by regulatory processes that alter gene expression in relationship to population density. Regulated processes are those that are adaptive only when the population density reaches a critical threshold number (the "quorum"). These sensing mechanisms are addressed in the following section on the metabolic dimension.

The Metabolic Dimension

The previous sections emphasized the role of diffusion and reaction in regulating the chemical structure of a microbial system. The metabolic dimension underlies the chemical reactions governing the flux of energy and matter through the system. Each microbial population must derive sufficient free energy of reaction for maintenance and growth. The biochemical explanation for how the total free energy available is parceled out among the populations is the metabolic dimension. This dimension encompasses factors regulating the biochemical response and activity of populations in any system. We now have only a sketchy understanding of the metabolic dimension and limit our discussion to selected examples of how quorum sensing, syntrophy, and symbiosis govern the metabolic dimension to shape microbial structure and function.

Quorum Sensing: A Language for Inter- and Intrapopulation Communication

It is increasingly apparent that specific signaling molecules modulate reactions within microbial communities (Ng and Bassler 2009; Fuqua et al. 2001; Fuqua and Greenberg 2002; Xavier and Bassler 2003). The term "quorum sensing" was introduced to describe signaling systems mediated by diffusible molecules (autoinducers, primarily different forms of peptides and acyl homoserine lactones [AHLs]) that regulate expression of genes that are most beneficial when a critical number of microorganisms (the quorum) are present in a locale. The peptide and AHL types of autoinducers primarily control reactions within a single population, for example, in the production of extracellular hydrolases during tissue invasion or light generation when colonizing particles or inhabiting specialized light organs of certain marine animals. However, a described furanone autoinducer (AI-2) has been implicated in signaling between disparate species of bacteria (Ng and Bassler 2009). A role of AI-2 in interspecies communication is receiving experimental support, such as a recent study by McNab and associates (2003) showing that interspecies autoinduction is required for the coordinated development of a biofilm comprised of two species of oral microbes, *Porphyromonas gingivalis* and *Streptococcus gordonii*, as well as cross domain biofilm development of *S. gordonii* and *Candida albicans* (Bamford et al. 2009).

Recognition of the widespread occurrence of quorum sensing among microbial species suggests there is frequently an active and rich dialogue between cells comprising one or more populations (Fuqua et al. 1994, 1996; Hastings and Greenberg 1999). To date, most examples have been defined in the context of disease or symbiosis and include such diverse processes as bioluminescence, antibiotic biosynthesis and resistance, production of EPS, swarming, plasmid conjugal transfer, and production of a variety of virulence determinants. Several studies have revealed the significance of quorum sensing in regulating the formation of biofilms (Davies et al. 1998; Huber et al. 2001; Fuqua and Greenberg 2002; Singh et al. 2000; ❯ *Fig. 1.6*). An especially fruitful lab model for understanding the possible role of quorum sensing has been the genetically tractable pathogen, *Pseudomonas aeruginosa*, which forms biofilms in the lungs of cystic fibrosis patients, marking the advancement of a serious and recalcitrant infection of the host. Mutations in the AHL receptors and gene regulation have shown that an active quorum sensing pathway is required for the proper development of the characteristic *P. aeruginosa* biofilm comprised of columns of cells embedded in a polysaccharide matrix with open channels between them. Mutants deficient in the production of the specific AHL formed a flat, undifferentiated biofilm. Thus, the more open biofilm structure, controlled at least in part by quorum sensing, presumably enhances nutrient exchange with the bulk liquid phase. The induction of biofilm formation is also associated with an increase in resistance to antibiotics.

Aggregated microbial communities, regulated in part through quorum sensing, are increasingly viewed as highly adaptive and resilient systems of organization. Exploration of the marine snow population revealed the production of AHLs by Roseobacter (Gram et al. 2002). Further work revealed that AHLs serve to regulate hydrolytic enzyme expression in these systems (Hmelo et al. 2011). While there have not been many studies of the possible environmental functions of quorum sensing, there is a growing consensus that this and other chemical signaling systems are a fundamental element of community structure and activity (Fuqua et al. 1996, 2001; Hastings and Greenberg 1999; Xavier and Bassler 2003).

Syntrophic Associations

The metabolic connections among different populations can range from highly specific to generic. An example of a more generic association may be simply the use of a vitamin or other nutrient released by several other cohabiting populations, so-called cross feeding. This type of interaction would not be expected to exert as much control over the organization of

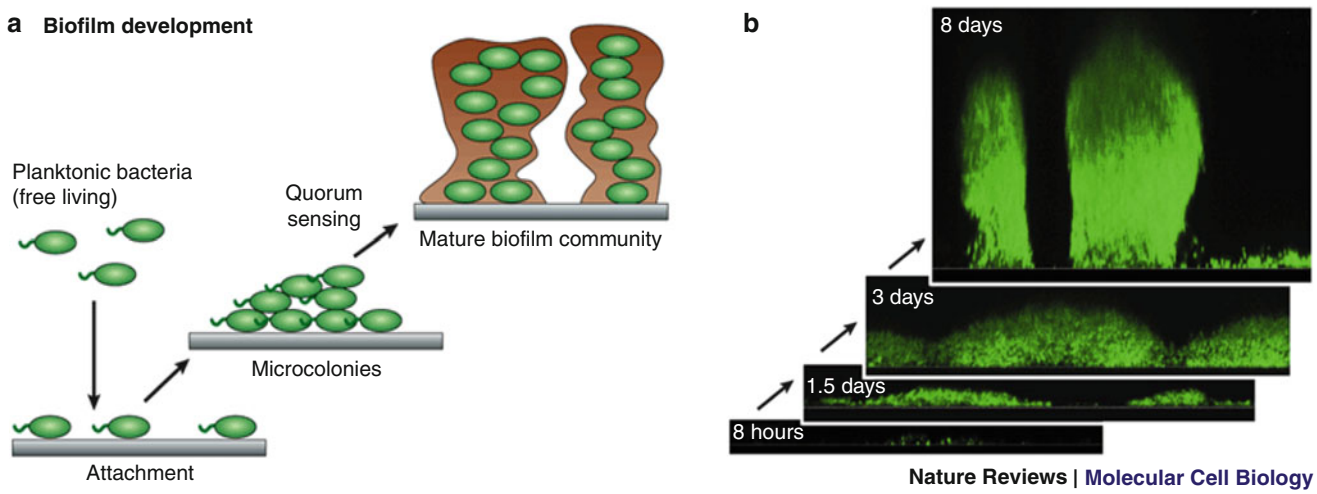


Fig. 1.6

Biofilm development and quorum sensing. (a) The steps involved in a biofilm development. (b) Confocal-microscope images of a *P. aeruginosa* biofilm developing over time on a microscope slide. The cells are producing green fluorescent protein. The mushroom- and towerlike structures that appear by 8 days are 100 μm high (The images in b were kindly provided by M. Welsh, P. Singh, and E.P. Greenberg at University of Washington, USA)

a microbial system or community as connections that specifically link energy recovery between two metabolic pathways. The best examples of systems structured by the parceling of available free energy among interacting populations are found in anaerobic habitats such as the sediment and mat systems described earlier. The anaerobic decomposition of organic material is carried out by the cooperation of several general physiological groups of microorganisms (Schink 1988). Although a variety of other functional assemblages are present in anaerobic habitats, for the purpose of discussing syntrophic associations in the metabolic dimension, only four main groups are considered here. In the absence of electron acceptors used for anaerobic respiration (e.g., oxyanions of sulfate and nitrate, and oxidized metals), the following four microbial groups are generally recognized to participate synergistically in the anaerobic degradation of organic matter: (1) hydrolytic and fermentative bacteria, which degrade complex biopolymers (e.g., plant cell wall components), monomers (sugars), and oligomers into acetate, hydrogen, carbon dioxide, and a mixture of short chain fatty acids, alcohols, succinate, and lactate; (2) proton-reducing acetogenic bacteria, which convert fatty acids, alcohols, succinate, and lactate to acetate, hydrogen, and carbon dioxide; (3) hydrogen-oxidizing methanogens, which convert hydrogen and carbon dioxide (as well as other quantitatively less important compounds) to methane and water; and (4) acetoclastic methanogens, which convert acetate into methane and carbon dioxide.

The complete mineralization of organic matter is dependent upon the cooperative growth of all four groups and is sustained by the microbially mediated (via methanogen groups 3 and 4) removal of hydrogen (and acetate). Interdependent growth (syntrophy) was first observed by Bryant and associates (Bryant et al. 1967, 1977; Wolin and Miller 1982). Notably, this discovery was a consequence of a failure of the pure culture technique. An organism thought capable of methanogenic growth on ethanol (*Methanobacillus omelianskii*) was found to be a syntrophic association of two species of prokaryotes. One bacterium oxidized ethanol to hydrogen, acetate, and CO_2 (group 2 type). The second was a hydrogenotrophic methanogen (group 3 type). Neither organism was capable of growth on ethanol alone. The energetic basis of this obligate association is the relationship between free energy and hydrogen concentration. The fermentation of ethanol is only favorable at low hydrogen concentrations (negative free energy). Thus, although the ethanol-oxidizing bacterium could initiate fermentation in a closed system (the test tube), hydrogen accumulation soon made this reaction energetically unfavorable. Enter the methanogen—its consumption of hydrogen permitted a continuous fermentation of ethanol. The methanogen was later formally described (*Methanobacterium bryantii*), but its syntrophic bacterial partner has since been lost.

Although interspecies hydrogen transfer was discovered in a closed system, the test tube, it is now recognized to determine higher order structure and activity in both natural and engineered systems (reviewed in Stams and Plugge 2009; McInerney et al. 2009). Establishment of a syntrophic association between hydrogenotrophic methanogens and H_2-evolving *Prevotella* in the human gut is hypothesized to facilitate fermentation of dietary fiber and has been suggested to contribute to obesity (Zhang et al. 2009). A dramatic macroscopic example of syntrophy is the granule structure that develops in anaerobic reactors designed to treat industrial waste streams (Sekiguchi et al. 1999). Within upflow anaerobic sludge blanket (UASB) reactors, the four functional assemblages promote the anaerobic degradation of organic industrial waste. These microbiota are retained in the reactor by formation of dense granules of

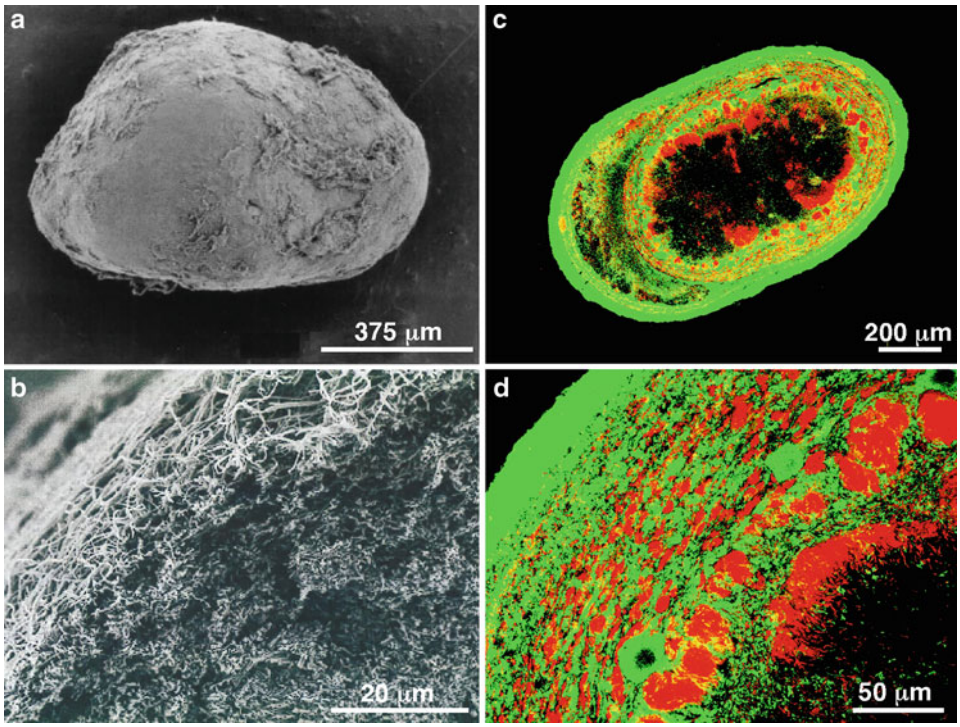


Fig. 1.7

Sections from mesophilic and thermophilic granules viewed by SEM and confocal laser scanning microscopy (CLSM). (a) SEM of surface of granule, (b) SEM of internal structure illustrating filaments and rods within layers of the granule, and (c) and (d) are sections simultaneously hybridized with Cy-5-labeled bacterial-domain probe (EUB338) (*green*) and rhodamine-labeled archaeal-domain probe (ARC915) (*red*) (Sekiguchi et al.1999)

millimetric size, their size controlled by balancing sedimentation with metered upward fluid flow in the reactor. Initial studies of the UASB granules using molecular probes to resolve the distribution revealed fine layering of population types, suggesting tight metabolic connections. The general architecture of each granule consists of a methanogenic core (*Archaea*), serving to consume hydrogen, CO_2, and acetate, surrounded by outer shells (layers) of fermentative bacteria (groups 1 and 2) that sustain the oxidation of the waste stream components such as propionate, sucrose, and acetate (*Fig. 1.7*).

In addition to promoting methane formation, syntrophic microbial alliances are also responsible for methane oxidation. For example, it has been estimated that the annual rate of methanogenesis in ocean sediments is about 85–300 $TgCH_4$ $year^{-1}$ (reviewed in Knittel and Boetius 2009). However, most (ca. 80–90%) of this biologically produced methane is oxidized anaerobically by other sediment microbiota. Anaerobic methane oxidation (AOM) is another example of a layered syntrophic association, generally consisting of an inner core of one of several novel and specialized groups of *Archaea* related to cultured *Methanosarcina* that is surrounded by sulfate-reducing bacteria (*Desulfosarcina-Desulfococcus* and *Desulfobulbus* groups; Knittel and Boetius 2009; Boetius et al. 2000; Orphan et al. 2001; Michaelis et al. 2002). These extremely slow-growing marine sediment consortia, coupling methane oxidation to sulfate reduction, are sustained near the energetic limits of life (ca. -11 kJ mol^{-1} at conditions typical of sediments) (Nauhaus et al. 2002). Although it was earlier hypothesized that the basis for this syntrophic association (like the layered granules described above) was methane oxidation to hydrogen and carbon dioxide, as yet, there is no evidence to support interspecies hydrogen exchange, or the exchange of other intermediates that might originate from a reversal of the methanogenesis pathway (Kruger et al. 2003; Nauhaus et al. 2002). For example, in the absence of added exogenous methane to a sediment sample obtained from a marine gas hydrate area, addition of hydrogen, formate, acetate, or methanol failed to sustain rates of sulfate reduction comparable to the addition of methane alone (Nauhaus et al. 2002). Another notable aspect of this globally significant metabolic interaction is the sharing of nitrogen fixed by the methane oxidizer with its sulfate-reducing partner (Dekas et al. 2009).

These relatively simple, but globally significant, syntrophic pairings are also very amenable to experimental study. Thus, an interaction first identified in the test tube is again being more fully characterized in defined laboratory systems. Defined laboratory cocultures ("synthetic communities") offer a reductionist format to more fully resolved the regulatory, metabolic, and evolutionary dimensions of microbial community structure (Conrad et al. 2011; Klitgord and Segre 2010; Stolyar et al. 2007). Studies of a simple laboratory pairing of a methanogen (*Methanothermobacter*) and bacterial syntroph (*Pelotomaculum*) revealed that flagella expressed by the bacterial partner initiate

both a close physical coupling and the expression of genes associated with syntrophic growth, suggesting coadapted mechanisms for fostering the formation of this partnership (Shimoyama et al. 2009). The pairing of a *Desulfovibrio* and a *Methanococcus* (growing as a syntrophic community of two in the absence of sulfate) was used as a basis to construct a metabolic model for syntrophic growth, identify an electron transfer pathway dedicated to syntrophy, and demonstrate the capacity for even simple communities to rapidly improve in stability and productivity through adaptive evolution (Stolyar et al 2007; Walker et al. 2009; Hillesland and Stahl 2010).

Symbiotic Systems

Multicellular eukaryotes originated in a microbial world. Thus, a complete understanding of the multicellular condition cannot be separated from pervasive and intimate associations with prokaryotes. The phenomenon of symbiosis was defined by De Bary (1879) as two dissimilar organisms living in close association ranging from the beneficial (mutualistic) to neutral (commensal) and pathogenic. Interactions between prokaryotes and their hosts have influenced evolution, development, and physiology of all multicellular life forms (Margulis and Fester 1991; McFall-Ngai 2001, 2002; McFall-Ngai et al. 2005). In turn, microbial interactions with multicellular eukaryotic hosts have had dramatic effects on evolution of prokaryotic physiology, structures, and genome architecture and content. The host habitat imposes evolutionary pressures, and close contact between microbes has stimulated exchange of essential genes. Recognition within the medical community that so-called normal flora of humans are important to both health and disease has resulted in large-scale efforts to understand the diversity of human microbiomes and their influence on human health and disease (Relman and Falkow 2001; Arumugam et al. 2011; Kau et al. 2011; Proctor 2011; Greenblum et al. 2012; Li et al. 2009). Growing acceptance that studies of mutualisms among nonhuman models contribute to our understanding of disease has led to symbiosis focus issues in leading high-impact journals (see Nature Reviews Microbiology Volume 6 2008 "Focus on symbiosis"). Over the past decade, studies of prokaryotic contributions to the evolution, development, and functioning of plant and animal systems has become a highly active area of investigation (Baumann 2005; Woyke et al. 2006; Herbert and Goodrich-Blair 2007; Dubilier et al. 2008; Moran et al. 2008; Ruby 2008; Werren et al. 2008; Hooper 2009; McInerney et al. 2009; Bright and Bulgheresi 2010; Gibson and Hunter 2010; Medina and Sachs 2010; Sachs et al. 2011).

In response to the need for microbiologists and animal/plant biologists to work in concert to enable progress in understanding the details of these interactions, a discipline of cellular microbiology was suggested in 1996 (Cossart et al. 1996) to encompass research devoted to characterizing bacterial interactions with eukaryotic cells. In 1999 the journal Cellular Microbiology published its first volume, initially with focus on pathogens, but later the scope expanded to include the benign and beneficial microorganisms. Today, most journals reporting research in microbiology include a section on host-microbe interactions: mutualistic, commensal, and pathogenic. Here, we focus our discussion on beneficial bacterial associations and emphasize that examples presented here are by no means the only or the most important associations, but are fairly well-studied systems that have provided insights into mechanisms.

Multicellular eukaryotic organisms form relationships with bacteria that span in complexity from hundreds of species comprising the populations of the mammalian gut to the simplified monospecific cultures in specialized organs such as root nodules of legumes and light organs of marine animals. Bacterial symbionts of eukaryotes confer unique abilities that enable their host to access resources that would otherwise be unavailable. Examples include the following: the chemoautotrophs within large worms, nematodes, bivalves, and shrimp living on sulfide or methane in a variety of marine habitats (Cary et al. 1997; Deming et al. 1997; Streams et al. 1997; Polz et al. 1998; Fisher 1990; Fujiwara et al. 2001; Woyke et al. 2006; Dubilier et al. 2008); the production of amino acids by the symbionts of sap-sucking insects (Baumann 2005; McCutcheon et al. 2009) to compensate for the missing amino acids in the diet; the degradation of cellulose and fixation of nitrogen by bacteria in shipworm bivalves and terrestrial termites to allow their hosts to live on wood (Douglas 2009; Breznak 1982; Waterbury et al. 1983; Lilburn et al. 2001; Distel et al. 2002); and the associations of cyanobacteria with a variety of fungi to fix carbon and nitrogen (lichens). The bacterial production of light (Hastings et al. 1987; Haygood 1993) by several marine predators enables either camouflage in the form of counter illumination, a source of light for hunting at night, or lures to attract prey (Hastings 1971; Hastings and Mitchell 1971; Morin et al. 1975; Young and Roper 1976). The production of toxins and antibiotics by bacteria (Kobayashi and Ishibashi 1993; Faulkner et al. 1994; Walls et al. 1995; Kaufman et al. 1998; Davidson et al. 2001) in sessile invertebrates, such as sponges and ascidians, or their eggs as for squid, allows them to avoid predation without the ability to escape. Likewise, insects use members of Streptomyces to help defend their burrows and food sources as in the predatory wasps, attine ants, and bark beetles (Currie et al. 1999, 2006; Scott et al. 2008; Brownlie and Johnson 2009; Schoenian et al. 2011; Kaltenpoth et al. 2012).

The activities of microbe-host associations influence the structure and function of entire ecosystems. The nitrogen-fixing *Rhizobium* spp. of legumes has allowed nitrogen-poor soils, not only to support plant growth, but also to become enriched in nitrogen. This has profound influence on agricultural practices and ecosystems. Likewise, the microbial community within the gut of termites enabling the breakdown and utilization of wood cellulose has shaped terrestrial ecosystems and may provide new enzymatic pathways enabling biofuel production (Brauman 2000; Brune and Friedrich 2000; Warnecke et al. 2007; Douglas 2009). Wood and leaf litter degradation and recycling by numerous arthropods (insects, isopods, and millipedes/centipedes) are enabled by cellulose-degrading microbe communities in the gut (Kukor and Martin 1986; Zimmer and Topp 1998; Zimmer and

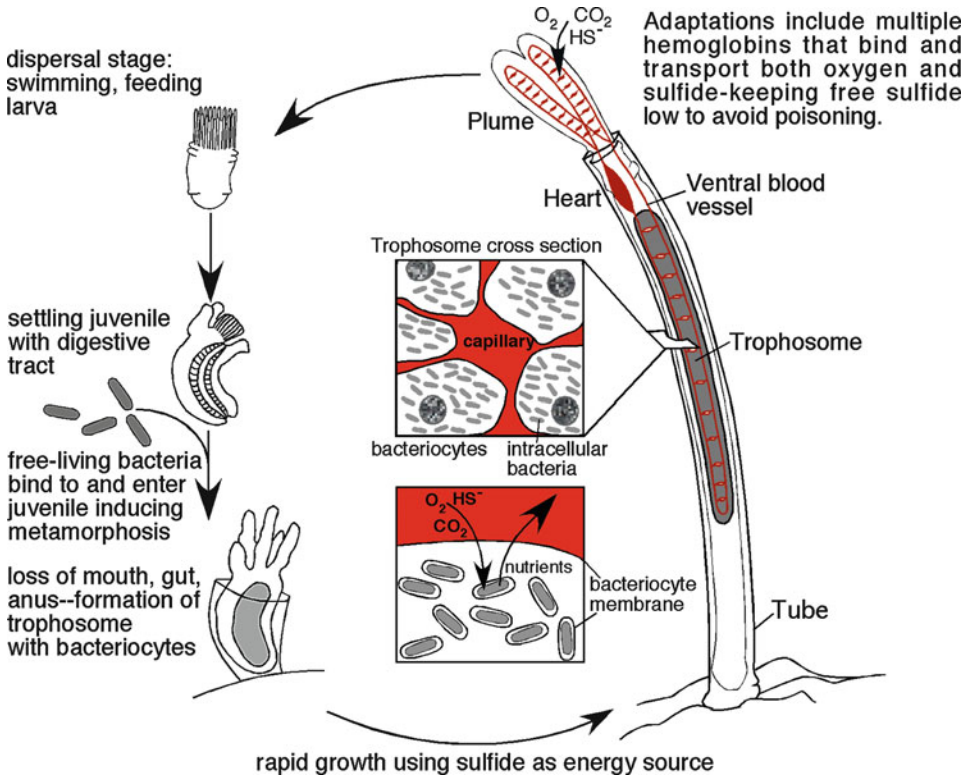


Fig. 1.8

Bacterial induction of morphological and biochemical adaptations in animals: Riftia pachyptila as an example. The dispersal stage resembles other annelid larvae, but upon acquisition of bacterial symbionts, the juvenile loses the digestive tract to form a specialized organ, the trophosome, that houses bacterial cells within host bacteriocytes. In addition to obvious morphological changes, the worm has evolved hemoglobin specialized for transport of both oxygen and sulfide (Childress et al. 1987; Southward 1999; Bright and Sorgo 2003; Nussbaumer et al. 2006)

Brune 2005; Douglas 2009). The leafcutter attine ants form extensive burrows and galleries to house their fungal gardens that are defended by the bioactive compounds produced by the ant Streptomyces symbionts (Schoenian et al. 2011; Currie et al. 1999). Extensive and diverse communities based solely on the energy gained by oxidation of reduced compounds by bacterial symbionts evolved in the deep sea at the hydrothermal vents (Fisher 1990; Dubilier et al. 2008). The activity of chemoautotrophic bacteria has taken the place of plants and photosynthesis as the base of the food chain. In all, a wide range of ecosystem services are provided by symbiotic systems that would not be possible by either the bacteria or the animal/plant alone.

This benefit of partnering with prokaryotes has led to mutual adaptations that necessarily involve adaptations to different stages of the host life cycle and alterations of host and symbiont to allow persistence of the association. Numerous examples exist of specific organs and even host body forms that have been altered over the evolution of the host-microbe relationship. Even subtly, as in the attine ants with a special cup in their exoskeleton to house the bacterial partner (Currie et al. 2006), prokaryotes have exerted evolutionary pressure for the eukaryotic form to adapt and maintain them. Especially, extreme modifications have occurred among annelids that house chemoautotrophic bacteria (Giere et al. 1998; Dubilier et al. 2001; Goffredi et al. 2004; Bright and Bulgheresi 2010). The vent annelid *Riftia pachyptila* has been profoundly modified from its ancestral form to take advantage of the capabilities of its bacterial partner. This annelid has replaced its entire digestive system (no mouth, gut, anus) with a specialized organ (the trophosome) harboring carbon-fixing sulfide-oxidizing bacteria (Reviewed in Fisher 1990, 1995) (*Fig. 1.8*). Early development of *Riftia* is similar to other marine annelids; swimming larvae are broadcast into the deep ocean and feed until they find a suitable settling site at a hydrothermal vent. These vents are ephemeral, and this association has adapted by allowing the juvenile host to be colonized by the local thioautotrophic strain of symbiont. The bacteria recognize, bind to, and invade the tissue of the developing worm, inducing a metamorphosis that results in the loss of the digestive tract (cell death) and formation of the trophosome, highly vascularized and packed with intracellular gamma-proteobacterial symbionts (Nussbaumer et al. 2006). A multihemoglobin system found in the red blood and coelomic fluid of these worms accomplishes the delivery of both oxygen and sulfide in a manner that keeps free sulfide levels low but supplies enough to maintain the demands of the symbiont (Fisher et al. 1988; Goffredi et al. 1997; Zal et al. 1998).

The free-living bacteria have been found at vents, and the roles they play in the ecosystem nutrient cycling outside of the tubeworms, or how they transition to an intracellular lifestyle, are not known (Harmer, et al. 2008).

The host environment in turn impacts the adaptations of the bacterial partner, which may live, divide, and grow either extracellularly (usually associated with specific tissue or organ systems) or intracellularly. There is a continuum of dependence on the partnership ranging from bacteria that are free-living and opportunistically colonize the host (i.e., *Vibrio fischeri*, the thioautotrophic *Riftia* symbiont, and *Rhizobium meliloti*) to obligate intracellular symbionts that are unable to live outside of host cells, having lost genes essential for independent function as a result of their lifestyle (Baumann et al. 1995; Douglas 1997; Werren 1997; Tamas et al. 2002; Baumann 2005). The smallest genomes (0.6–1.5 Mb) among bacteria are found in obligate intracellular parasites and symbionts (Casjens 1998; Moran et al. 2008). The severe reduction of genomes in *Buchnera* and *Wolbachia* is a well-studied example (Ochman and Moran 2001; Wilcox et al. 2003; Moran et al. 2008, 2009). The discovery of severely reduced genomes, such as *Carsonella* with only 160 kb, has challenged our concept of gene content requirements for cell survival (Spaulding and von Dohlen 1998; Nakabachi et al. 2006; Moran et al. 2008). Expanding genome sequence data sets have revealed a striking pattern of genome architecture changes that occur as bacteria become increasingly specific and dependent on their host (McCutcheon and Moran 2012). Initially, there is an expansion, as repetitive elements and pseudogenes accumulate. This accumulation of dysfunctional DNA is thought to be a result of decreased selective pressure to retain essential functions necessary for life outside the host. Because the host environment tends toward highly stable, and likely provides necessary nutrients, there is less regulation of genome content. However, in some ways, this idea contradicts the next stage of genome changes that involve the loss of the pseudogenes and large fragments, ending with a streamlined genome with limited metabolism and synthetic capabilities that serve the functions needed by the host.

Adaptations of microbe to host and vice versa must occur for various life stages of the host, not only in the mature association, because the free-living bacteria must gain entrance and find their location within their hosts. Early recruitment and colonization of a bacterial symbiont presents a challenge to both partners. The bacteria must evade, or tolerate, host immune defenses, move to and recognize the proper colonization site, and then persist without harming the host. In turn, the host must selectively recruit and encourage growth of its necessary partner without compromising its own health. Several systems have provided some understanding of the multiple levels of interaction involved in forming and sustaining a symbiotic association. Examples of well-established experimental systems include the *Vibrio*/squid and *Rhizobium*/legume symbioses (McFall-Ngai et al. 2012; Ruby 1999; Stougaerd 2000; Lum and Hirsch 2003). These have yielded insight into the biochemistry and genes involved in the initiation, negotiations, colonization, and persistence of the bacterial cells in the host. Key sets of characters that are important for colonization of metazoan and metaphytan hosts include motility, chemotaxis, adhesion, biofilm formation, and quorum sensing and defenses against the host innate immune system (Hirsch and McFall-Ngai 2000; Ruby 1999). Many of these features are also important in pathogenic associations and represent characters necessary for finding and persisting in a eukaryotic host. In *E. scolopes*, mechanisms preventing colonization by the wrong bacteria include innate immunity (e.g., oxidative stress produced by the host) and surface adhesins enabling only specific bacteria to enter and bind to host tissue (Weis et al. 1996; Small and McFall-Ngai 1999; Aeckersberg et al. 2001; Davidson et al. 2004; Nyholm and McFall-Ngai 2003, 2004; Nyholm et al. 2009). To avoid overgrowth, the squid has evolved a behavior of ejecting most of the bacteria at the end of the night, when light is no longer needed, and allowing the remaining 5% to grow up during the day while the squid is concealed in the sand. The ecological influence of localized increase in numbers of *Vibrio fischeri* in the water column is not known (Lee and Ruby 1994; Ruby and Lee 1998).

This field will continue to be enriched and enabled by development of additional model systems, offering comparative frameworks in which to study theme and variation in symbiotic systems. Only a handful of symbiotic systems have proven especially amenable to experimental study of cellular and molecular mechanisms, sharing the following critical characteristics: (1) the bacterial partner can be cultivated separately from the host, (2) the host can be studied without the symbiont, (3) genetic manipulation of the bacteria is possible, and (4) the intact life cycle (or at least early colonization events) can be maintained in the lab (reviewed in McFall-Ngai 2002; Seckbach 2002; Ruby 2008). There are exceptions to this, and much advancement in our understanding of obligate symbionts has been enabled by studies of aposymbiotic hosts, genomic and metagenomic analyses, and tissue culture (Douglas 1996; Wilkinson and Douglas 1996; Dubilier et al. 2001; Darby et al. 2005; Moran et al. 2008). Several systems that have become established as tractable experimental systems for study of bacterial associations with their hosts include *Photorhabdus* and *Xenorhabdus* in nematode hosts (Martens et al. 2003; Heungens et al. 2002; Vivas and Goodrich-Blair 2001), *Aeromonas*/leech (Braschler et al. 2003; Graf et al. 2006; Rio et al. 2007; Silver et al. 2007), *Verminephrobacter*/earthworm (Davidson and Stahl 2008; Pinel et al. 2008; Dulla et al. 2012; *Fig. 1.9*), and Zebra fish gut as model for both pathogen and mutualist interactions with the immune system and host developmental programs (Rawls et al. 2004; Bates et al. 2006; Cheesman and Guillemin 2007). Genetic examination of nematode symbionts *Xenorhabdus* spp. and *Photorhabdus* spp. has identified genes important for the association and the switch between the pathogenic phase in the insect prey and the mutualistic phase within the nematode host (Ffrench-Constant et al. 2003; Ciche et al. 2006; Goodrich-Blair and Clarke 2007).

Features that enable bacteria to associate stably with a eukaryotic host and maintain balance between mutualism and pathogenesis are still elusive. Elucidation and

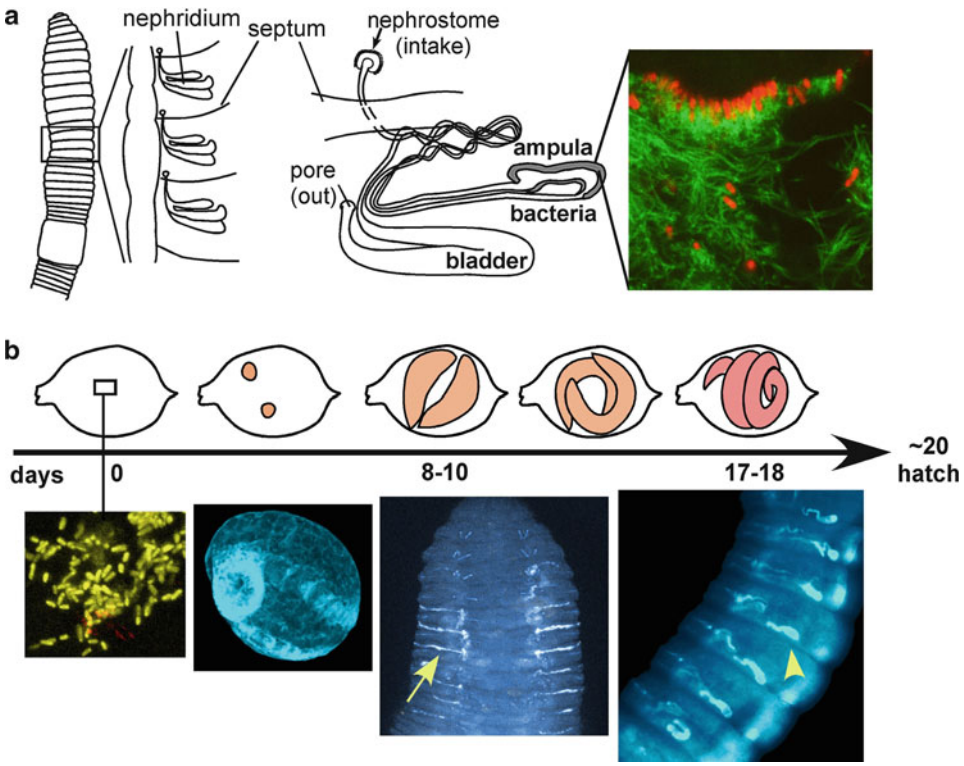


Fig. 1.9
Bacterial symbionts within the earthworm system. (a) Diagram of the location of nephridia and basic nephridial structure. Bacteria are found specifically bound to the surface within the ampulla of the nephridia and osmoregulatory organs of the earthworms. Fluorescence in situ hybridization image shows *Verminephrobacter eiseniae* (*red*) and a *Flexibacter*-related symbiont (*green*). (b) Developmental time line of embryo colonization by bacteria transmitted to the egg capsules by the parent earthworm. *Arrow* indicates labeled bacterial cells within a colonization canal. *Arrowhead* indicates the nephridia of a prehatchling worm

understanding of the complex genetic and biochemical systems involved is the challenge in symbiosis research. The developments in metagenomics have enabled researchers to mine these diverse animal-associated communities for novel enzymatic pathways and secondary metabolite pathways, providing both evidence for their microbial functions and potential for development of new technologies and therapeutics for treatment of human disease. With increasing numbers of available genome sequences, comparisons are possible that may reveal commonalties between bacteria able to associate with hosts and those that do not. The majority of sequenced genomes are from pathogens of humans or symbionts dependent on close interaction with a eukaryotic host (Ochman and Moran 2001). Although this is a bias reflective of the human desire to understand disease, it offers comparative opportunities to examine which genes confer the ability to associate stably with a eukaryotic host. Certainly, there are pathogenicity islands, sets of genes that confer virulence to normally nonpathogenic bacteria such as *E. coli.* However, there are also examples of symbiosis islands that confer the ability to invade and associate with the host in a positive way as in the Rhizobium-legume interaction (500 kb inserted at a tRNA locus in the plant symbiont *Mesorhizobium loti*) (Sullivan and Ronson 1998). The growing diversity of model systems with tools for detailed biochemical and genetic study of the interactions of prokaryotes with eukaryotic hosts is critical for, and will continue to deepen, our understanding of the balance between pathogenesis and mutualisms.

The Temporal System

The temporal dimension of microbial community structure and activity is determined by the complex interaction of physical, chemical, and biological parameters that often vary on regular daily, seasonal, interannual, and possibly decadal time scales. For example, in aquatic habitats, the seasonal stratification of the water column driven by temperature or salinity changes in water density influences nutrient distribution that ultimately alters microbial activity, community structure, and interactions with higher trophic levels. How the community is shaped by these episodic changes ultimately influences the productivity of the microbial community. Since microorganisms are the only biota capable of recovering the dilute organic substrates generally present in aquatic systems ("the microbial loop"; ❯ *Fig. 1.10*), their responses to predictable diel, seasonal, or annual variables are fundamental parameters governing the flux of carbon and energy in aquatic habitats (Pomeroy 1974; Azam et al. 1983; Williams 2000). The most obvious environmental variables that

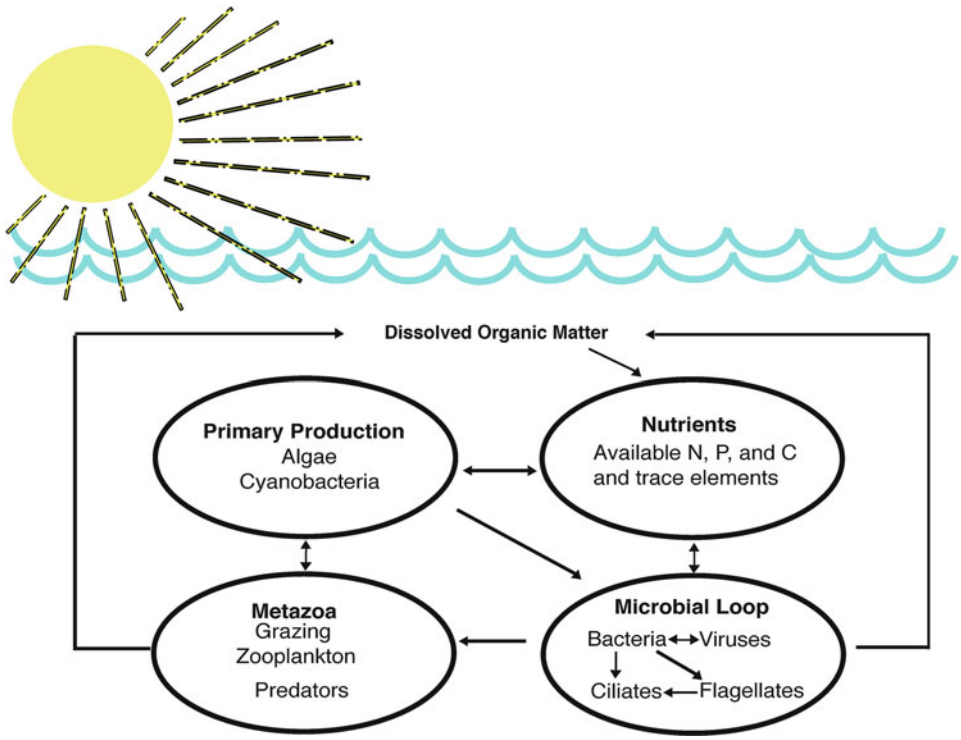


Fig. 1.10
Microbial loop of the upper water column showing the connections between different trophic levels (After Azam et al. 1983)

change on diel and seasonal cycles are light (>400 nm) and temperature (Giovannoni and Vergin 2012; Fuhrman et al. 2008, Fuhrman 2009; Murray et al. 1998; Pernthaler et al. 1998; Crump et al. 2003). Light positively influences primary production among cyanobacteria but also inflicts UV radiation damage and creates oxidative stress that reduces both primary and secondary production (Llabres and Agusti 2006; Garcia-Pichel et al. 1994; Herndl et al. 1993; Ramsing et al. 1997; Pakulski et al. 1998; Booth et al. 2001). Other variables influencing the seasonal distribution of bacterial species are nutrients (LeBaron et al. 2001; MacGregor et al. 2001; Ovreas et al. 2003; Simek et al. 2003), changes in organic matter quantity and quality (van Hannen et al. 1999a, b; Crump et al. 2009), abundance of grazers (Pernthaler et al. 2001; Simek et al. 2001a, 2003; Calbet 2008), and episodic viral lysis (van Hannen et al. 1999b; Suttle 2002, 2005, 2007; Rohwer and Thurber 2009; Fuhrman 2000; Hahn and Hofle 2001). Interannual climatic changes also impact the physical structure of the oceanic water column which influences nutrient cycling and leads to shifts in the microbial community structure. Resolving the key variables controlling microbial community structure and activity is also receiving greater attention because of ongoing climate change. For example, a greater average temperature is predicted to result in increasing isolation of marine surface waters from deeper water nutrients as a consequence of greater stratification (Behrenfeld et al. 2006).

In contrast to the increasingly well-documented annually reoccurring bacteria communities in oceanic and riverine systems, soil habits present much more variable patterns of periodicity. This variability is related primarily to geography and interactions of the soil microbiota with plants. For example, clear seasonal changes in microbial community structure were associated with snow cover melt in alpine soils (Lipson and Schmidt 2004). Seasonal changes in arbuscular mycorrhizal communities were shown to be correlated with changes in the flux of plant-derived carbon to root systems (Dumbrell et al. 2011). In contrast, microbial populations in a coastal grassland field in California appeared much more resilient to natural or imposed climate variation over a 7-year study period (Cruz-Martinez et al. 2009). Thus, we here restrict our discussion to a few examples from aquatic systems that illustrate the complex interaction of physics and biology on diel, seasonal, and greater time scales that influence microbial community structure and activity.

Diel Changes in Gene Expression

During daily fluctuations in light, temperature, and other physical parameters, there is potential for damage as well as the need to take advantage of optimal conditions for nutrient and energy acquisition. The ability to anticipate diel cycles of these parameters and adjust gene expression on a regular schedule may have an adaptive advantage. The most well-studied diel rhythm is the circadian clockwork, defining a periodicity of approximately (*circa* = about) 1 day (*dies* = day). First studied in eukaryotes, these rhythms are attributed to autoregulation of gene

expression in eukaryotic species. The unexpected recognition that some bacteria also display a true circadian rhythm was the discovery that nitrogen fixation in cyanobacteria is regulated by a circadian clock having maximum expression at night when oxygen levels are low and conditions then more favorable for the oxygen sensitive nitrogenase (Grobbelaar et al. 1986). A true circadian rhythm is defined by three properties: (1) persistence in the absence of environmental cues, the light-dark cycle, (2) temperature independence (the period length is only slightly altered by temperature change), and (3) the cycle can be reset (entrained) to a new environmental cycle of light and dark. The precision of the cycle in cyanobacteria is determined by a posttranslational clockwork ("nanoclockwork") composed of three proteins (KaiA, KaiB, and KaiC). Remarkably, an oscillating 24-h cycle of KaiC phosphorylation and dephosphorylation can be reconstituted in vitro in the absence of a light-day cycle with only these three proteins and ATP (Nakajima et al. 2005). This nanoclockwork is the primary cyanobacterial timekeeper, receiving input from a system of transcriptional regulation to mediate, for example, entrainment into a new light-dark cycle (Johnson et al. 2011). Although homologues of the Kai proteins are widely distributed among *Bacteria* and *Archaea*, those now recognized to function in a circadian rhythm are restricted to cyanobacteria (Dvornyk and Knudsen 2005). Synchrony of the endogenous clock and the environmental temporal cycle has been shown to increase the fitness of cyanobacteria (Ouyang et al. 1998).

Diel changes in expression also occur in direct response to an environmental variable rather than governed by a true circadian clockwork. For example, RecA expression occurs on a diel cycle but in response to DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) resulting from absorbance of light in the ultraviolet wavelength (as reviewed in Holm-Hansen et al. 1993; Mitchell and Kerntz 1993; Moran and Zepp 2000; and Jeffrey et al. 2000). The number of CPD lesions increases over the course of a day, causing mutations if not repaired. In response, bacterioplankton have coordinated RecA-mediated DNA repair and replication over the diel cycle (Herndl et al. 1993; Pakulski et al. 1998) with RecA protein induction peaking at dusk (Booth et al. 2001). Since the efficiency of DNA repair varies among bacterial species (Arrieta et al. 2006), diel fluctuation in UV is expected to influence the population structure of microbial communities in the euphotic zone of aquatic systems (Abboudi et al. 2008). These are just two examples of strategies employed by bacteria in response to environmental change on a diel scale and emphasize that activities and structure oscillate over time in often predictable patterns.

Seasonally Recurring Microbial Community Structure

The potential impact of extreme seasonal changes in chemical and physical variables on a microbial system is well illustrated by a study of the high-mountain lake Gossenkollesee (Pernthaler et al. 1998). Changes in light temperature, nutrients, and organic matter quantity and quality resulted in bacterial populations that were annually recurrent and seasonally variable. Stratification of the water column occurred from June through September with warming of the surface layers, followed by thermal mixing as temperatures cooled in November, and then ice covers throughout the winter and spring. Algae demonstrated a seasonal peak in productivity from December through February as an under-ice bloom (*Fig. 1.11a*) and was correlated with bacterial productivity (Sommaruga et al. 1997). During autumnal thermal mixing, the total microbial biomass declined (*Fig. 1.11a*) followed by a peak during December, once ice formation had occurred. In contrast, *Alcaligenes* sp. showed an annual maximum in November indicating that total biomass estimates often mask fluxes in individual populations (*Fig. 1.11b*). Following ice cover, succession continued to occur beneath the ice at temperatures ranging from 0 °C to 4.2 °C. For example, *Rhodoferax* spp. decreased at the onset of the under-ice algal bloom and then increased after the under-ice algae declined (*Fig. 1.11b*). During the ice-cover melt, bacterial populations responded to associated inputs of organic carbon from the melting ice, thermal mixing of the water column, and increased temperature. The tight coupling of seasonal variables and microbial population succession in these lake systems offers opportunities to analyze and better resolve the dynamic interactions between biology, chemistry, and physics in natural systems. Similar seasonal fluctuations have been for variety of other lake systems (reviewed in Newton et al. 2011) as well as in streams and rivers (Hullar et al. 2006; Crump et al. 2009; Findlay et al. 2008).

Ocean time-series studies are also revealing remarkable and predictable periodicity of microbial community structure and activity (reviewed in Fuhrman 2009; Giovannoni and Vergin 2012). Biotic inventories have been one component of these time-series data sets and have revealed predictable rhythmic seasonal oscillations in community structure using high-resolution molecular methods for fine-grained community analyses extending over multiple years. These studies include to date the 4.5-year San Pedro Ocean Time series (SPOTS), the 6-year western English Channel times series (Gilbert et al. 2010, 2012), the longer-term Bermuda Atlantic Time series (BATS) (Morris et al. 2005; Treusch et al. 2009), and the Hawaii Ocean Time series (HOT) (Karl and Lukas 1996; Karl 2010). The SPOTS study revealed monthly turnover in microbial community structure that is predictable on the basis of changing season and environmental conditions, including temperature and salinity, and abundances of chlorophyll, nutrients, viruses, and bacteria (Fuhrman 2009; Fuhrman et al. 2008). The BATS data set has clearly resolved three microbial communities, corresponding to the spring bloom (featuring eukaryotic picophytoplankton, marine Actinobacteria, and SAR11 and OCS116 clades), a summertime community associated with water column stratification and the formation of an upper euphotic zone (featuring *Pelagibacter*, *Puniceispirillum*, and SAR86 and SAR92 clades), and a deeper more stable upper mesopelagic community

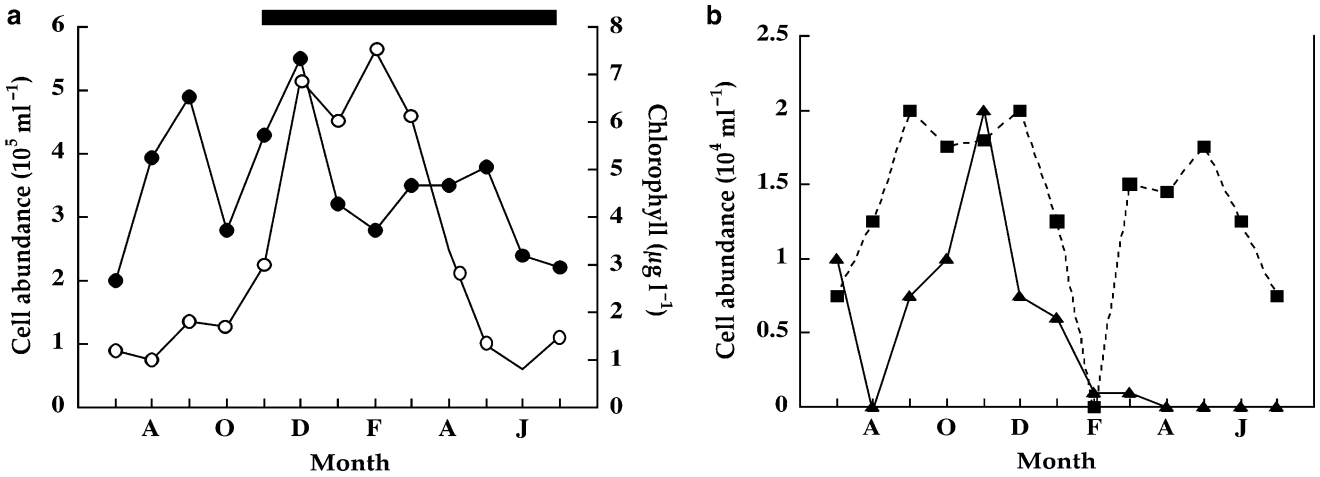


Fig. 1.11

Seasonal dynamics of primary producers and bacteria at 4 m in lake Gossenkollesee, an oligotrophic high-mountain lake in the Central Alps of Austria. (a) Se asonal fluctuation in chlorophyll a (○) and bacterial cells stained with 4′,6′-diamidino-2- phenylindole (DAPI) (●) at 4 m. The *horizontal bar* indicates the period of ice cover. (b) Population dynamics of cells hybridizing with probes GKS16 (Rhodoferax) (■) and GKS98 (Alcaligenes) (▲) (Pernthaler et al. 1998)

(featuring *Nitrosopumilus* and the SAR202, SAR324, SAR406, and SAR11 clades) (reviewed in Giovannoni and Vergin 2012). These long-term observations are beginning to provide data sets sufficiently large to resolve the relative influence of physical/chemical factors, as opposed to biotic interactions, on shaping microbial community composition and activity (Steele et al. 2011).

Interaction with higher trophic levels through grazing also influences the seasonal distribution of bacterial species in aquatic systems (as reviewed by Pernthaler 2005; Jurgens and Matz 2002). For example, a seasonal change in nutrient inputs can stimulate rapid increase of a heterotrophic microbial populations which in turn stimulate grazing and growth of protists in aquatic systems (Simek et al. 2003). Grazing by nanoflagellates and protists can be responsible for changes in morphological structure, physiological status of the bacteria (Posch et al. 1999), and taxonomic shifts in the bacterial populations (Hahn and Hofle 2001; Simek et al. 2001b) affecting overall cell number and secondary productivity (Simek et al. 2001b, 2003; Massana 2011).

Viruses also influence the temporal dynamics of community structure and function (as reviewed by Suttle 2005, 2007; Fuhrman 2000). While viral infections do not lead to extinction, they can shift the relative abundance and growth rates of bacteria on a seasonal time scale and are suggested to be relevant to better understand the impact of climate change on ocean function (Danovaro et al. 2011). Since viruses require contact with a host cell, the density of the bacterial cells affects the probability of infection. And since the more competitive and numerically dominant microorganisms are therefore more prone to attack and the rare escape attack, this scenario implies viruses influence the number of species that can coexist in a resource-limited environment (Fuhrman 1999, 2000). However, a study in which viral infection was alleviated using virus-depleted water resulted in large increases in naturally low abundance populations, implicating host characteristics and not simply host density, in viral-induced mortality (Bouvier and del Giorgio 2007). Phage immunity systems, for example, those associated with clustered regularly interspaced short palindromic repeats (CRISPRs), have more recently been recognized to shape virus and microbial population structure (Andersson and Banfield 2008; Avrani et al. 2011). Finally, viruses represent a vast reservoir of genetic diversity increasingly recognized to influence the gene content and diversity of cellular microbiota (Kettler et al. 2007).

Longer Time Scale Oscillations in Community Structure. The record of global sea surface temperature (SST) for the past 100 years has revealed interannual- to decade-scale variation in SST correlated with modes of climate variability. Although the drivers of the different modes and their phasing are mostly unknown, they are associated with large fluctuations in oceanic primary production (reviewed in Chavez et al. 2011). With increasing recognition of ongoing climate change, there is growing interest in how this will influence longer time scale oscillations in climate patterns and how, in turn, this alters the microbial activities in the oceans and soils globally. The "El Nino-Southern Oscillation" (ENSO) is the better understood of climatic modes, occurring every 3–8 years (Chavez et al. 2011; Wang and Fiedler 2006; Fiedler 2002). In non-ENSO years, the trade winds blow in an easterly direction along the equator resulting in increased sea surface height in the eastern Pacific (about 0.5 m). In the western equatorial Pacific, water upwells from the deep ocean, causing a decrease in sea surface temperature (about 8 °C) and inputs nutrients (NO_3) that fuel algal blooms in an otherwise nutrient-limited sea. During ENSO, the trade winds relax, reducing upwelling, nutrient

enrichment (NO_3), and primary productivity (Matsumoto and Furuya 2011; Le Bourgne et al. 2002). These changes then influence secondary productivity and food web structure. The warming of the upper surface in the western Pacific enhances thermal stratification, decreasing the influx of inorganic nutrients (NO_3 and Si) and selecting for N_2-fixing cyanobacteria (as opposed to Si-dependent diatoms). Thus, the gradual increase in sea and atmospheric temperatures has the potential to alter the time scale and intensity of the ENSO, along with other climatic cycles, influencing the oscillations in microbial ecosystems controlling major biogeochemical cycles (reviewed in Chavez et al. 2011: Philander and Fedorov 2003).

Concluding Thoughts

While the fundamental factors that influence population structure are beginning to be elucidated, the extensive diversity in both the environmental as well as biotic space still leaves us with a thus far limited view of these processes. Clearly, a multitude of factors including environmental conditions, horizontal gene transfer, selection, and predation at both the global and local scales influence the structure and function of microbial communities, as they have since the emergence of life on earth. The astounding diversity of microbial life being revealed by molecular characterization, a discovery process now being vastly accelerated by next-generation high-throughput sequencing, suggests that the reductionist paradigm so successfully applied to the study of microorganisms in pure culture will not enable a deeper understanding of the workings of complex environmental systems (Woese 2004). First, as emphasized in this chapter, only a limited spectrum of environmental conditions naturally experienced by an organism can be replicated in the laboratory. Thus, further development of methods for directly characterizing specific genetic, metabolic, and behavioral activities in the environment is needed to expand understanding of the essential biological units of selection (provisionally a microbial "species") and how environment and biology together shape community structure and organismal function. Secondly, organisms are small, and their diversity and activity must be studied by characterizing relatively small samples. In contrast, their impacts are global, for example, controlling or influencing the export of carbon to the deep ocean and altering atmospheric chemistry. Thus, the development of more predictive models will also be an essential tool for untangling the feedbacks and complex interconnections between ecology, biogeochemistry, and physiological diversity. In this regard, the development of diversity-based modeling is an attractive advance in modeling approaches (Follows and Dutkiewicz 2011). Briefly, this approach seeds a model environment with a set of virtual organisms; each organism randomly assigned a physiological profile consistent with the range of traits established within a class of organisms (e.g., cyanobacteria). The system is then allowed to evolve in silico. Relative fitness in relation to the recent history of the local environment leads to selection for a particular subset of physiologies. Other physiologies are excluded in that environment but may be fitter in other regions or seasons. Thus, there is an emergent ecosystem structure and function that, in turn, modifies the environment (*Fig. 1.12*). This approach has been remarkably successful in predicting the environmental distribution of marine cyanobacterial species that closely match the physiological types verified by direct sampling (Barton et al. 2010; Follows et al. 2007).

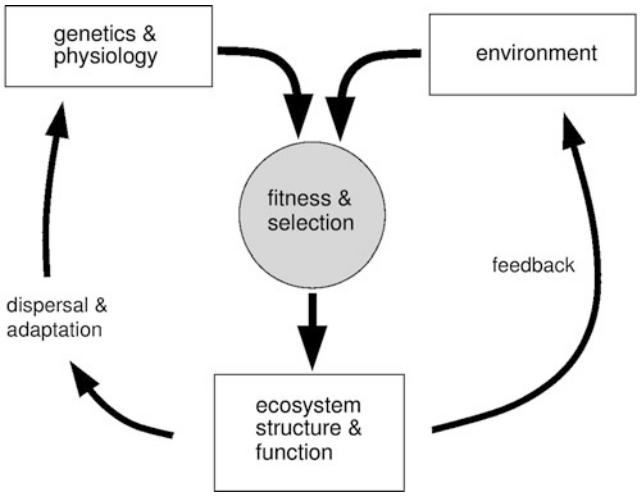


Fig. 1.12
Diagram of biological and environmental feedbacks employed in diversity-based modeling to predict microbial community structure. Following the seeding of a modeled environment with a set of virtual organisms, each representing the range of physiological features characteristic of a specific functional guild (e.g., substrate affinities or optimum growth temperatures of cyanobacterial species), their fitness in relation to the recent history of the local environment leads to selection for a particular subset of physiologies. Other physiologies are excluded in that environment but may be fitter in other regions or seasons. Over time, there is an emergent ecosystem structure and function that, in turn, modifies the environment (Figure courtesy of Mick Follows, as adapted from Follows and Dutkiewicz 2011)

Acknowledgments

We are grateful to grants from NSF Oceanography (OCE-0623174) and Molecular and Cellular Biosciences (MCB-0604448 & MCB-0920741); the dimensions of biodiversity program in Biological Oceanography (OCE-1046017); ENIGMA, a scientific focus area program supported by the US Department of Energy, Office of Science, and Office of Biological and Environmental Research; and Genomics: GTL Foundational Science through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US Department of Energy for partial support of the studies reported in this chapter.

References

Abboudi M, Jeffrey WH, Ghiglione JF, Pujo-Pay M, Oriol L, Sempere R, Charriere B, Joux F (2008) Effects of photochemical transformations of dissolved organic matter on bacterial metabolism and diversity in three contrasting coastal sites in the Northwestern Mediterranean sea during summer. Microb Ecol 55:344–357

Aeckersberg F, Lupp C, Feliciano B, Ruby EG (2001) *Vibrio fischeri* outer membrane protein OmpU plays a role in normal symbiotic colonization. J Bacteriol 183:6590–6597

Alldredge AL, Cohen Y (1987) Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. Science 235: 689–691

Alldredge AL, Silver MW (1988) Characteristics, dynamics and significance of marine snow. Prog Oceanogr 20:41–82

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in-situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Amann R, Ludwig W (2000) Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiol Rev 24:555–565

Andersson AF, Banfield JF (2008) Virus population dynamics and acquired virus resistance in natural microbial communities. Science 320:1047–1050

Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM et al (2011) Enterotypes of the human gut microbiome. Nature 473:174–180

Avrani S, Wurtzel O, Sharon I, Sorek R, Lindell D (2011) Genomic island variability facilitates Prochlorococcus-virus coexistence. Nature 474: 604–608

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

Bahr M, Hobbie JE, Sogin ML (1996) Bacterial diversity in an Arctic lake: a freshwater SAR11 cluster. Aquat Microb Ecol 11:271–277

Bamford CV, D'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF (2009) Streptococcus gordonii modulates *Candida albicans* biofilm formation through intergeneric communication. Infect Immun 77:3696–3704

Barton AD, Dutkiewicz S, Flierl G, Bragg J, Follows MJ (2010) Patterns of diversity in marine phytoplankton. Science 327:1509–1511

Bates JM, Mittge E et al (2006) Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Dev Biol 297:374–386

Baumann P (2005) Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu Rev Microbiol 59:155–189

Baumann P, Baumann L, Lai CY, Roubakhsh D, Moran NA, Clark MA (1995) Genetics, physiology, and evolutionary relationships of the genus Buchnera intracellular symbionts of aphids. Annu Rev Microbiol 49:55–94

Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, Milligan AJ, Falkowski PG, Letelier RM, Boss ES (2006) Climate-driven trends in contemporary ocean productivity. Nature 444:752–755

Beijerinck MW (1913) De infusies en de ontdekking der backteriën. In Jaarboek van de Koninklijke Akademie v. Wetenschappen. Amsterdam, Müller

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

Blackburn N, Fenchel T (1999) Influence of bacteria, diffusion and sheer on microscale nutrient patches, and implications for bacterial chemotaxis. Mar Ecol Prog Ser 189:1–7

Blackburn N, Fenchel T, Mitchell J (1998) Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science 282:2254–2256

Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Booth MG, Jeffrey WH, Miller RV (2001) RecA expression in response to solar UVR in the marine bacterium *Vibrio natriegens*. Microb Ecol 42:531–539

Bouvier T, del Giorgio PA (2007) Key role of selective viral-induced mortality in determining marine bacterial community composition. Environ Microbiol 9:287–297

Braschler TR, Merino S, Tomas JM, Graf J (2003) Complement resistance is essential for colonization of the digestive tract of *Hirudo medicinalis* by Aeromonas strains. Appl Environ Microbiol 69:4268–4271

Brauman A (2000) Effect of gut transit and mound deposit on soil organic matter transformations in the soil feeding termite: a review. Eur J Soil Biol 36:117–125

Breznak JA (1982) Intestinal microbiota of termites and other xylophagous insects. Annu Rev Microbiol 36:323–343

Bright M, Bulgheresi S (2010) A complex journey: transmission of microbial symbionts. Nat Rev Microbiol 8:218–230

Bright M, Sorgo A (2003) Ultrastructural reinvestigation of the trophosome in adults of *Riftia pachyptila* (Annelida, Siboglinidae). Invertebr Biol 122:347–368

Brochier-Armanet C, Gribaldo S, Forterre P (2012) Spotlight on the thaumarchaeota. ISME J 6:227–230

Brownlie JC, Johnson KN (2009) Symbiont-mediated protection in insect hosts. Trends Microbiol 17:348–354

Brune A, Friedrich M (2000) Microecology of the termite gut: structure and function on a microscale. Curr Opin Microbiol 3:263–269

Brusseau GA, Rittmann BE, Stahl DA (1998) Addressing the microbial ecology of marine biofilms. In: Cooksey KE (ed) Molecular approaches to the study of the ocean. Chapman and Hall, New York

Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*: a symbiotic association of two species of bacteria. Arch Microbiol 59:20–31

Bryant MP, Campbell LL, Reddy CA, Crabill MR (1977) Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl Environ Microbiol 33:1162–1169

Buttner M, Xie DL, Nelson H, Pinther W, Hauska G, Nelson N (1992) Photosynthetic reaction center genes in green sulfur bacteria and in photosystem-1 are related. Proc Natl Acad Sci 89:8135–8139

Button DK (1994) The physical base of marine bacterial ecology. Microb Ecol 28:273–285

Calbet A (2008) The trophic roles of microzooplankton in marine systems. Ices J Marine Sci 65:325–331

Canfield DE, DeMarais DJ (1991) Aerobic sulfate reduction in microbial mats. Science 251:1471–1473

Canfield DE, Marais DJD (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim Cosmochim Acta 57:3971–3984

Cantalupo PG, Calgua B, Zhao GY, Hundesa A, Wier AD, Katz JP, Grabe M, Hendrix RW, Girones R, Wang D, Pipas JM (2011) Raw sewage harbors diverse viral populations. Mbio 2

Carlson CA, Morris R, Parsons R, Treusch AH, Giovannoni SJ, Vergin K (2009) Seasonal dynamics of SAR11 populations in the euphotic and mesopelagic zones of the northwestern Sargasso Sea. ISME J 3:283–295

Cary SC, Cottrell MT, Stein JL, Camacho F, Desbruyeres D (1997) Molecular identification and localization of filamentous symbiotic bacteria associated with the hydrothermal vent annelid *Alvinella pompejana*. Appl Environ Microbiol 63:1124–1130

Casamayor EO, Pedros-Alio C, Muyzer G, Amann R (2002) Microheterogeneity in 16 S ribosomal DNA defined bacterial populations from a stratified planktonic environment is related to temporal changes and to ecological adaptations. Appl Environ Microbiol 68:1706–1714

Casjens S (1998) The diverse and dynamic structure of bacterial genomes. Annu Rev Genet 32:339–445

Characklis WG, Marshall KC (1990) Biofilms. Wiley, New York

Chavez FP, Messie M, Pennington JT (2011) Marine primary production in relation to climate variability and change. Ann Rev Marine Sci 3:227–260

Cheesman SE, Guillemin K (2007) We know you are in there: conversing with the indigenous gut microbiota. Res Microbiol 158:2–9

Chen L, Liu MY, Legall J, Fareleira P, Santos H, Xavier AV (1993) Rubredoxin oxidase, a new flavo-hemo-protein, is the site of oxygen reduction to water by the strict anaerobe desulfovibrio-gigas. Biochem Bioph Res Commun 193:100–105

Childress JJ, Felbeck H, Somero GN (1987) Symbiosis in the deep-sea. Sci Am 256:114–130

Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the oceans interior. Nature 332:441–443

Ciche TA, Darby C et al (2006) Dangerous liaisons: the symbiosis of entomopathogenic nematodes and bacteria. Biol Control 38:22–46

Cohen Y, Rosenberg E (1989) Microbial mats: physiological ecology of benthic microbial communities. American Society of Microbiology, Washington, DC

Coleman ML, Sullivan MB, Martiny AC, Steglich C, Barry K, DeLong EF, Chisholm SW (2006) Genomic islands and the ecology and evolution of Prochlorococcus. Science 311:1768–1770

Conrad TM, Lewis NE, Palsson BO (2011) Microbial laboratory evolution in the era of genome-scale science. Mol Syst Biol 7

Cossart P, Boquet P, Normark S, Rappuoli R (1996) Cellular microbiology emerging. Science 271:315–316

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69:2253–2268

Crump BC, Peterson BJ, Raymond PA, Amon RMW, Rinehart A, McClelland JW, Holmes RM (2009) Circumpolar synchrony in big river bacterioplankton. Proc Natl Acad Sci USA 106:21208–21212

Cruz-Martinez K, Suttle KB, Brodie EL, Power ME, Andersen GL, Banfield JF (2009) Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME J 3:738–744

Currie CR, Scott JA, Summerbell RC, Malloch D (1999) Fungus growing ants use antibiotic producing bacteria to control garden parasites. Nature 398: 701–704

Currie CR, Poulsen M et al (2006) Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Science 311:81–83

Danovaro R, Corinaldesi C, Dell'Anno A, Fuhrman JA, Middelburg JJ, Noble RT, Suttle CA (2011) Marine viruses and global climate change. FEMS Microbiol Rev 35:993–1034

Darby AC, Chandler SM et al (2005) Aphid-symbiotic bacteria cultured in insect cell lines. Appl Environ Microbiol 71:4833–4839

Darwin C (1969) On the origin of species: facsimile edition. Harvard University Press, Cambridge, MA

Daubin V, Moran NA, Ochman H (2003) Phylogenetics and the cohesion of bacterial genomes. Science 301:829–832

Davidson SK, Allen SW, Lim GE, Anderson CM, Haygood MG (2001) Evidence for the biosynthesis of bryostatins by the bacterial symbiont Candidatus endobugula sertula of the bryozoan *Bugula neritina*. Appl Environ Microbiol 67:4531–4537

Davidson SK, Koropatnick TA, Kossmehl R, Sycuro L, McFall-Ngai MJ (2004) No means 'yes' in the squid-vibrio symbiosis: nitric oxide during the initial stages of a beneficial association. Cell Microbiol 6:1139–1151

Davidson SK, Stahl DA (2008) Selective recruitment of bacteria during embryogenesis of an earthworm. ISME J 2:510–518

Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298

De Bary A (1879) Die erscheinumg der symbiose: Vortrag gehalten auf der Versammlung Deutscher Naturforscher und Aerzte zu Cassel. Verlag von Karl J, Trübner, Strasburg

Decho AW, Herndl GJ (1995) Microbial activities and the transformation of organic matter within mucilaginous material. Sci Total Environ 165: 33–42

Dekas AE, Poretsky RS, Orphan VJ (2009) Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326:422–426

Delong EF, Pace NR (2001) Environmental diversity of Bacteria and Archaea. Syst Biol 50:470–481

Delong EF, Frankel RB, Bazylinski DA (1993) Multiple evolutionary origins of magnetotaxis in bacteria. Science 259:803–806

Delong EF, Wu KY, Prézelin BB, Jovine RVM (1994) High abundance of archaea in Antarctic marine picoplankton. Nature 371:695–697

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martinez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496–503

Deming JW, Reysenbach AL, Macko SA, Smith CR (1997) Evidence for the microbial basis of a chemoautotrophic invertebrate community at a whale fall on the deep seafloor: bone colonizing bacteria and invertebrate endosymbionts. Microsc Res Techniq 37:162–170

Dermott R, Legner M (2002) Dense mat-forming bacterium *Thioploca ingrica* (Beggiatoaceae) in eastern Lake Ontario. Implications to the benthic food web. J Great Lakes Res 28:688–697

Devereux R, Delaney M, Widdel F, Stahl DA (1989) Natural relationships among sulfate reducing eubacteria. J Bacteriol 171:6689–6695

Devereux R, He SH, Doyle CL, Orkland S, Stahl DA, Legall J, Whitman WB (1990) Diversity and origin of desulfovibrio specie: phylogenetic definition of a family. J Bacteriol 172:3609–3619

Dillon JG, Miller S, Bebout B, Hullar M, Pinel N, Stahl DA (2009) Spatial and temporal variability in a stratified hypersaline microbial mat community. FEMS Microbiol Ecol 68:46–58

Distel DL, Beaudoin DJ, Morrill W (2002) Coexistence of multiple proteobacterial endosymbionts in the gills of the wood-boring bivalve *Lyrodus pedicellatus* (Bivalvia: Teredinidae). Appl Environ Microbiol 68:6292–6299

Dobrindt U, Agerer F, Michaelis K, Janka A, Buchrieser C, Samuelson M, Svanborg C, Gottschalk G, Karch H, Hacker J (2003) Analysis of genome plasticity in pathogenic and commensal *Escherichia coli* isolates by use of DNA arrays. J Bacteriol 185:1831–1840

Doolittle WF (1999) Phylogenetic classification and the universal tree. Science 284:2124–2128

Douglas AE (1996) Reproductive failure and the free amino acid pools in pea aphids (*Acyrthosiphon pisum*) lacking symbiotic bacteria. J Insevt Physiol 42:247–255

Douglas AE (1997) Parallels and contrasts between symbiotic bacteria and bacterial-derived organelles: evidence from Buchnera, the bacterial symbiont of aphids. FEMS Microbiol Ecol 24:1–9

Douglas AE (2009) The microbial dimension in insect nutritional ecology. Funct Ecol 23:38–47

Dubilier N, Mulders C et al (2001) Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature 411:298–302

Dubilier N, Bergin C et al (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat Rev Microbiol 6:725–740

Dulla G, Go R et al (2012) Verminephrobacter eiseniae type IV pili and flagella are required to colonize earthworm nephridia. ISME J 6:1166–1175

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 190:794–804

Dvornyk V, Knudsen B (2005) Functional divergence of the circadian clock proteins in prokaryotes. Genetica 124:247–254

Elena SF, Lenski RE (2003) Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. Nat Rev Genet 4:457–469

Faulkner J, Unson MD, Bewley CA (1994) The chemistry of some sponges and their symbionts. Pure Appl Chem 66:1983–1990

Fenchel T, Finlay BJ (1995) Ecology and evolution in anoxic worlds. Oxford University Press, Oxford

Fenchel T, Glud RN (1998) Veil architecture in a sulphide-oxidizing bacterium enhances countercurrent flux. Nature 394:367–369

Fenchel T (2002) Microbial behavior in a heterogeneous world. Science 296:1068–1071

Ferdelman TG, Lee C, Pantoja S, Harder J, Bebout BM, Fossing H (1997) Sulfate reduction and methanogenesis in a thioploca dominated sediment off the coast of Chile. Geochim Cosmochim Acta 61:3065–3079

Ffrench-Constant R, Waterfield N, Daborn P, Joyce S, Bennett H, Au C, Dowling A, Boundy S, Reynolds S, Clarke D (2003) Photorhabdus: towards a functional genomic analysis of a symbiont and pathogen. FEMS Microbiol Rev 26:433–456

Fiedler PC (2002) Environmental change in the eastern tropical Pacific Ocean: review of ENSO and decadal variability. Mar Ecol Prog Ser 244:265–283

Field KG, Gordon D, Wright T, Rappe M, Urbach E, Vergin K, Giovannoni SJ (1997) Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. Appl Environ Microbiol 63:63–70

Findlay RH, Yeates C, Hullar MA, Stahl DA, Kaplan LA (2008) Biome-level biogeography of streambed microbiota. Appl Environ Microbiol 74: 3014–3021

Fisher CR, Childress JJ, Sanders NK (1988) The role of Vestimentiferan hemoglobin in providing an environment suitable for chemoautotrophic sulfide-oxidizing endosymbionts. Symbiosis 5:229–246

Fisher CR (1990) Chemoautotrophic and methanotrophic symbioses in marine-invertebrates. Rev Aquat Sci 2:399–436

Fisher CR (1995) Towards and appreciation of hydrothermal-vent animals: their environment, physiological ecology, and tissue stable isotope values. In: Humphris SE, Zierenberg RA, Multineaux LS, Thompson RE (ed) Seafloor hydrothermal systems: physical, chemical, biological, and geological interactions. AGU, Washington DC, pp 297–316

Follows MJ, Dutkiewicz S (2011) Modeling diverse communities of marine microbes. Ann Rev Mar Sci 3(3):427–451

Follows MJ, Dutkiewicz S, Grant S, Chisholm SW (2007) Emergent biogeography of microbial communities in a model ocean. Science 315:1843–1846

Fossing H, Gallardo VA, Jorgensen BB, Huttel M, Nielsen LP, Schulz H, Canfield DE, Forster S, Glud RN, Gundersen JK, Kuver J, Ramsing NB, Teske A, Thamdrup B, Ulloa O (1995) Concentration and transport of nitrate by the mat-forming sulfur bacterium Thioploca. Nature 374: 713–715

Fuhrman JA, McCallum K, Davis AA (1992) Novel major archaebacterial group from marine plankton. Nature 356:148–149

Fuhrman JA, Mccallum K, Davis AA (1993) Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl Environ Microbiol 59:1294–1302

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Fuhrman JA (2000) Impact of viruses on bacterial processes. In: Kirchman DL (ed) Microbial ecology of the sea. Wiley-Liss, New York, pp 327–350

Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown MV, Green JL, Brown JH (2008) A latitudinal diversity gradient in planktonic marine bacteria. Proc Natl Acad Sci USA 105:7774–7778

Fuhrman JA (2009) Microbial community structure and its functional implications. Nature 459:193–199

Fujiwara Y, Kato C, Masui N, Fujikura K, Kojima S (2001) Dual symbiosis in the cold seep thyasirid clam Maorithyas hadalis from the hadal zone in the Japan Trench, western Pacific. Mar Ecol-Prog Ser 214:151–159

Fukui M, Teske A, Assmus B (1999) Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus Desulfonema). Arch Microbiol 172:193–203

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density responsive transcriptional regulators. J Bacteriol 176:269–275

Fuqua C, Winans SC, Greenberg EP (1996) Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu Rev Microbiol 50:727–751

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Fuqua C, Greenberg EP (2002) Listening in on bacteria: acyl-homoserine lactone signaling. Nature Rev Mole Cell Biol 3:685–695

Gallardo CS (1977) Two modes of development in morph-species Crepidula dilatata (Gastropoda: Calyptraeidae) from Southern Chile. Mar Biol 39:241–251

Garcia-Pichel F, Mechling M, Castenholz RW (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl Environ Microbiol 60:1500–1511

Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, Duthoy S, Grondin S, Lacroix C, Monsempe C, Simon S, Harris B, Atkin R, Doggett J, Mayes R, Keating L, Wheeler PR, Parkhill J, Barrell BG, Cole ST, Gordon SV, Hewinson RG (2003) The complete genome sequence of *Mycobacterium bovis*. Proc Natl Acad Sci USA 100:7877–7882

Gibson CM, Hunter MS (2010) Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. Ecol Lett 13:223–234

Giere O, Erseus C et al (1998) A new species of olavius (Tubificidae) from the Algarve coast in Portugal, the first East Atlantic gutless oligochaete with symbiotic bacteria. Zool Anz 237:209–214

Gilbert JA, Field D, Swift P, Thomas S, Cummings D, Temperton B, Weynberg K, Huse S, Hughes M, Joint I, Somerfield PJ, Muhling M (2010) The taxonomic and functional diversity of microbes at a temperate coastal site: a 'multi-omic' study of seasonal and diel temporal variation. Plos one 5(11): e15545. doi:10.1371/journal.pone.0015545

Gilbert JA, Steele JA, Caporaso JG, Steinbrueck L, Reeder J, Temperton B, Huse S, McHardy AC, Knight R, Joint I, Somerfield P, Fuhrman JA, Field D (2012) Defining seasonal marine microbial community dynamics. ISMEJ 6:298–308

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 345:60–63

Giovannoni SJ, Cary SC (1993) Probing marine systems with ribosomal RNAs. Oceanography 6:95–104

Giovannoni SJ, Rappe MS, Vergin KL, Adair NL (1996) 16 S rRNA genes reveal stratified open ocean bacterioplankton populations related to the Green Non-Sulfur bacteria. Proc Natl Acad Sci USA 93:7979–7984

Giovannoni SJ, Vergin KL (2012) Seasonality in ocean microbial communities. Science 335:671–676

Glockner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl Environ Microbiol 65:3721–3726

Goffredi SK, Childress JJ, Desaulniers NT, Lallier FH (1997) Sulfide acquisition by the vent worm *Riftia pachyptila* appears to be via uptake of HS-, rather than H_2S. J Exp Biol 200:2609–2616

Goffredi SK, Paull CK et al (2004) Unusual benthic fauna associated with a whale fall in Monterey Canyon. Deep-Sea Res Pt I 51:1295–1306, California

Goodrich-Blair H, Clarke DJ (2007) Mutualism and pathogenesis in Xenorhabdus and Photorhabdus: two roads to the same destination. Mol Microbiol 64:260–268

Graf J, Kikuchi Y et al (2006) Leeches and their microbiota: naturally simple symbiosis models. Trends Microbiol 14:365–371

Gram L, Grossart HP, Schlingloff A, Kiorboe T (2002) Possible quorum sensing in marine snow bacteria: production of acylated homoserine lactones by Roseobacter strains isolated from marine snow. Appl Environ Microbiol 68:4111–4116

Greenblum S, Turnbaugh PJ et al (2012) Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci USA 109:594–599

Grobbelaar N, Huang T-C, Lin H-Y, Chow T-J (1986) FEMS Microbiol Lett 37:173–177

Grotzinger JP, Knoll AH (1999) Stromatolites in precambrian carbonates: evolutionary mileposts or environmental dipsticks? Ann Rev Earth Planet Sci 27:313–358

Hahn MW, Hofle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113–121

Harmer TL, Rotjan RD et al (2008) Free-living tube worm endosymbionts found at deep-sea vents. Appl Environ Microbiol 74:3895–3898

Hastings JW (1971) Light to hide by – ventral luminescence to camouflage silhouette. Science 173:1016

Hastings JW, Mitchell G (1971) Endosymbiotic bioluminescent bacteria from light organ of pony fish. Biol Bull 141:261–268

Hastings JW, Makemson J, Dunlap PV (1987) Are growth and luminescence regulated independently in light organ symbionts? Symbiosis 4:3–24

Hastings JW, Greenberg EP (1999) Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria. J Bacteriol 181:2667–2668

Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M, Shinagawa H (2001) Complete genome sequence of enterohemorrhagic *Escherichia coli* O157: H7 and genomic comparison with a laboratory strain K-12. DNA Res 8:11–22

Haygood MG (1993) Light organ symbioses in fishes. Crit Rev Microbiol 19:191–216

Head IM, Hiorns WD, Embley TM, McCarthy AJ, Saunders JR (1993) The phylogeny of autotrophic ammonia oxidizing bacteria as determined by analysis of 16 s ribosomal-RNA gene-sequences. J Gen Microbiol 139:1147–1153

Heidelberg KB, Gilbert JA, Joint I (2010) Marine genomics: at the interface of marine microbial ecology and biodiscovery. Microb Biotechnol 3:531–543

Herbert EE, Goodrich-Blair H (2007) Friend and foe: the two faces of *Xenorhabdus nematophila*. Nat Rev Microbiol 5:634–646

Herndl GJ, Mullerniklas G, Frick J (1993) Major role of ultraviolet in controlling bacterioplankton growth in the surface layer of the ocean. Nature 361:717–719

Heungens K, Cowles CE, Goodrich-Blair H (2002) Identification of *Xenorhabdus nematophila* genes required for mutualistic colonization of *Steinernema carpocapsae* nematodes. Mol Microbiol 45:1337–1353

Hillesland KL, Stahl DA (2010) Rapid evolution of stability and productivity at the origin of a microbial mutualism. Proc Natl Acad Sci USA 107:2124–2129

Hirsch AM, McFall-Ngai MJ (2000) Fundamental concepts in symbiotic interactions: light and dark, day and night, squid and legume. J Plant Growth Reg 19:113–130

Hmelo LR, Mincer TJ, Van Mooy BAS (2011) Possible influence of bacterial quorum sensing on the hydrolysis of sinking particulate organic carbon in marine environments. Environ Microb Rep 3:682–688

Holm-Hansen O, Lubin D, Hebling EW (1993) Ultraviolet radiation and its effect on organisms in aquatic environments. In: Young AR, Bjorn L, Moan J, Nultsch W (eds) Environmental UV photobiology. Plenum, New York, pp 379–425

Hooper LV (2009) OPINION Do symbiotic bacteria subvert host immunity? Nat Rev Microbiol 7:367–374

Huber B, Riedel K, Hentzer M, Heydorn A, Gotschlich A, Givskov M, Molin S, Eberl L (2001) The cep quorum sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. Microbiology 47: 2517–2528

Huettel M, Forster S, Kloser S, Fossing H (1996) Vertical migration in the sediment-dwelling sulfur bacteria Thioploca spp in overcoming diffusion limitations. Appl Environ Microbiol 62:1863–1872

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774

Hullar MAJ, Kaplan LA, Stahl DA (2006) Recurring seasonal dynamics of microbial communities in stream habitats. Appl Environ Microbiol 72:713–722

Huse SM, Dethlefsen L, Huber JA, Welch DM, Relman DA, Sogin ML (2008) Exploring microbial diversity and taxonomy using SSU rRNA Hypervariable Tag Sequencing. Plos Genet 4

Iverson V, Morris RM, Frazar CD, Berthiaume CT, Morales RL, Armbrust EV (2012) Untangling genomes from metagenomes: revealing an uncultured class of marine euryarchaeota. Science 335:587–590

Jannasch HW, Nelson DC, Wirsen CO (1989) Massive natural occurrence of unusually large bacteria (*Beggiatoa sp*) at a hydrothermal deep-sea vent site. Nature 342:834–836

Jeffrey W, Kase JP, Wilhelm SW (2000) UV radiation effects on heterotrophic bacterioplankton and viruses in marine ecosystems. In: Mora SD (ed) The effects of UV radiation in the marine environment. Cambridge University Press, New York, pp 206–236

Johnson CH, Stewart PL, Egli M (2011) The cyanobacterial circadian system: from biophysics to bioevolution. Annu Rev Biophys 40:143–167

Jorgensen BB, Cohen Y (1977) Solar Lake (Sinai) sulfur cycle of benthic cyanobacterial mats. Limnol Oceanogr 22:657–666

Jorgensen BB, Revsbech NP (1983) Colorless sulfur bacteria, Beggiatoa Spp and Thiovulum Spp in O_2 and H_2S microgradients. Appl Environ Microbiol 45:1261–1270

Jorgensen BB (1994) Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbiol Ecol 13:303–312

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic, New York, pp 21–132

Jurgens K, Matz C (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Anton Leeuw Int J G 81: 413–434

Kaltenpoth M, Yildirim E et al (2012) Refining the roots of the beewolf-Streptomyces symbiosis: antennal symbionts in the rare genus Philanthinus (Hymenoptera, Crabronidae). Appl Environ Microb 78:822–827

Karl DM (1994) Accurate estimation of microbial loop processes and rates. Microb Ecol 28:147–150

Karl DM, Lukas R (1996) The Hawaii Ocean time-series (HOT) program: background, rationale and field implementation. Deep-Sea Res Pt II 43:129–156

Karl DM (2010) Oceanic ecosystem time-series programs: ten lessons learned. Oceanography 23:104–125

Karner MB, Delong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507–510

Kau AL, Ahern PP et al (2011) Human nutrition, the gut microbiome and the immune system. Nature 474:327–336

Kaufman MR, Ikeda Y, Patton C, Van Dykhuizen G, Epel D (1998) Bacterial symbionts colonize the accessory nidamental gland of the squid Loligo opalescens via horizontal transmission. Biol Bull-Us 194:36–43

Kettler GC, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferriera S, Johnson J, Steglich C, Church GM, Richardson P, Chisholm SW (2007) Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. PLoS Genet 3:2515–2528

Kiorboe T, Tang K, Grossart HP, Ploug H (2003) Dynamics of microbial communities on marine snow aggregates: colonization, growth, detachment, and grazing mortality of attached bacteria. Appl Environ Microbiol 69:3036–3047

Klitgord N, Segre D (2010) Environments that induce synthetic microbial ecosystems. PLOS Comput Biol 6

Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. Ann Rev Microbiol 63:311–334

Kobayashi J, Ishibashi M (1993) Bioactive metabolites of symbiotic marine microorganisms. Chem Rev 93:1753–1769

Kolenbrander PE (2000) Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol 54:413–437

Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546

Koonin EV (2009) Darwinian evolution in the light of genomics. Nucleic Acids Res 37:1011–1034

Kruger M, Meyerdierks A, Glockner FO, Amann R, Widdel F, Kube M, Reinhardt R, Kahnt J, Bocher R, Thauer RK, Shima S (2003) A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. Nature 426:878–881

Kukor JJ, Martin MM (1986) The effect of acquired microbial enzymes on assimilation efficiency in the common woodlouse, Tracheoniscus-Rathkei. Oecologia 69:360–366

Lane DJ, Harrison AP, Stahl D, Pace B, Giovannoni SJ, Olsen GJ, Pace NR (1992) Evolutionary relationships among sulfur-oxidizing and iron-oxidizing eubacteria. J Bacteriol 174:269–278

Le Bourgne R, Barber RT, Delacroix T, Inoue HY, Mackey DJ, Rodier M (2002) Pacific warm pool and divergence: temporal and zonal variations on the equator and their effects on the biological pump. Deep-Sea Res Pt II 49:2471–2512

Lebaron P, Servais P, Troussellier M, Courties C, Muyzer G, Bernard L, Schafer H, Pukall R, Stackebrandt E, Guindulain T, Vives-Rego J (2001) Microbial community dynamics in Mediterranean nutrient enriched seawater mesocosms: changes in abundances, activity and composition. FEMS Microbiol Ecol 34:255–266

Lee KH, Ruby EG (1994) Effect of the squid host on the abundance and distribution of symbiotic *Vibrio fischeri* in nature. Appl Environ Microbiol 60:1565–1571

Ley RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72:3685–3695

Li F, Hullar MAJ, Schwarz Y, Lampe JW (2009) Human gut bacterial communities are altered by addition of cruciferous vegetables to a controlled fruit- and vegetable-free diet. J Nutr 139:1685–1691

Lilburn TC, Kim KS, Ostrom NE, Byzek KR, Leadbetter JR, Breznak JA (2001) Nitrogen fixation by symbiotic and free-living spirochetes. Science 292:2495–2498

Lipson DA, Schmidt SK (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl Environ Microbiol 70:2867–2879

Llabres M, Agusti S (2006) Picophytoplankton cell death induced by UV radiation: evidence for oceanic Atlantic communities. Limnol Oceanogr 51:21–29

Lum MR, Hirsch AM (2003) Roots and their symbiotic microbes: strategies to obtain nitrogen and phosphorus in a nutrient limiting environment. Am J Plant Growth Reg 21:368–382

MacGregor BJ, Moser DP, Baker BJ, Alm EW, Maurer M, Nealson KH, Stahl DA (2001) Seasonal and spatial variability in Lake Michigan sediment small-subunit rRNA concentrations. Appl Environ Microbiol 67:3908–3922

Maier S, Volker H, Beese M, Gallardo VA (1990) The fine structure of *Thioploca aruacae* and *Thioploca chileae*. Can J Microbiol 36:438–448

Margulis L, Fester R (eds) (1991) Symbiosis as a source of evolutionary innovation: speciation and morphogenesis. The MIT Press, Cambridge, MA

Martens EC, Heungens K, Goodrich-Blair H (2003) Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus nematophila* bacteria. J Bacteriol 185:3147–3154

Martin HG, Ivanova N, Kunin V, Warnecke F, Barry KW, McHardy AC, Yeates C, He SM, Salamov AA, Szeto E, Dalin E, Putnam NH, Shapiro HJ, Pangilinan JL, Rigoutsos I, Kyrpides NC, Blackall LL, McMahon KD, Hugenholtz P (2006) Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nat Biotechnol 24:1263–1269

Massana R, Murray AE, Preston CM, Delong EF (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl Environ Microbiol 63:50–56

Massana R, Taylor LJ, Murray AE, Wu KY, Jeffrey WH, Delong EF (1998) Vertical distribution and temporal variation of marine planktonic archaea in the Gerlache Strait, Antarctica, during early spring. Limnol Oceanogr 43:607–617

Massana R, Delong EF, Pedros-Alio C (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl Environ Microbiol 66:1777–1787

Massana R (2011) Eukaryotic picoplankton in surface oceans. Annu Rev Microbiol 65:91–110

Matsumoto K, Furuya K (2011) Variations in phytoplankton dynamics and primary production associated with ENSO cycle in the western and central equatorial Pacific during 1994–2003. J Geophys Res Oceans 116

Mayr E (1982) The growth of biological thought: diversity, evolution, and inheritance. Belknap Press of Harvard University Press, Cambridge, MA

McCutcheon JP, Moran NA (2012) Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol 10:13–26

McCutcheon JP, McDonald BR et al (2009) Convergent evolution of metabolic roles in bacterial co-symbionts of insects. Proc Natl Acad Sci USA 106:15394–15399

McFall-Ngai MJ (2001) Identifying "prime suspects": symbioses and the evolution of multicellularity. Comp Biochem Phys B 129:711–723

McFall-Ngai MJ (2002) Unseen forces: The influence of bacteria on animal development. Dev Biol 242:1–14

McFall-Ngai MJ, Henderson B, Ruby E (eds) (2005) The influence of cooperative bacteria on animal host biology. Cambridge University Press, New York

McFall-Ngai M, Heath-Heckman EA, Gilette AA, Peyer SM, Harvie EA (2012) The Secret Language of coevolved symbioses: insights from the Euprymna scolopes-*Vibrio fischeri* symbiosis. Semin Immunol 24:3–8

McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. Curr Opin Biotechnol 20:623–632

McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ (2003) LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. J Bacteriol 185:274–284

Medina M, Sachs JL (2010) Symbiont genomics, our new tangled bank. Genomics 96:377–378

Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, Blumenberg M, Knittel K, Gieseke A, Peterknecht K, Pape T, Boetius A, Amann R, Jorgensen BB, Widdel F, Peckmann JR, Pimenov NV, Gulin MB (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297: 1013–1015

Minz D, Flax JL, Green SJ, Muyzer G, Cohen Y, Wagner M, Rittmann BE, Stahl DA (1999a) Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl Environ Microbiol 65: 4666–4671

Minz D, Fishbain S, Green SJ, Muyzer G, Cohen Y, Rittmann BE, Stahl DA (1999b) Unexpected population distribution in a microbial mat community: sulfate reducing bacteria localized to the highly oxic chemocline in contrast to an eukaryotic preference for anoxia. Appl Environ Microbiol 65:4659–4665

Mitchell DL, Kerntz D (1993) The induction and repair of DNA photodamage in the environment. In: Young AR, Bjorn L, Moan J, Nultsch W (eds) Environmental UV photobiology. Plenum, New York, pp 379–425

Monds RD, O'Toole GA (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. Trends Microbiol 17:73–87

Moore LR, Rocap G, Chisholm SW (1998) Physiology and molecular phylogeny of coexisting Prochlorococcus ecotypes. Nature 393:464–467

Moran MA, Zepp RG (2000) UV radiation effects on microbes and microbial processes. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 201–228

Moran NA, McCutcheon JP et al (2008) Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet 42:165–190

Moran NA, McLaughlin HJ et al (2009) The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. Science 323:379–382

Morin JG, Harrington A et al (1975) Light for all reasons – versatility in behavioral repertoire of flashlight fish. Science 190:74–76

Morris RM, Rappe MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420:806–810

Morris RM, Vergin KL, Cho JC, Rappe MS, Carlson CA, Giovannoni SJ (2005) Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic time-series Study site. Limnol Oceanogr 50:1687–1696

Mulkidjanian AY, Koonin EV, Makarova KS, Mekhedov SL, Sorokin A, Wolf YI, Dufresne A, Partensky F, Burd H, Kaznadzey D, Haselkorn R, Galperin MY (2006) The cyanobacterial genome core and the origin of photosynthesis. Proc Natl Acad Sci USA 103:13126–13131

Murray AE, Preston CM, Massana R, Taylor LT, Blakis WK, Delong EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island Antarctica. Appl Environ Microbiol 64:2585–2595

Nakabachi A, Yamashita A et al (2006) The 160-kilobase genome of the bacterial endosymbiont Carsonella. Science 314:267

Nakajima M, Imai K, Ito H, Nishiwaki T, Murayama Y, Iwasaki H, Oyarna T, Kondo T (2005) Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. Science 308:414–415

Nauhaus K, Boetius A, Kruger M, Widdel F (2002) In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. Environ Microbiol 4:296–305

Nealson KH, Stahl D (1997) Biogeochemical cycling and bacterial metabolism: what can we learn from layered microbial communities? In: Banfield J (ed) Geomicrobiology: interactions between microbes and minerals. Mineralogical Society of America, New York

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Molec Biol Rev 75: 14–49

Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. Annu Rev Genet 43:197–222

Nussbaumer AD, Fisher CR et al (2006) Horizontal endosymbiont transmission in hydrothermal vent tubeworms. Nature 441:345–348

Nyholm SV, McFall-Ngai MJ (2003) Dominance of *Vibrio fischeri* in secreted mucus outside the light organ of Euprymna scolopes: the first site of symbiont specificity. Appl Environ Microbiol 69:3932–3937

Nyholm SV, Mcfall-Ngai MJ (2004) The winnowing: establishing the squid-vibrio symbiosis. Nat Rev Microbiol 2:632–642

Nyholm SV, Stewart JJ et al (2009) Recognition between symbiotic *Vibrio fischeri* and the haemocytes of Euprymna scolopes. Environ Microbiol 11:483–493

Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304

Ochman H, Moran NA (2001) Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. Science 292:1096–1098

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365

Orphan VJ, House CH, Hinrichs KU, McKeegan KD, Delong EF (2001) Methane consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science 293:484–487

Ouyang Y, Andersson CR, Kondo T, Golden SS, Johnson CH (1998) Resonating circadian clocks enhance fitness in cyanobacteria. Proc Natl Acad Sci USA 95:8660–8664

Ovreas L, Bourne D, Sandaa RA, Casamayor EO, Benlloch S, Goddard V, Smerdon G, Heldal M, Thingstad TF (2003) Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. Aquat Microb Ecol 31:109–121

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276:734–740

Pace NR (2009) Mapping the tree of life: progress and prospects. Microbiol Mol Biol R 73:565–576

Pace NR, Sapp J, Goldenfeld N (2012) Phylogeny and beyond: scientific, historical, and conceptual significance of the first tree of life. Proc Natl Acad Sci USA 109:1011–1018

Paerl HW, Prufert LE (1987) Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen depleted aerobic marine waters. Appl Environ Microbiol 53:1078–1087

Pakulski JD, Aas P, Jeffrey W, Lyons M, van Waasbergen LG, Mitchell D, Coffin R (1998) Influence of light on bacterioplankton production and respiration in a subtropical coral reef. Aquat Microb Ecol 15:137–148

Papke RT, Ramsing NB, Bateson MM, Ward DM (2003) Geographical isolation in hot spring cyanobacteria. Environ Microbiol 5:650–659

Passow U (2002) Transparent exopolymer particles (TEP) in aquatic environments. Prog Oceanogr 55:287–333

Pennisi E (2001) Microbial genomes: sequences reveal borrowed genes. Science 294:1634–1635

Perna NT, Plunkett G, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Postal G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin JY, Yen G, Schwartz DC, Welch RA, Blattner FR (2001) Genome sequence of enterohaemorrhagic *Escherichia coli* O157: H. Nature 410:240–245

Pernthaler J, Glockner FO, Unterholzner S, Alfreider A, Psenner R, Amann R (1998) Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. Appl Environ Microbiol 64:4299–4306

Pernthaler J, Posch T, Simek K, Vrba J, Pernthaler A, Glockner FO, Nubel U, Psenner R, Amann R (2001) Predator-specific enrichment of actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. Appl Environ Microbiol 67:2145–2155

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

Philander SG, Fedorov A (2003) Is El Nino sporadic or cyclic? Ann Rev Earth Planet Sci 31:579–594

Pinel N, Davidson SK et al (2008) *Verminephrobacter eiseniae* gen. nov., sp nov., a nephridial symbiont of the earthworm Eisenia foetida (Savigny). Int J Syst Evol Micr 58:2147–2157

Polz MF, Robinson JJ, Cavanaugh CM, Van Dover CL (1998) Trophic ecology of massive shrimp aggregations at a mid-Atlantic ridge hydrothermal vent site. Limnol Oceanogr 43:1631–1638

Pomeroy LR (1974) The ocean's foodweb: a changing paradigm. Bioscience 24:499–504

Posch T, Simek K, Vrba J, Pernthaler S, Nedoma J, Sattler B, Sonntag B, Psenner R (1999) Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. Aquat Microb Ecol 18:235–246

Proctor LM (2011) The human microbiome project in 2011 and beyond. Cell Host Microbe 10:287–291

Purcell EM (1977) Life at low Reynolds-number. Am J Phys 45:3–11

Ramsing NB, Ferris MJ, Ward DM (1997) Light-induced motility of thermophilic Synechococcus isolates from octopus spring, Yellowstone National Park. Appl Environ Microbiol 63:2347–2354

Rappe MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633

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

Raskin L, Stromley JM, Rittman BE, Stahl DA (1994) Group specific 16 S ribosomal RNA hybridization probes to describe natural communities of methanogens. Appl Environ Microbiol 60:1232–1240

Rawls JF, Samuel BS et al (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proc Natl Acad Sci USA 101:4596–4601

Relman DA, Falkow S (2001) The meaning and impact of the human genome sequence for microbiology. Trends Microbiol 9:206–208

Reno ML, Held NL, Fields CJ, Burke PV, Whitaker RJ (2009) Biogeography of the Sulfolobus islandicus pan-genome. Proc Natl Acad Sci USA 106:8605–8610

Revsbech NP, Jorgensen BB (1983) Photosynthesis of benthic microflora measured with high spatial-resolution by the oxygen microprofile method – capabilities and limitations of the method. Limnol Oceanogr 28: 749–756

Riemann L, Farnelid H, Steward GF (2010) Nitrogenase genes in non-cyanobacterial plankton: prevalence, diversity and regulation in marine waters. Aquat Microb Ecol 61:225–237

Rio RVM, Anderegg M et al (2007) Characterization of a catalase gene from *Aeromonas veronii*, the digestive-tract symbiont of the medicinal leech. Microbiol-SGM 153:1897–1906

Risatti JB, Capman WC, Stahl DA (1994) Community structure of a microbial mat – the phylogenetic dimension. Proc Natl Acad Sci USA 91:10173–10177

Rocap G, Distel DL, Waterbury JB, Chisholm SW (2002) Resolution of Prochlorococcus and Synechococcus ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl Environ Microbiol 68:1180–1191

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER, Chisholm SW (2003) Genome divergence in two Prochlorococcus ecotypes reflects oceanic niche differentiation. Nature 424:1042–1047

Rodrigues JLM, Serres MH, Tiedje JM (2011) Large-scale comparative phenotypic and genomic analyses reveal ecological preferences of Shewanella species and identify metabolic pathways conserved at the genus level. Appl Environ Microbiol 77:5352–5360

Rohwer F, Thurber RV (2009) Viruses manipulate the marine environment. Nature 459:207–212

Ruby EG, Lee KH (1998) The *Vibrio fischeri* Euprymna scolopes light organ association: current ecological paradigms. Appl Environ Microbiol 64: 805–812

Ruby EG (1999) The Euprymna scolopes-*Vibrio fischeri* symbiosis: a biomedical model for the study of bacterial colonization of animal tissue. J Molec Microbiol Biotech 1:13–21

Ruby EG (2008) Symbiotic conversations are revealed under genetic interrogation. Nat Rev Microbiol 6:752–762

Sachs JL, Skophammer RG et al (2011) Evolutionary transitions in bacterial symbiosis. Proc Natl Acad Sci USA 108:10800–10807

Sassen R, Roberts HH, Aharon P, Larkin J, Chinn EW, Carney R (1993) Chemosynthetic bacterial mats at cold hydrocarbon seeps, Gulf of Mexico continental slope. Org Geochem 20:77–85

Schaecter M (2003) Integrative microbiology: the third golden age. J Biosci 28:149–154

Schink B (1988) Principles and limits of anaerobic degradation, environmental and technological aspects. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 771–846

Schoenian I, Spiteller M et al (2011) Chemical basis of the synergism and antagonism in microbial communities in the nests of leaf-cutting ants. Proc Natl Acad Sci USA 108:1955–1960

Schulz HN, Jorgensen BB (2001) Big bacteria. Ann Rev Microbiol 55:105–137

Scott JJ, Oh DC et al (2008) Bacterial protection of beetle-fungus mutualism. Science 322:63

Seckbach J (2002) Symbiosis: mechanisms and model systems. In: Seckbach J (ed) Cellular origin and life in extreme habitats. Kluwer, Norwell

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (1999) Fluorescence in situ hybridization using 16S rRNA-targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules. Appl Environ Microbiol 65:1280–1288

Shimoyama T, Kato S, Ishii S, Watanabe K (2009) Flagellum mediates symbiosis. Science 323:1574

Sigalevich P, Meshorer E, Helman Y, Cohen Y (2000) Transition from anaerobic to aerobic growth conditions for the sulfate-reducing bacterium desulfovibrio oxyclinae results in flocculation. Appl Environ Microbiol 66:5005

Silver AC, Kikuchi Y et al (2007) Interaction between innate immune cells and a bacterial type III secretion system in mutualistic and pathogenic associations. Proc Natl Acad Sci USA 104:9481–9486

Simek K, Armengol J, Comerma M, Garcia JC, Kojecka P, Nedoma J, Hejzlar J (2001a) Changes in the epilimnetic bacterial community composition, production, and protist induced mortality along the longitudinal axis of a highly eutrophic reservoir. Microbial Ecol 42:359–371

Simek K, Pernthaler J, Weinbauer MG, Hornak K, Dolan JR, Nedoma J, Masin M, Amann R (2001b) Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appl Environ Microbiol 67:2723–2733

Simek K, Hornak K, Masin M, Christaki U, Nedoma J, Weinbauer MG, Dolan JR (2003) Comparing the effects of resource enrichment and grazing on a bacterioplankton community of a mesoeutrophic reservoir. Aquat Microb Ecol 31:123–135

Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 407:762–764

Small AL, McFall-Ngai MJ (1999) Halide peroxidase in tissues that interact with bacteria in the host squid Euprymna scolopes. J Cell Biochem 72: 445–457

Smillie CS, Smith MB, Friedman J, Cordero OX, David LA, Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci USA 103: 12115–12120

Sommaruga R, Obernosterer I, Herndl GJ, Psenner R (1997) Inhibitory effect of solar radiation on thymidine and leucine incorporation by freshwater and marine bacterioplankton. Appl Environ Microbiol 63:4178–4184

Southward EC (1999) Development of Perviata and Vestimentifera (Pogonophora). Hydrobiologia 402:185–202

Spaulding AW, von Dohlen CD (1998) Phylogenetic characterization and molecular evolution of bacterial endosymbionts in psyllids (Hemiptera: Sternorrhyncha). Mol Biol Evol 15:1506–1513

Stahl DA, Lane DJ, Olsen GJ, Pace NR (1984) Analysis of hydrothermal vent-associated symbionts by ribosomal-RNA sequences. Science 224:409–411

Stahl D (1993) The natural history of microorganisms. ASM News 59:609–613

Staley JT, Gosink JJ (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. Annu Rev Microbiol 53:189–215

Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol 7:568–577

Steele JA, Countway PD, Xia L, Vigil PD, Beman JM, Kim DY, Chow CET, Sachdeva R, Jones AC, Schwalbach MS, Rose JM, Hewson I, Patel A, Sun FZ, Caron DA, Fuhrman JA (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME J 5:1414–1425

Stern A, Sorek R (2011) The phage-host arms race: shaping the evolution of microbes. Bioessays 33:43–51

Stolyar S, Van Dien S, Hillesland KL, Pinel N, Lie TJ, Leigh JA, Stahl DA (2007) Metabolic modeling of a mutualistic microbial community. Mol Syst Biol 3:92

Stougaerd J (2000) Regulators and regulation of legume root nodule development. Plant Physiol 124:531–539

Streams ME, Fisher CR, Fiala-Medioni A (1997) Methanotrophic symbiont location and fate of carbon incorporated from methane in a hydrocarbon seep mussel. Mar Biol 129:465–476

Sullivan JT, Ronson CW (1998) Evolution of Rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. Proc Natl Acad Sci USA 95:5145–5149

Suttle CA (2002) Community structure: viruses. In: Hurst CJ, Crawford RL, Knudsen GR, McInerney MJ, Stetzenbach LD (eds) Manual of environmental microbiology. ASM press, Washington, DC, pp 364–370

Suttle CA (2005) Viruses in the sea. Nature 437:356–361

Suttle CA (2007) Marine viruses – major players in the global ecosystem. Nat Rev Microbiol 5:801–812

Taillefert M, MacGregor BJ, Gaillard JF, Lienemann CP, Perret D, Stahl DA (2002) Evidence for a dynamic cycle between Mn and Co in the water column of a stratified lake. Environ Sci Technol 36:468–476

Tamas I, Klasson L, Canback B, Naslund AK, Eriksson AS, Wernegreen JJ, Sandstrom JP, Moran NA, Andersson SGE (2002) 50 million years of genomic stasis in endosymbiotic bacteria. Science 296:2376–2379

Teske A, Alm E, Regan JM, Toze S, Rittmann BE, Stahl DA (1994) Evolutionary relationships among ammonia-oxidizing and nitrite-oxidizing bacteria. J Bacteriol 176:6623–6630

Teske A, Ramsing NB, Habicht K et al (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl Environ Microbiol 64:2943–2951

Thamdrup B, Canfield DE (1996) Fate of elemental sulfur in an intertidal sediment. FEMS Microbiol Ecol 19:95–103

Thar R, Fenchel T (2001) True chemotaxis in oxygen gradients of the sulfur-oxidizing bacterium *Thiovulum majus*. Appl Environ Microbiol 67:3299–3303

Torsvik V, Ovreas L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245

Torsvik V, Ovreas L, Thingstad TF (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. Science 296:1064–1066

Treusch AH, Vergin KL, Finlay LA, Donatz MG, Burton RM, Carlson CA, Giovannoni SJ (2009) Seasonality and vertical structure of microbial communities in an ocean gyre. ISME J 3:1148–1163

Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen K, Chang HW, Podar M, Short JM, Mathur EJ, Detter JC, Bork P, Hugenholtz P, Rubin EM (2005) Comparative metagenomics of microbial communities. Science 308:554–557

Turley CM, Mackie PJ (1995) Bacterial and cyanobacterial flux to the deep NE Atlantic on sediment particles. Deep-Sea Res Pt I 42:1453–1474

Turner JT (2002) Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat Microbial Ecol 27:57–102

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43

van Hannen EJ, Mooij W, van Agterveld MP, Gons HJ, Laanbroek HJ (1999a) Detritus-dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. Appl Environ Microbiol 65:2478–2484

van Hannen EJ, Zwart G, van Agterveld MP, Gons HJ, Ebert J, Laanbroek HJ (1999b) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl Environ Microbiol 65:795–801

van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB (1990) Influence of interfaces on microbial activity. Microbiol Rev 54:75–87

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu DY, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers YH, Smith HO (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74

Vivas EI, Goodrich-Blair H (2001) *Xenorhabdus nematophilus* as a model for host-bacterium interactions: rpoS is necessary for mutualism with nematodes. J Bacteriol 183:4687–4693

Walker CB, He ZL, Yang ZK, Ringbauer JA, He Q, Zhou JH, Voordouw G, Wall JD, Arkin AP, Hazen TC, Stolyar S, Stahl DA (2009) The electron transfer

system of syntrophically grown *Desulfovibrio vulgaris*. J Bacteriol 191: 5793–5801

Walls JT, Blackman AJ, Ritz DA (1995) Localization of the amathamide alkaloids in surface bacteria of Amathia-Wilsoni Kirkpatrick, 1888 (Bryozoa, Ctenostomata). Hydrobiologia 297:163–172

Wang CZ, Fiedler PC (2006) ENSO variability and the eastern tropical Pacific: a review. Prog Oceanogr 69:239–266

Ward DM, Weller R, Bateson MM (1990) 16S ribosomal-RNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345:63–65

Warnecke F, Luginbuhl P et al (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450:560–U517

Waterbury JB, Calloway CB, Turner RD (1983) A cellulolytic nitrogen-fixing bacterium cultured from the gland of Deshayes in shipworms (Bivalvia, Teredinidae). Science 221:1401–1403

Weis VM, Small AL, McFallNgai MJ (1996) A peroxidase related to the mammalian antimicrobial protein myeloperoxidase in the Euprymna-Vibrio mutualism. Proc Natl Acad Sci USA 93:13683–13688

Werren JH (1997) Biology of Wolbachia. Annu Rev Entomol 42:587–609

Werren JH, Baldo L et al (2008) Wolbachia: master manipulators of invertebrate biology. Nat Rev Microbiol 6:741–751

West NJ, Scanlan DJ (1999) Niche-partitioning of Prochlorococcus populations in a stratified water column in the eastern North Atlantic Ocean. Appl Environ Microbiol 65:2585–2591

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301:976–978

Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP (2001) Gene expression in Pseudomonas aeruginosa biofilms. Nature 413:860–864

Wilcox JL, Dunbar HE, Wolfinger RD, Moran NA (2003) Consequences of reductive evolution for gene expression in an obligate endosymbiont. Mol Microbiol 48:1491–1500

Wilhelm LJ, Tripp HJ, Givan SA, Smith DP, Giovannoni SJ (2007) Natural variation in SARII marine bacterioplankton genomes inferred from metagenomic data. Biol Direct 2

Wilkinson TL, Douglas AE (1996) The impact of aposymbiosis on amino acid metabolism of pea aphids. Entomol Exp Appl 80:279–282

Williams LB (2000) Heterotrophic bacteria and the dynamics of dissolved organic material. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 153–200

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

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms – proposal for the domains archaea, bacteria, and eucarya. Proc Natl Acad Sci USA 87:4576–4579

Woese CR (2000) Interpreting the universal phylogenetic tree. Proc Natl Acad Sci USA 97:8392–8396

Woese CR (2004) A new biology for a new century. Microbiol Mol Biol R 68:173–186

Wolin MJ, Miller TL (1982) Interspecies hydrogen transfer: 15 years later. ASM News 48:561–565

Woyke T, Teeling H et al (2006) Symbiosis insights through metagenomic analysis of a microbial consortium. Nature 443:950–955

Wren BW (2000) Microbial genome analysis: Insights into virulence, host adaptation and evolution. Nat Rev Genet 1:30–39

Wright TD, Vergin KL, Boyd PW, Giovannoni SJ (1997) A novel delta-subdivision proteobacterial lineage from the lower ocean surface layer. Appl Environ Microbiol 63:1441–1448

Xavier KB, Bassler BL (2003) LuxS quorum sensing: more than just a numbers game. Curr Opin Microbiol 6:191–197

Young RE, Roper CFE (1976) Bioluminescent counter-shading in midwater animals – evidence from living squid. Science 191:1046–1048

Zal F, Leize E, Lallier FH, Toulmond A, Van Dorsselaer A, Childress JJ (1998) S-sulfohemoglobin and disulfide exchange: the mechanisms of sulfide binding by Riftia pachyptila hemoglobins. Proc Natl Acad Sci USA 95:8997–9002

Zaura E, Keijser BJF, Huse SM, Crielaard W (2009) Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol 9:259

Zengler K, Toledo G, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. Proc Natl Acad Sci USA 99:15681–15686

Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, Parameswaran P, Crowell MD, Wing R, Rittmann BE, Krajmalnik-Brown R (2009) Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA 106:2365–2370

Zimmer M, Brune A (2005) Physiological properties of the gut lumen of terrestrial isopods (Isopoda: Oniscidea): adaptive to digesting lignocellulose? J Comp Physiol B 175:275–283

Zimmer M, Topp W (1998) Microorganisms and cellulose digestion in the gut of the woodlouse Porcellio scaber. J Chem Ecol 24:1397–1408

Zuckerkandl E, Pauling L (1965) Molecules as documents of evolutionary history. J Theoret Biol 8:357–366

Zwart G, Hiorns WD, Methe BA, Van Agterveld MP, Huismans R, Nold SC, Zehr JP, Laanbroek HJ (1998) Nearly identical 16 S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. Syst Appl Microbiol 21:546–556

2 Quantitative and Theoretical Microbial Population Biology

Martin F. Polz[1] · *William P. Hanage*[2]
[1]Parsons Laboratory, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[2]Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

Introduction

While intuition tells us that, like all living things, bacteria and archaea live in populations, in practice it has remained difficult to define and identify their properties. Populations in animal and plant biology are broadly defined as coexisting members of a species. However, for bacteria and archaea, as for most eukaryotic microbes, it has been nearly impossible to agree upon what a species is.

Observations of frequent horizontal gene transfer across wide phylogenetic distances challenge the definition of species as interbreeding units, a concept derived largely from vertebrate biology. Nor is it easy to know what coexistence entails because microbial habitats are difficult to sample at relevant scales, and dispersal and other important population parameters are poorly constrained. These difficulties notwithstanding, it is of great theoretical and practical importance to search for population-like units among microbes. Evolution is commonly defined as changes in the genetic makeup of populations, and in ecology, basic estimates of community diversity, dynamics, and assembly rely on counts of populations or species coexisting in a particular habitat. Hence, the quest for bacterial and archaeal populations is one for defining and identifying fundamental players in ecology and evolution.

Populations, regardless of the underlying species definition, represent groups of organisms that are highly clustered on both the genotypic and phenotypic levels, that is, the variance within populations is far less than between them. So we can say that closely related organisms within the same population are likely to share such important phenotypic qualities as virulence, the ability to metabolize certain sugars, colonize certain particles or body sites, or make defined contributions to community ecology. In spite of the ongoing problems with a theory-based definition of populations, such clustering is observed in bacteria and archaea (Goodfellow et al. 1997), and particularly genotypic clustering has been extensively documented in recent years by multilocus sequence analysis (MLSA) (Gevers et al. 2005). The discovery of these clusters offers a practicable solution to the species dilemma, that is, to sidestep it for the moment and to adopt a pragmatic definition of populations that emphasizes the existence and definition of clusters of coexisting strains that are consistently similar on a genetic and phenotypic basis. Such clusters may be defined from multiple different sources of genetic data (core gene sequences, microarrays, or whole genomes) and may form tractable units to address evolutionary and ecological questions. Hence, the goal of this chapter is to provide an up-to-date summary of clusters as organizational units within microbial communities, and how such clusters may be identified and related to relevant aspects of the environment in which bacteria and archaea have evolved. Finally, mechanisms of evolution of clusters are discussed with an outlook toward population genomics, which is enabled by advances in sequencing and computational techniques.

Bacteria Are Organized into Genotypic and Phenotypic Clusters

The question of whether bacteria are organized into genotypic clusters has primarily been addressed by comparing sequences of multiple protein-coding genes for groups of closely related organisms, often within the same-named species but not necessarily so. This approach, termed multilocus sequence typing (MLST), originated from epidemiology, where the need to provide a reliable and portable classification scheme for pathogens led to the assessment of allelic variation at multiple protein-coding genes (Maiden et al. 1998; Maiden 2006). However, an exciting recent development is the analysis of genotypic clusters using whole genome (Croucher et al. 2011; Cadillo-Quiroz et al. 2012; Shapiro et al. 2012) and metagenomic sequences

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_35,

(Denef et al. 2010b), enabled by the decreasing sequencing cost and development of environmentally relevant model systems with limited diversity, respectively.

While the amount of variation at a single protein-coding locus is typically far short of that required for a discriminatory means of classification, combining multiple loci scattered around the genome allows for fine-scale differentiation. It can also mitigate against the distorting signal of homologous recombination (see below), because even if one gene has been recently imported from a distantly related lineage, the others give a more reliable signal of recent ancestry.

The usefulness of this MLST approach for population genetic studies was immediately apparent. MLST data have shown the relative importance of mutation versus homologous recombination in generating diversity for many different bacteria (Feil et al. 2000, 2001; Spratt et al. 2001b; Fraser et al. 2005; Hanage et al. 2006a). In some cases, recombination is very rare, whereas in others it greatly outstrips mutation as the driver of clonal diversification, and hence cluster formation (Fraser et al. 2007). Subsequently, the approach was extended to explicitly compare the genotypic structure at higher taxonomic levels, namely, the clusters associated with named species (Hanage et al. 2005, 2006b), and to distinguish between different groups of organisms and, to reflect the changed scope, the method was called multilocus sequence analysis (MLSA) (Gevers et al. 2005).

As MLSA has become widely used for the characterization of both clinical isolates and environmental bacteria, the pervasive nature of genotypic clustering at multiple levels has become evident. An example of such clustering is shown in *Fig. 2.1*, which shows hierarchical genotypic clusters formed by streptococci at levels from species clusters, to individual clones within a species. In all cases, the figure shows trees constructed from concatenated sequences of loci found in all strains. However, moving from left to right, the samples under analysis range from an MLSA analysis of viridans streptococci, to MLST of more than 8,000 individual and unique genotypes of a single streptococcal species, to trees constructed from whole genomes of just one of those genotypes. Clustering is evident at all levels. Moreover, it is clear that the clusters in question can vary in their characteristics. For example, the cluster associated with *Streptococcus pneumoniae* is notably less diverse than those of its sister species *Streptococcus mitis* and *Streptococcus pseudopneumoniae*.

Such differences in cluster structure may be caused by differences in the evolutionary age of the cluster, or demographic factors: for example, the epidemic life-style (Smith et al. 2000) of many pathogens may result in relatively clonal populations, while environmental bacteria are generally more diverse (Thompson et al. 2005; Wilmes et al. 2009). The latter is reflected in large allelic diversity for protein coding genes, making it necessary to obtain fairly large numbers of sequences from each population for robust analysis of structure.

Similarly, divergence between clusters can differ. It has been noted that, in terms of the loci used for MLST/MLSA, two unrelated *S. mitis* are on average as similar to the pathogen *S. pneumoniae* as they are to each other (see Hanage et al. 2006b and *Fig. 2.1*). While this observation underlines differences between clusters, it is important to appreciate that our understanding and characterization of these clusters is incomplete. MLSA uses relatively small numbers of loci to assay the differences between strains. As a result, MLST can only speak to variation at the loci used. We would hope that they are representative of the variation across much of the rest of the genome, but we cannot be certain this is always the case. Indeed, both MLST and MLSA specifically require that loci with intermediate variation be used: neither too high to be likely the result of diversifying selection, nor so low that they offer no discriminatory power. Where genomic data have been obtained from multiple isolates of closely related lineages (as estimated by MLST/MLSA) considerable diversity has been evident (e.g., Harris et al. 2010; Croucher et al. 2011; Price et al. 2012; Shapiro et al. 2012). As more whole genomes become available from a wider variety of taxa, our goal should be to calibrate how divergence in the core genome relates to variation in gene content and ecological association.

It is important to note that a major factor in clustering can also be sample bias. Unfortunately, bias is difficult to constrain since the relevant population and its environmental distribution is typically unknown so that it is impossible to truly anticipate sample bias at the beginning of a study. It is therefore important to collect as many metadata as possible and explicitly link them to the sequence data so that potential biases can be discovered in follow-up studies with different sampling scope.

A final consequence of the different genetic diversity of clusters (evident in *Fig. 2.1*) is that there is no universal sequence cutoff that can be used to delineate natural clusters. This view is in contrast to orthodox microbial taxonomy, which describes species by set criteria of genetic relatedness. Accordingly, isolates fall within a species if they share at least 70% DNA hybridization. Attempts to correlate this measure with rRNA gene sequencing for microbial classification have resulted in the oft-quoted 97% rRNA sequence similarity cutoff for species designation (Stackebrandt and Goebel 1994; Rosselló-Mora and Amann 2001). However, the relationship is not clear-cut and later comparison suggested that 99% rRNA sequence similarity cutoff more closely mimics 70% DNA hybridization (Keswani and Whitman 2001). These uncertainties notwithstanding, some natural clusters have been shown to be congruent with taxonomic species, but because of the large variation in cluster structure, this cannot be *a priori* assumed (Preheim et al. 2011b). A recent trend to incorporate multilocus sequencing into species descriptions may improve the matter considerably and is conceptually similar to the phylogenetic species concept (Taylor et al. 2000). However, it may be that no single set of rules will apply to all bacteria and archaea. The matter is further complicated by the possibility that horizontal gene transfer can lead to variation in gene content that is not captured by MLSA. If such transferred genes are ecologically adaptive, they may lead to different environmental

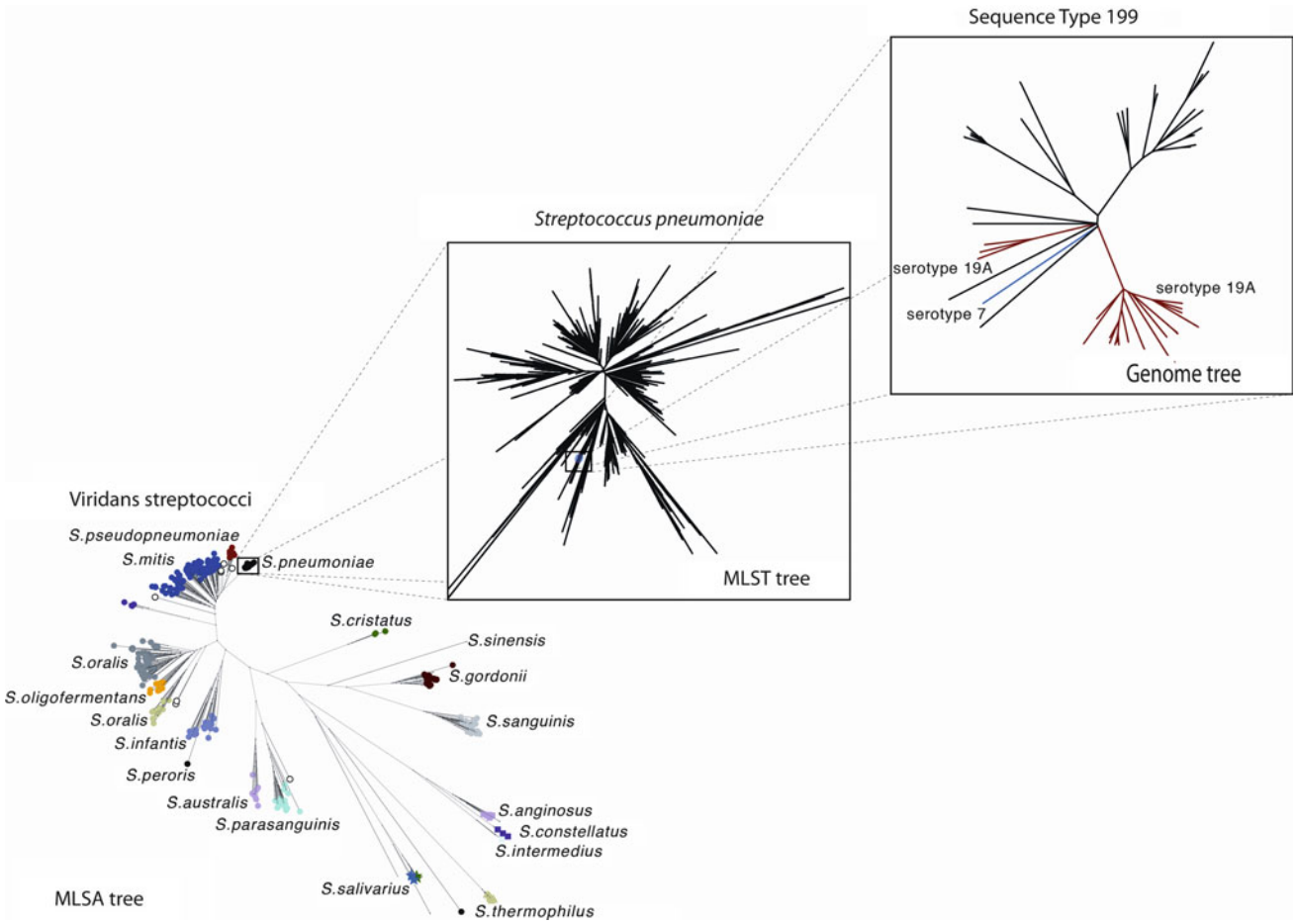


Fig. 2.1

MLSA tree. Clusters at different evolutionary scales in streptococci. In the bottom left hand corner, the relationship between members of the viridans group streptococci is displayed, calculated using an alignment of core gene sequences stored at viridans.emlsa.net. Taxa are colored and labeled in accordance with accepted species nomenclature. The second panel in the series represents a NJ tree generated using concatenated DNA sequences from 8010 genotypes in the spneumoniae.mlst.net database. Each tip is a single sequence type; a sample of the genomic diversity present in a single tip of this tree is displayed in the top right corner. This shows a maximum likelihood tree based on mapping Illumina sequence reads from over forty strains to the *S. pneumoniae* CDC3059-06 reference genome. The phylogeny was constructed using core genome polymorphisms, with recombinant sites removed by the method of Croucher et al. (2011). Even over the short timescales over which such closely related isolates diverge, there is time for structure to emerge within the population

associations and thus population structure. Whether such populations will be on independent evolutionary trajectories or transiently split depends on the strength of recombination versus selection and is dealt with more explicitly later in this chapter.

Although the majority of work on sequence clusters has studied bacteria that can be isolated and cultured in the laboratory, culture-independent analysis has suggested that microbial communities are also organized into clusters. This was first shown for coastal ocean water using 16S rRNA sequencing, which suggested that clusters contained sequences of 99% identity (Acinas et al. 2004). Since then, metagenomics for ocean water and acid mine drainage (AMD) biofilms has confirmed hierarchical genetic clustering (Konstantinidis and DeLong 2008; Denef et al. 2010a). The AMD biofilms are currently the only example of a whole community analysis where metagenomics has produced multiple closed or nearly closed genomes (see *Box 2.1*). Importantly, these fell into closely related but distinct clusters with different diversity within. For example, the dominant cluster, *Leptospirillum* Group II, only displayed 0.08% polymorphism frequency, while the archaeal *Ferroplasma* Type II had a polymorphism rate of 2.2%. Hence, cluster structure observed for cultured isolates and derived from metagenomic data appear to agree well.

Identification of Clusters

While it is evident that groups of more or less related strains do exist in Fig. 2.1, it is by no means clear where we should draw the line to state that they are different clusters or populations. An attractive approach to this problem is the use of computational statistical genetic tools to define populations. These characterize a population in terms of the frequencies of allelic variation within it, and given a sample, can then partition it into clusters. Two frequently used tools for prokaryotic data are STRUCTURE (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) and BAPS (Nazer 1992; Corander et al. 2003, 2008; Corander and Tang 2007), the latter of which has recently been incorporated into some of the online MLST databases to allow users to compare the relation of new data to the existing curated sequence (Cheng et al. 2011). In some cases, we must define the number of populations expected in advance, while in others the number of populations that best fit the data is recovered during the analysis. However, the groups or clusters found by such methods can be biased by assumptions made in the analysis, and given the limitations of available samples, it is possible that important elements of clustering could be missed or artifactually grouped together (this is not only the case for prokaryotic data (for an example from *Drosophila*, see Orozco-terWengel et al. (2011)). Hence, while results from such analyses are frequently very informative, they are not definitive and it is best to treat them as hypotheses of population structure.

If we can define clusters or populations distinguished by some set of polymorphisms or allele frequencies, then we can detect recombination in the form of admixture between clusters. Although it is important to recall that the accuracy of such analyses depends on successfully defining populations in the first place. In at least one case (BAPS), there is good evidence that the analysis successfully defines deep branching lineages even from MLSA data (WPH unpublished observations). An important factor in the future use of these methods will be whether they can be scaled for use with genomic data.

Definitions of clusters and other units important to bacterial populations are often frustratingly vague. This is partially because there is no consistent and standardized approach to the problem and as a result different data are used to characterize different samples, in such a way that the results may not be comparable (for an excellent empirical comparison of the performance of different methods, see Waples and Gaggiotti 2006). Moreover, varying rates of recombination may well mean that no universally applicable definition exists. Whichever means have been used to define clusters, it is important to remember that the results will be to a degree contingent on the sample under test (both the isolates in question and the genetic data used to characterize them) and whatever assumptions have been made regarding distance measures. As such, defining clusters is not a straightforward or automatic process. Rather, it is an exercise in data exploration where it may be necessary to use several methods and parameter settings in an iterative process. Importantly, as detailed in the next section, some examples now exist that show clusters behaving cohesively with regard to relevant ecological factors.

Environmental Association of Clusters

How microbial diversity can be linked to environmental parameters is a central issue. Among sexually reproducing organisms, the population, that is, locally coexisting members of a species, form such units because the sexual process ensures cohesion by allowing spread of ecologically adaptive genes within a population. For bacteria and archaea, the issue is more complicated due to pervasive horizontal gene transfer, which has, in the extreme, led to a gene-centric view where adaptive genes move freely among genomes and thus continuously erode population structure beyond the clonal lineage that has received the adaptive gene (Doolittle and Papke 2006; McInerney et al. 2011). Even if there is population structure, it may not be easily recognized due to the vast genomic diversity, even among closely related isolates (e.g., see Croucher et al. 2011 and Fig. 2.1), and the difficulty of sampling microbial habitats at appropriate scales. For example, isolates with many identical protein-coding genes across the genome can differ in the total size of their genomes by as much as 20% (Medini et al. 2005; Tettelin et al. 2008). Only a small fraction of these genes need be ecologically relevant and expressed, for even apparently closely related organisms to exhibit considerable ecological variation. Although individual variation is also a property of sexual eukaryotic populations, among microbes, it remains difficult to reconcile with a natural, ecologically informative definition of populations.

These theoretical issues notwithstanding, we can approach the issue empirically by posing the tractable hypothesis that clusters (at some level) represent ecologically differentiated units. If clusters do represent such units, they should show a cohesive response to environmental parameters that distinguish them from closely related clusters. The distinction from close relatives is important if one wishes to identify the phylogenetic boundaries of ecologically cohesive populations, hence the preferable use of the more variable protein-coding genes over rRNA marker genes. However, it has to be kept in mind that even protein-coding genes, which in the MLST/MLSA scheme are chosen not to be under diversifying environmental selection, may not provide enough resolution for recognition of very recently differentiated populations. As detailed in the next section, in cases such as these only the genes under environmental selection may provide sufficient signal to differentiate populations and may require whole genome analysis (Shapiro et al. 2012).

A second issue is that populations represent organisms coexisting in a habitat and hence having the potential to interact locally. This distinguishes populations from lineages, but also suggests that sampling scale is relevant. Environmental microbiologists have worked hard to address these issues, which are complicated by the difficulty of resolving ecological parameters at appropriate spatial and temporal scales. For example, while it has been relatively easy to show differential microbial

association with temperature and salinity gradients, which vary over relatively large temporal and spatial scales, documentation of resource partitioning of different types of organic material has remained more challenging since these are often distributed in microscale patches. Such patches are often relatively labile due to their origins from, for example, algal or root exudates, cellular material released by viral lysis or autolysis, and particles produced by sloppy animal feeding and fecal pellets (Azam and Long 2001; Polz et al. 2006). Generally, ecological gradients are more easily resolved in aquatic environments than in soil and sediment where resolution of patch structure remains challenging. In the cases of pathogens and symbionts, an obvious ecological distinction is differential host association and the shifting landscape of host immunity (e.g., see Sheppard et al. 2008).

One recent example of resolution of fine-scale resource partitioning among closely related bacteria is provided by a study differentiating *Vibrionaceae* coexisting within coastal water. Partitioning was discovered because isolates were collected from ecologically informative environmental samples (sequential filter-fractions containing organic particles and zoo- and phytoplankton of different sizes and types), followed by MLSA and mathematical modeling of both sequence evolution and environmental history of clades (Hunt et al. 2008). This demonstrated fine-scale resource partitioning even among very closely related groups of isolates.

The example of fine-scale ecological analysis of *Vibrionaceae* represents a case where phylogenetic and habitat structure is co-discovered (Hunt et al. 2008; Preheim et al. 2011a, b); however, prior described clusters have also been successfully mapped onto environmental gradients, in some cases supported by mathematical models of cluster evolution (Koeppel et al. 2008). For example, different clades of the *Bacillus subtilis-licheniformis* group were shown to be differentially distributed according to solar exposure and soil texture, and this was consistent with some physiological properties of isolates (Koeppel et al. 2008; Connor et al. 2010). In model-free approaches, it was shown that closely related cyanobacteria (differentiated by their 16S-23S rRNA internal transcribed spacer) are differentially distributed in light and temperature microenvironments in hot spring mats (Ferris et al. 2003; Becraft et al. 2011). Similarly, in the cyanobacterium *Prochlorococcus*, sequence clusters containing high- and low-light-adapted strains show distinct depth distributions (Moore et al. 1998; Rocap et al. 2003). Additionally, six clades denoted by differences in internal transcribed spacer (ITS) sequences displayed distinct distribution patterns on ocean-scale gradients (Johnson et al. 2006). In particular, temperature correlated to occurrence and tolerance limits of different isolates from within the clusters, but other ecological factors also showed a relationship (Bouman et al. 2006; Johnson et al. 2006). Finally, for uncultured microbes, analysis of the AMD community has shown that closely related groups, originally defined as clusters in whole genome analysis, show cohesive behavior in biofilm succession and toward temperature where a combination of metagenomics, proteomics, and metabolomics suggested fine-scale differentiation, including differentially regulated expression (*Box 2.1*).

There are several more examples of such mapping studies (and we explicitly do not consider in this context the vast literature on differential distribution of rRNA genes in the environment since clusters on that phylogenetic level are poorly constrained). However, the general procedure always first defines genotypic clusters by various techniques and ideally using as much sequence information as affordable (16 S rRNA, ITS, protein coding genes, whole genomes). These clusters are then mapped onto environmental samples, which have been collected according to some systematic scheme, testing ecological parameters of interest. Fraser et al. (2009) proposed that such studies should ideally involve explicit modeling and simulations of population structure and empirical tests of their prediction to arrive at species concepts (Fraser et al. 2009); however, the same applies to populations where one of the main issues remains how to judge the high potential for horizontal gene flow eroding population structure.

Box 2.1

A pioneering effort of characterizing a microbial community in its entirety is a long running study of acid mine drainage (AMD) biofilms in the Richmond mine in CA carried out by Banfield and colleagues (Denef et al. 2010b). These biofilms are complex and dynamical entities but display low enough diversity to be comprehensively analyzed by "omics" techniques. Importantly, this series of studies has demonstrated that it is possible to ask a broad spectrum of ecological and evolutionary questions in (near) absence of cultivated organisms. The comparatively low diversity in the AMD system is likely due to the extremely low pH and high metal concentrations under which they develop. A typical sample contains about 4–6 abundant taxa and overall, about 20 predominant taxa have been identified across different samples (Denef et al. 2010b). Early work used metagenomics to demonstrate that it is possible to obtain complete genomes from the dominant members of the community and convincingly demonstrated coexisting microdiverse populations that are distinct in terms of environmental association and patterns of gene flow (Denef et al. 2010b). For example, the dominant *Leptospirillum* Group II population had low nucleotide polymorphism (0.08%), while the archaeal *Ferroplasma* Type II population was much more diverse with 2.2% (Tyson et al. 2004). Gene flow patterns were also analyzed and showed much higher rates of homologous recombination within populations than between consistent with the idea that the core genomes of populations are cohesive, albeit subject to occasional import of foreign alleles (Eppley et al. 2007). Similarly, effects of horizontal gene transfer were evident (Allen et al. 2007) and documented, for example, the rapid evolution of virus resistance via acquisition of CRISPR elements (Andersson and Banfield 2008; Tyson and Banfield 2008). More recent efforts concentrated on assembling genomic information of more rare community members and on development of proteomic and metabolomic approaches to analyze and interpret metabolic capabilities of the biofilm communities. Strain-resolved proteomics also documented the exchange of

large blocs of sequence (10–100 s of kbs) among genomes (Lo et al. 2007) and provided evidence that adaptive genes may be sorted into populations by homologous recombination (Denef et al. 2010a). Such comprehensive "omic" analysis of a single microbial community remains unique, largely because current techniques are not mature enough to tackle the often orders of magnitude higher complexity. Although it is hard to guess how long it will take to assemble a reasonable number of genomes from various environmental samples, the AMD example, nonetheless, stresses that cultivation may not be necessary for in-depth analysis of ecology and evolution of microbial populations.

Emergence of Clusters

The question of whether clusters reliably represent ecologically relevant units requires consideration of the evolutionary processes that create them. To date, this has primarily been addressed from a theoretical standpoint; however, some observations on recently diverged or currently diverging populations provide some empirical insights into the process of cluster formation and maintenance (Cadillo-Quiroz et al. 2012; Shapiro et al. 2012).

The first important question is whether clusters can arise in sympatry via neutral processes, that is, as a result of the mutation and recombination processes without environmental selection. This may be addressed using a neutral model of mutation and drift to delineate the conditions under which populations do or do not separate into distinct genotypic clusters (Hanage et al. 2006c; Fraser et al. 2007). This requires us to take into account two important properties of recombination: first that its relative importance varies among different bacterial taxa (Spratt et al. 2001a), and second that empirical observations suggest homologous recombination drops in log-linear fashion with sequence divergence (although this may not be true for some archaea Grogan & Stengel 2008) (Majewski 2001) (*Fig. 2.2*). The overall question was whether in any population the combination of mutational and recombinational process could create strains that are different enough from the parent population to become genetically isolated.

The general conclusion of the above model was that clusters can form neutrally but that strong and permanent clustering is unlikely in the absence of selection (Fraser et al. 2007). For near clonal populations, in which point mutation is less or roughly equally important to homologous recombination in diversifying loci, neutral clustering was observed; however, clusters contained only low sequence diversity and were unstable, being subject to a continuous dynamic of formation by chance, neutral drift and eventual extinction. On the other hand, for sexual populations, in which homologous recombination outweighs mutation in generating nucleotide diversity, the model produced clusters only when unrealistically steep decline in recombination rates with sequence divergence were assumed. Although the authors cautioned that more empirical

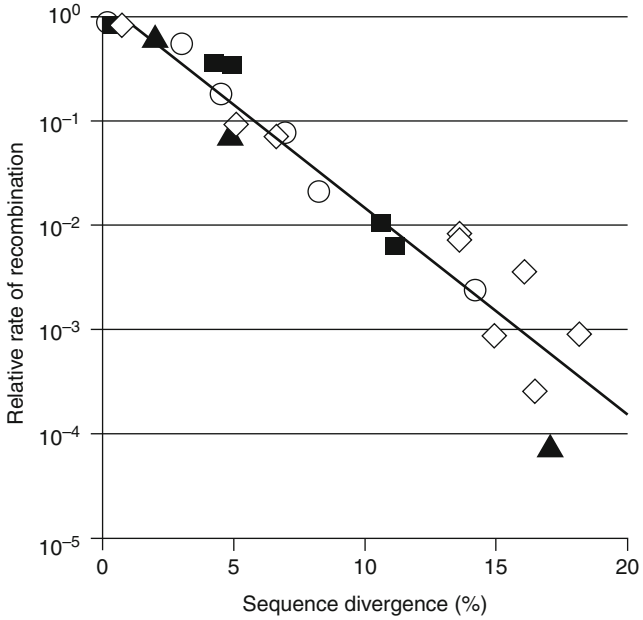


Fig. 2.2
Rate of recombination decreases loglinearly with sequence divergence between donor and recipient DNA

observations of the effect of low sequence divergence on recombination rates are needed, the results did overall highlight the importance of selection in creating permanently differentiated clusters in sympatry.

A central assumption of Mayr's biological species concept is that species frequently arise due to geographic isolation during which time they genetically diverge until they are reproductively isolated. If the physical barriers to gene flow are subsequently removed or overcome, reproductive isolation will ensure that the species remain distinct entities (Fraser et al. 2009). For microbes, the strongest evidence for geographic isolation triggering genotypic differentiation has been provided for globally dispersed populations of hot spring microbes (Papke et al. 2003; Whitaker et al. 2003). In addition, cluster formation during periods of geographic isolation followed by renewed coexistence has been inferred for two closely related, coexisting clusters of *Ferroplasma* in AMD biofilms (Denef et al. 2010b) and for animal-associated *Campylobacter* species (Sheppard et al. 2008). In the first case, failure to detect strong signs of positive selection across genomes was interpreted as clusters having arisen by drift during geographic isolation rather than by selection in sympatry. In the second case, evidence that recent but not more ancient recombination events crossed species boundaries was taken to indicate an ongoing "despeciation" process, blending previously established clusters (though this has been strongly critiqued on the grounds of the choice of loci (Caro-Quintero et al. 2009)). These examples highlight that geographic isolation, especially among differentially host-associated microbes, is a plausible driver for the formation of genotypic clusters; however, it is difficult to establish as a mechanism of formation for contemporary, coexisting clusters. Moreover, strong geographic

isolation is generally regarded as the exception due to the potential for efficient dispersal of environmental microbes.

The considerations above suggest the fundamental question: Do clusters arise as a consequence of ecological specialization? In other words: Does the spread of adaptive genes within populations trigger formation of genotypic clusters? The answer to this question must lie in the balance between recombination and selection and is illustrated by a simple comparison of rates. Because observed rates of homologous recombination are orders of magnitude lower than even moderate rates of selection, it follows that gene flow should not be high enough to unlink a gene under selection from the rest of the genome (Cohan 1994; Shapiro et al. 2009). Consequently, as the gene under selection increases in frequency within the population, so should the genome it resides in until it has outcompeted other genomes sharing a niche. This relationship is most saliently expressed by the "ecotype" theory, which posits that such selective sweeps may periodically purge genetic variation from populations (Cohan 2002). Subsequent to a sweep, the successful genome is free to diversify until similar patterns of clustering should be apparent at all loci, except for occasional discordant alleles introduced by homologous recombination from other populations (Hanage et al. 2005).

Although the ecotype theory is consistent with the observation of widespread clustering among microbes, it is strongly at odds with observations of environment-specific genes (Coleman and Chisholm 2010) and alleles (Papke et al. 2007), and reduced diversity at single loci amid high genome-wide polymorphism (Guttman and Dykhuizen 1994; Denef et al. 2010a). All of these suggest that genes can sweep independent of entire genomes and that genotypic clusters should, therefore, not arise as a consequence of environmental specialization. How to reconcile the two observations of gene-specific sweeps and widespread clustering is a fascinating subject for future research.

Recent observation of genome-wide events accompanying differentiation of two recently diverged populations of ocean bacteria demonstrates how ecological differentiation may drive genotypic differentiation and may help reconcile gene-specific sweeps with formation of genotypic clusters (Shapiro et al. 2012). These populations of *Vibrio cyclotrophicus* were originally identified by their divergence in a fast-evolving protein-coding gene and differential occurrence within the large and small-size fraction of filtered seawater, containing different types of organic particles and zoo- and phytoplankton (Hunt et al. 2008). This population structure was reproduced in an independent sampling 3 years later giving confidence that although the populations are nearly indistinguishable in most genes across the genomes, they represent distinguishable ecological units (Szabo et al. 2012).

Analysis of whole genome sequences from each of the differentially associated populations provided clear evidence that genome regions and not whole genomes have swept through each ecologically differentially associated population (Shapiro et al. 2012). Moreover, recent homologous and nonhomologous recombination events tended to be population-specific, while older events were not. Despite this differentiation in terms of recent gene flow, these nascent populations remained phylogenetically indistinguishable in most neutral marker genes due to the history of extensive within-population recombination.

Similarly, recent analysis of patterns of gene flow among very closely related populations of hot spring archaea suggested sympatric differentiation without genome-wide selective sweeps (Cadillo-Quiroz et al. 2012). Like in the *Vibrio* example, genomic comparison indicated decreasing homologous recombination over time, consistent with diverging populations; however, it was less clear which genes or genome regions may have been under environmental selection, thus initiating ecological separation.

Taken together, the above examples suggest a model where an ancestral, ecologically uniform and recombining population undergoes a split triggered by acquisition of habitat-specific genes (e.g., via horizontal gene transfer) by one or a few strains. These adaptive genes subsequently spread within a subpopulation via homologous or nonhomologous recombination. This may result in sufficiently different habitat preferences to limit recombination, thus founding a new, ecologically derived population. If differential associations remain stable, accumulation of population-specific mutations would further enhance genetic isolation due to the sharp decline in the efficiency of homologous recombination with sequence divergence (Majewski 2001) (*Fig. 2.2*). Horizontal gene transfer may further enhance this isolation since it has been postulated that this process may depress homologous recombination in the vicinity of acquired, habitat-specific genes (Lawrence 2002). Importantly, these observations on recently diverged populations suggest a mechanism of how gene-centered sweeps may eventually lead to genotypic clustering—a pattern characteristic of genome-wide sweeps. It is, however, not fully understood what factors might depress the effect of selection versus recombination. An obvious and likely candidate is negative frequency-dependent selection, for example, by viral and protozoan predators, but more research will be needed to address this issue.

Although it is far from clear how generally applicable the above mechanism of ecological differentiation is, the proposed mechanism is strikingly similar to models of speciation in sexual eukaryotes (Cadillo-Quiroz et al. 2012; Shapiro et al. 2012). However, this does not mean that bacteria will form biological species akin to animals and plants. Their genomes remain open to horizontal gene acquisition, which likely plays a more important role in adaptation than point mutation. Recent findings demonstrate that horizontal gene transfer among coexisting members of distinct species can occur at very high rates (Boucher et al. 2011; Smilllie et al. 2011). As illustrated in the next section, horizontal gene transfer happens via a variety of vehicles.

Horizontal Gene Transfer Processes

That bacterial inheritance is not strictly clonal, meaning that each lineage diversifies solely by the inheritance of vertically

transmitted mutations, has been known since Joshua Lederberg showed the horizontal inheritance of plasmids (Lederberg 1947, 1952). Plasmids, meaning extrachromosomal DNA molecules, were very early on recognized to be capable of carrying genes for diverse functions that were not necessarily needed by all the cells of the species in which they were found. Well-known examples of such genes include antibiotic resistance or heavy metal resistance. Additionally, they can carry genes for their own mobilization and transfer between cells, and have long been seen to some degree as selfish elements hitch-hiking a ride in the bacteria that propagate them. In this view, the additional functions carried by the plasmid act to mitigate against loss from the infected cell, by offering selective benefits in return for their maintenance.

Because the study of plasmids is nearly as old as the science of molecular biology, it is somewhat misleading to suggest that the importance of horizontal gene transfer (HGT) has only recently been recognized. However, the *extent* of horizontal gene transfer has been demonstrated to a degree that was quite unexpected only a few decades ago and which can still surprise the unwary.

As well as plasmids, bacteria contain a host of other mobile genetic elements (MGEs). These are too diverse to permit anything but the simplest summary here. In brief, MGEs at their most basic configuration contain the genes for their mobility (a transposase and integrase, e.g.) and exist as parasites on their bacterial hosts. However, multiple mechanisms exist by which MGEs can transfer host genetic material along with their own. While some MGEs transfer along with only a few genes—such as those that carry resistance determinants among lineages of various species and sometimes between species—the horizontal transfer that MGEs facilitate is capable of assembling large segments of DNA termed "genomic islands," which encode multiple genes/operons and usually code for entire additional functions (Juhas et al. 2009). Often termed "pathogenicity islands" because they were first identified by comparison of virulent and avirulent isolates within named species (Hacker and Carniel 2001; Gal-Mor and Finlay 2006), they in fact can encode adaptation to many different environments. Genomic islands bear many signs of being horizontally transferred: they typically show a GC content quite at odds with the rest of the genome; they insert at conserved tRNA genes; and they bear mobility genes. Non-plasmid MGEs include bacteriophage (Canchaya et al. 2003), integrons (Cambray et al. 2010), integrative conjugative elements (ICE) (Wozniak and Waldor 2010), transposons (conjugative and non-conjugative), and insertion sequence (IS) elements.

IS elements are composed only of the transposase necessary for their movement, but can exert a large impact on evolution through homologous recombination. This is the transfer of DNA without specific mobility genes. If two DNA molecules are sufficiently similar, then homologous recombination can occur between them, replacing one with the other (note the process is not reciprocal). The importance of homologous recombination arises because many bacteria actively take up DNA from the environment, for purposes that are not clear. Once the DNA is inside the cell, homologous recombination can occur. This has been a mechanism by which antibiotic resistance determinants and different antigenic types have spread throughout and between species. And in general, homologous recombination has the potential to spread a fit allele at any locus among those lineages with sufficient homology.

The synergy between IS elements and homologous recombination arises because although the efficiency of homologous recombination declines rapidly with increasing sequence divergence (*Fig. 2.2*), all that is required is a short region of near-perfect homology at the start and end of the transferred segment. This is how homologous recombination can transfer things like the capsular locus in the pneumococcus, which ranges in size from 10,337 (serotype 3) to 30,298 bp (serotype 38) (Bentley et al. 2006), and contains a large number of loci, many of which are not homologous to one another (Mavroidi et al. 2007). Conserved flanking regions permit transfer among otherwise not closely related strains. Two IS elements in a genome can and do act as such conserved flanking regions, effectively mobilizing whatever genes lie between them and recombining with other flanking regions. Moreover, if a MGE transfers a novel set of loci into a new genetic background, provided there is sufficiently conserved flanking sequence, those loci may readily be donated to other strains through homologous recombination. Similarly MGEs could be removed by recombination with the ancestral sequence.

Despite the diverse means by which DNA may be transferred between lineages, it should be appreciated that in almost all cases bacteria remain in significant linkage disequilibrium (Smith et al. 1993). Also the amounts and predominant mechanisms of HGT vary greatly. In some cases, for example *Neisseria gonorrhoeae*, homologous recombination is so common the organism is virtually panmictic, while in species like *Staphylococcus aureus*, the great majority of change is mutational, but transducing phage and similar elements are capable of generating significant evolutionary innovation (Feil et al. 2003).

It seems increasingly clear that recombination rates not only vary between species, they also vary within them. This can be seen from direct experiment and also population genetic analyses. Different lineages of pneumococcus exhibit different transformation rates in the lab (Lee et al. 2010). There is evidence that non-typable *Haemophilus influenzae* are more recombinogenic than those with a capsule (Meats et al. 2003), and the population structure of Group A meningococcus indicates far less homologous recombination than other serogroups (Bart et al. 2001). In the pneumococcus, statistical analysis of molecular epidemiological data showed that those strains showing evidence of recombination were significantly more likely to be resistant to all antibiotics for which data were available (Hanage et al. 2009). It is possible that recombination rates vary over the history of a lineage, and that at some point in the past they have been higher than now. If so, the distribution of recombination events will vary over time, with some regions of a phylogeny being marked by many events, after which the

normal background rate resumes. It is possible that such hyper-recombinogenic strains are being generated continuously (in much the same fashion as hyper-mutators (Denamur et al. 2000)) but only rarely benefit from sufficient genetic innovation to survive; those that do persist have restored their wild type recombination rates.

It should also be noted that further mechanisms likely remain to be discovered. Molecular epidemiology and genomics has shown conclusively that one of the most important MRSA clones is the result of a huge 0.5 Mb recombination event that combined the genomes of two other MRSA strains in a fashion more reminiscent of meiotic than bacterial processes (Robinson and Enright 2004). Moreover, recent analysis documented the likely horizontal acquisition and subsequent sweep through closely related strains of the smaller of two chromosomes in *V. cyclotrophicus* (Shapiro et al. 2012). And while it is known that HGT can transfer genetic material between bacterial species, it is increasingly clear that for bacteria that colonize eukaryotes, on occasion recombination can occur between superkingdoms. It is known that *Wolbachia* genes have inserted into the chromosome of their arthropod hosts (Dunning Hotopp et al. 2007), and the human long interspersed nuclear element L1 has been reported in the genome of some *N. gonorrhoeae* strains (Anderson and Seifert 2011).

Genomes and Clusters

Our ability to define the genetic structure of communities, populations, species, and genera has been intimately tied to technology, and in particular to DNA sequencing. The initial and groundbreaking study of conserved loci, such as 16S rRNA, has been extended in turn to housekeeping loci within and between species, and more recently to whole genomes. Genomic data from multiple isolates of the same species are becoming common. Given that "Next-Gen" methods are increasingly accessible and economical, in coming years genomic data are expected to become the standard currency among scientists studying these questions.

This is not to suggest that methods such as MLST and MLSA are already irrelevant or superseded. Both are likely to be useful to researchers for some time to come, and even as next-Gen sequencing becomes routine, the choices of which isolates should be sequenced to provide context from existing strains collections will likely be motivated by MLST/MLSA analysis. Two early examples of such population genomics (Harris et al. 2010; Croucher et al. 2011) have examined lineages in the major pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae*. In each, a lineage or clone of known clinical importance was selected on the basis of MLST, and then a global collaboration of scientists supplied isolates of that clone for sequencing. This approach has some major advantages. Notably, while homologous recombination scrambles phylogenetic signal in diverse datasets, when studying a single lineage, recombination can be readily identified by approaches that examine anomalous patterns of SNP variation (Didelot and Falush 2007; Tang et al. 2009; Didelot et al. 2010; Croucher et al. 2011; Marttinen et al. 2012). It was estimated that, in the case of the *S. pneumoniae* lineage, 75% of the genome had been changed by recombination in at least one isolate since the most recent common ancestor of the sample, which existed around 40 years previously. Such observations emphasize that recombination, homologous or not, cannot be ignored.

Conclusion

In conclusion, the profusion of genetic and genomic data is revolutionizing the study of prokaryote populations. It is becoming possible to lay out aspects of an approach to prokaryote population biology that should become standard. The following are, in our mind, essential components for the success of the field in the coming years.

First is the collection of a sample with systematic and well-documented stratification, together with an abundance of metadata on factors (such as physicochemical gradients) that may be relevant to strain success. It is reasonable to note that all appropriate metadata may not be evident at an early stage. However, the more that can be collected, the more valuable the study is likely to be in combination with future strain collections. For example, imagine a sample with the aim of identifying populations of a pathogen with a specific tropism for hosts of a certain age. If previous samples have also documented host age, these can be used to test the generality of the conclusions, or considered alongside the present sample. Any prior samples for which such data were not collected will be useless for the question under consideration.

For each isolate, as much sequence should be collected as is affordable. It is important to pay attention to differences between genome sequencing platforms in the types of data they provide, both in order to ensure samples can be compared between studies, and because some platforms have an intrinsically higher error rate. Sequencing errors may be of little consequence in some studies, but where very closely related strains are under analysis, they must be minimized. In practice, it is likely that the field will turn to genomic data. It should be appreciated that genomes need not be finished to yield valuable insights.

Because, at present, there is no standard computational approach to the problem of how populations should be identified, it is helpful to explore the data by applying multiple approaches to identify clusters and recombination between them, as well as selective processes. Empirical classification algorithms, that consider genetic and ecological data to jointly map isolates, should be favored.

To test whether the results of such tests are reasonable, the assumptions are justified, and the conclusions well supported, the results of these analyses should be used to parameterize evolutionary simulations. If the results of these recapitulate the observed patterns, then this supports both the model and the results. For the increasingly complex datasets that will be used

here, methods like approximate Bayesian computation (ABC) may be increasingly relevant.

The outcome of such an ambitious program of work, if successful, should be well-defined populations that are hypothesized to have arisen by well-understood means, which together define a model that can be applied to the study of other bacteria. It is by no means an easy task, but advances in technology and analytical capacity have made it a realistic goal.

Acknowledgements

We thank Nick Croucher for valuable discussions and suggestions. Funding was provided by the National Science Foundation, the National Institutes of Health and the Gordon and Betty Moore Foundation.

References

Acinas SG, Klepac-Ceraj V, Hunt DE, Pharino C, Ceraj I, Distel DL, Polz MF (2004) Fine-scale phylogenetic architecture of a complex bacterial community. Nature 430:551–554

Allen EE, Tyson GW, Whitaker RJ, Detter JC, Richardson PM, Banfield JF (2007) Genome dynamics in a natural archaeal population. Proc Natl Acad Sci USA 104:1883–1888

Anderson MT, Seifert HS (2011) Opportunity and means: horizontal gene transfer from the human host to a bacterial pathogen. mBio 2:e00005–e00011

Andersson AF, Banfield JF (2008) Virus population dynamics and acquired resistance in natural microbial communities. Science 320:1047–1050

Azam F, Long RA (2001) Oceanography – sea snow microcosms. Nature 414:495

Bart A, Barnabe C, Achtman M, Dankert J, van der Ende A, Tibayrenc M (2001) The population structure of *Neisseria meningitidis* serogroup A fits the predictions for clonality. Infect Genet Evol 1:117–122

Becraft ED, Cohan FM, Kühl M, Jensen SI, Ward DM (2011) Fine-scale distribution patterns of *Synechococcus* ecological diversity in microbial mats of Mushroom Springs, Yellowstone. Appl Environ Microbiol 77:7689–7697

Bentley SD, Aanensen DM, Mavroidi A, Saunders D, Rabbinowitsch E, Collins M, Donohoe K, Harris D, Murphy L, Quail MA, Samuel G, Skovsted IC, Kaltoft MS, Barrell B, Reeves PR, Parkhill J, Spratt BG (2006) Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. PLoS Genet 2:e31

Boucher Y, Cordero OX, Takemura A, Hunt DE, Schliep K, Bapteste E, Lopez P, Tarr CL, Polz MF (2011) Local mobile gene pools rapidly cross species boundaries to create endemicity within global *Vibrio cholerae* populations. mBio 2:e00335–00310

Bouman HA, Ulloa O, Scanlan DJ, Zwirglmaier K, Li WKW, Platt T, Stuart V, Barlow R, Leth O, Clementson L, Lutz V, Fukasawa M, Watanabe S, Sathyendranath S (2006) Oceanographic basis of the global surface distribution of *Prochlorococcus* ecotypes. Science 312:918–921

Cadillo-Quiroz H, Didelot X, Heid NL, Herrara A, Darling A, Reno ML, Krause DJ, Whitaker RJ (2012) Patterns of gene flow define species of thermophilic archaea. PLoS Biol 10:e1001265

Cambray G, Guerout AM, Mazel D (2010) Integrons. Annu Rev Genet 44: 141–166

Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann ML, Brussow H (2003) Phage as agents of lateral gene transfer. Curr Opin Microbiol 6:417–424

Caro-Quintero A, Rodriguez-Castano GP, Konstantinidis KT (2009) Genomic insights into the convergence and pathogenicity factors of *Campylobacter jejuni* and *Campylobacter coli* species. J Bacteriol 191:5824–5831

Cheng L, Connor TR, Aanensen DM, Spratt BG, Corander J (2011) Bayesian semi-supervised classification of bacterial samples using MLST databases. BMC Bioinformatics 12:302

Cohan FM (1994) The effects of rare but promiscuous genetic exchange on evolutionary divergence in prokaryotes. Am Nat 143:965–986

Cohan FM (2002) What are bacterial species. Annu Rev Microbiol 56:457–487

Coleman ML, Chisholm SW (2010) Ecosystem-specific selection pressures revealed through comparative population genomics. Proc Natl Acad Sci USA 107:18634–18639

Connor N, Sikorski J, Rooney AP, Kopac S, Koeppel AF, Burger A, Cole SG, Perry EB, Krizanc D, Field NC, Slaton M, Cohan FM (2010) Ecology of speciation in the genus Bacillus. Appl Environ Microbiol 76:1349–1358

Corander J, Marttinen P, Siren J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539

Corander J, Tang J (2007) Bayesian analysis of population structure based on linked molecular information. Math Biosci 205:19–31

Corander J, Waldmann P, Sillanpaa MJ (2003) Bayesian analysis of genetic differentiation between populations. Genetics 163:367–374

Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, McGee L, von Gottberg A, Song JH, Ko KS, Pichon B, Baker S, Parry CM, Lambertsen LM, Shahinas D, Pillai DR, Mitchell TJ, Dougan G, Tomasz A, Klugman KP, Parkhill J, Hanage WP, Bentley SD (2011) Rapid pneumococcal evolution in response to clinical interventions. Science 331:430–434

Denamur E, Lecointre G, Darlu P, Tenaillon O, Acquaviva C, Sayada C, Sunjevaric I, Rothstein R, Elion J, Taddei F, Radman M, Matic I (2000) Evolutionary implications of the frequent horizontal transfer of mismatch repair genes. Cell 103:711–721

Denef VJ, Kalnejais LH, Mueller RS, Wilmes P, Baker BJ, Thomas BC, VerBerkmoes NC, Hettich RL, Banfield JF (2010a) Proteogenomic basis for ecological divergence of closely related bacteria in natural acidophilic microbial communities. Proc Natl Acad Sci USA 107:2383–2390

Denef VJ, Mueller RS, Banfield JF (2010b) AMD biofilms: using model communities to study microbial evolution and ecological complexity in nature. ISME J 4:599–610

Didelot X, Falush D (2007) Inference of bacterial microevolution using multilocus sequence data. Genetics 175:1251–1266

Didelot X, Lawson D, Darling A, Falush D (2010) Inference of homologous recombination in bacteria using whole-genome sequences. Genetics 186:1435–1449

Doolittle WF, Papke RT (2006) Genomics and the bacterial species problem. Genome Biol 7:116

Dunning Hotopp JC, Clark ME, Oliveira DC, Foster JM, Fischer P, Munoz Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317:1753–1756

Eppley JM, Tyson GW, Getz WM, Banfield JF (2007) Genetic exchanges across a species boundary in the archaeal genus *Ferroplasma*. Genetics 177:407–416

Falush D, Wirth T, Linz B, Pritchard JK, Sephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Mégraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S (2003) Traces of human migrations in *Helicobacter pylori* populations. Science 299: 1582–1585

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes. doi:10.1111/j.1471-8286.2007.01758.x

Feil EJ, Enright MC, Spratt BG (2000) Estimating the relative contributions of mutation and recombination to clonal diversification: a comparison between *Neisseria meningitidis* and *Streptococcus pneumoniae*. Res Microbiol 151:465–469

Feil EJ, Holmes EC, Bessen DE, Chan MS, Day NP, Enright MC, Goldstein R, Hood DW, Kalia A, Moore CE, Zhou J, Spratt BG (2001) Recombination within natural populations of pathogenic bacteria: short-term empirical estimates and long-term phylogenetic consequences. Proc Natl Acad Sci USA 98:182–187

Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, Peacock SJ, Smith JM, Murphy M, Spratt BG, Moore CE, Day NP (2003) How clonal is *Staphylococcus aureus*? J Bacteriol 185:3307–3316

Ferris MJ, Kühl M, Wieland A, Ward DM (2003) Cyanobacterial ecotypes in different optical microenvironments of a 68 degrees hot spring mat

community revealed by 16 S-23 S rRNA internal transcribed spacer region variation. Appl Environ Microbiol 69:2893–2898

Fraser C, Hanage WP, Spratt BG (2005) Neutral microepidemic evolution of bacterial pathogens. Proc Natl Acad Sci USA 102:1968–1973

Fraser C, Hanage WP, Spratt BG (2007) Recombination and the nature of bacterial speciation. Science 315:476–480

Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP (2009) The bacterial species challenge: making sense of genetic and ecological diversity. Science 232:741–746

Gal-Mor O, Finlay BB (2006) Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell Microbiol 8:1707–1719

Gevers D, Cohan FM, Lawrence JG, Spratt BG, Coenye T, Feil EJ, Stackebrandt E, Van de Peer Y, Vandamme P, Thompson FL, Swings J (2005) Re-evaluating prokaryotic species. Nat Rev Microbiol 3:733–739

Goodfellow M, Manfio GP, Chun J (1997) Towards a practical species concept for cultivable bacteria. In: Claridge MF, Dawah HA, Wilson MR (eds) The units of biodiversity. Chapman and Hall, London

Grogan DW, Stengel KR (2008) Recombination of synthetic oligonucleotides with prokaryotic chromosomes: substrate requirements of the *Escherichia coli* 1 Red and *Sulfolobus acidocaldarius* recombination systems. Mol Microbiol 69:1255–1265

Guttman DS, Dykhuizen DE (1994) Detecting selective sweeps in naturally occurring *Escherichia coli*. Genetics 138:993–1003

Hacker J, Carniel E (2001) Ecological fitness, genomic islands and bacterial pathogenicity – a Darwinian view of the evolution of microbes. EMBO Rep 2:376–381

Hanage WP, Fraser C, Spratt BG (2005) Fuzzy species among recombinogenic bacteria. BMC Biol 3. doi:10.1186/1741-7007-1183-1186

Hanage WP, Fraser C, Spratt BG (2006a) The impact of homologous recombination on the generation of diversity in bacteria. J Theor Biol 239:210–219

Hanage WP, Fraser C, Spratt BG (2006b) Sequences, sequence clusters and bacterial species. Philos Trans R Soc Lond B 361:1917–1927

Hanage WP, Spratt BG, Turner KM, Fraser C (2006c) Modeling bacterial speciation. Philos Trans R Soc Lond B Biol Sci 361:2039–2044

Hanage WP, Fraser C, Tang J, Connor TR, Corander J (2009) Hyper-recombination, diversity, and antibiotic resistance in pneumococcus. Science 324:1454–1457

Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD (2010) Evolution of MRSA during hospital transmission and intercontinental spread. Science 327:469–474

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332

Hunt DE, David LD, Gevers D, Preheim SP, Alm EJ, Polz MF (2008) Resource partitioning and sympatric differentiation among closely related bacterioplankton. Science 320:1081–1085

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS, Chisholm SW (2006) Niche partitioning among *Prochlorococcus ecotypes* along ocean-scale environmental gradients. Science 311:1737–1740

Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW (2009) Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol Rev 33:376–393

Keswani J, Whitman WB (2001) Relationship of 16 S rRNA sequence similarity to DNA hybridization in prokaryotes. Int J Syst Evol Microbiol 51:667–678

Koeppel A, Perry EB, Sikorski J, Krizanc D, Warner A, Ward DM, Rooney AP, Brambilla E, Connor N, Ratcliff RM, Nevo E, Cohan FM (2008) Identifying the fundamental units of bacterial diversity: a paradigm shift to incorporate ecology into bacterial systematics. Proc Natl Acad Sci USA 105:2504–2509

Konstantinidis KT, DeLong EF (2008) Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. ISME J 10:1052–1065

Lawrence JG (2002) Gene transfer in bacteria: speciation without species? Theor Pop Biol 61:449–460

Lederberg J (1947) Gene Recombination and linked segregations in *Escherichia coli*. Genetics 32:505–525

Lederberg J (1952) Cell genetics and hereditary symbiosis. Physiol Rev 32: 403–430

Lee JY, Song JH, Ko KS (2010) Recombination rates of *Streptococcus pneumoniae* isolates with both erm(B) and mef(A) genes. FEMS Microbiol Lett 309: 163–169

Lo I, Denef VJ, VerBerkmoes NC, Shah M, Goltsman D, DiBartolo G, Tyson GW, Allen EE, Ram RJ, Detter JC, Richardson PM, Thelen MP, Hettich RL, Banfield JF (2007) Strain-resolved community proteomics reveals recombining genomes of acidophilic bacteria. Nature 446:537–541

Maiden MC (2006) Multilocus sequence typing of bacteria. Annu Rev Microbiol 60:561–588

Maiden MC, Bygraves JA, Feil E, Morelli G, Russel JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA 95:3140–3145

Majewski J (2001) Sexual isolation in bacteria. FEMS Microbiol Lett 199:161–169

Marttinen P, Hanage WP, Croucher NJ, Connor TR, Harris SR, Bentley SD, Corander J (2012) Detection of recombination events in bacterial genomes from large population samples. Nucleic Acids Res 40:e6

Mavroidi A, Aanensen DM, Godoy D, Skovsted IC, Kaltoft MS, Reeves PR, Bentley SD, Spratt BG (2007) Genetic relatedness of the *Streptococcus pneumoniae* capsular biosynthetic loci. J Bacteriol 189:7841–7855

McInerney JO, Pisani D, Bapteste E, O'Connell MJ (2011) The public goods hypothesis for the evolution of life on earth. Biol Direct 6:41

Meats E, Feil EJ, Stringer S, Cody AJ, Goldstein R, Kroll JS, Popovic T, Spratt BG (2003) Characterization of encapsulated and noncapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. J Clin Microbiol 41:1623–1636

Medini D, Donati C, Tettelin H, Masignani V, Rappuoli R (2005) The microbial pan-genome. Curr Opin Genet Dev 15:589–594

Moore LR, Rocap G, Chisholm SW (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393:464–467

Nazer HM (1992) Early diagnosis of cystic fibrosis in Jordanian children. J Trop Pediatr 38:113–115

Orozco-terWengel P, Corander J, Schlotterer C (2011) Genealogical lineage sorting leads to significant, but incorrect Bayesian multilocus inference of population structure. Mol Ecol 20:1108–1121

Papke RT, Ramsing NB, Bateson MM, Ward DM (2003) Geographical isolation in hot spring cyanobacteria. Environ Microbiol 5:650–659

Papke RT, Zhaxybayeva O, Feil EJ, Sommerfeld K, Muise D, Doolittle WF (2007) Searching for species in haloarchaea. Proc Natl Acad Sci USA 104:14092–14097

Polz MF, Hunt DE, Preheim SP, Weinreich DM (2006) Patterns and mechanisms of genetic and phenotypic differentiation in marine microbes. Phil Trans R Soc Lond B 361:2009–2021

Preheim SP, Boucher Y, Wildschutte H, David LA, Veneziano D, Alm EJ, Polz MF (2011a) Metapopulation structure of *Vibrionaceae* among coastal marine invertebrates. Environ Microbiol 13:265–275

Preheim SP, Timberlake S, Polz MF (2011b) Merging taxonomy with ecological population prediction: a case study of Vibrionaceae. Appl Environ Microbiol 77:7195–7206

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM (2012) *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3:e00305–e00311

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Robinson DA, Enright MC (2004) Evolution of *Staphylococcus aureus* by large chromosomal replacements. J Bacteriol 186:1060–1064

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER, Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature 424:1042–1047

Rosselló-Mora R, Amann R (2001) The species concept for prokaryotes. FEMS Microbiol Rev 25:39–67

Shapiro BJ, David LA, Friedman J, Alm EJ (2009) Looking for Darwin's footprints in the microbial world. Trends Microbiol 17:196–204

Shapiro BJ, Friedman J, Cordero OX, Preheim SP, Timberlake SC, Szabo G, Polz MF, Alm EJ (2012) Population genomics of early events in the ecological differentiation of bacteria. Science 336:48–51

Sheppard SK, McCarthy ND, Falush D, Maiden MCJ (2008) Convergence of Campylobacter species: implications for bacterial evolution. Science 320:237–239

Smilllie CS, Smith MB, Friedman J, Cordero OX, Alm EJ (2011) Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480:241–244

Smith JM, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? Proc Natl Acad Sci USA 90:4384–4388

Smith JM, Feil EJ, Smith NH (2000) Population structure and evolutionary dynamics of pathogenic bacteria. BioEssays 22:1115–1122

Spratt BG, Hanage WP, Feil EJ (2001a) The relative contribution of recombination and point mutation to the diversification of bacterial clones. Curr Opin Microbiol 4:602–606

Spratt BG, Hanage WP, Feil EJ (2001b) The relative contributions of recombination and point mutation to the diversification of bacterial clones. Curr Opin Microbiol 4:602–606

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

Szabo G, Preheim SP, Kauffman AKM, David LA, Shapiro BJ, Alm EJ, Polz MF (2012) Reproducibility of *Vibrionaceae* population structure in coastal bacterioplankton (Submitted)

Tang J, Hanage WP, Fraser C, Corander J (2009) Identifying currents in the gene pool for bacterial populations using an integrative approach. PLoS Comput Biol 5:e1000455

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concept in fungi. Fungal Genet Biol 31:21–32

Tettelin H, Riley D, Cattuto C, Medini D (2008) Comparative genomics: the bacterial pan-genome. Curr Opin Microbiol 12:472–477

Thompson JR, Pacocha S, Pharino C, Klepac-Ceraj V, Hunt DE, Benoit J, Sarma-Rupavtarm R, Distel DL, Polz MF (2005) Genotypic diversity within a natural coastal bacterioplankton population. Science 307: 1311–1313

Tyson GW, Banfield JF (2008) Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms. Environ Microbiol 10:200–207

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301:976–978

Wilmes P, Simmons SL, Denef VJ, Banfield JF (2009) The dynamic genetic repertoire of microbial communities. FEMS Microbiol Rev 33:109–132

Wozniak RA, Waldor MK (2010) Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat Rev Microbiology 8:552–563

3 Alpine and Arctic Soil Microbial Communities

M. Rhodes · J. Knelman · R. C. Lynch · J. L. Darcy · D. R. Nemergut · S. K. Schmidt
Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA

Introduction

Cold environments, where average daily air temperatures are below 5 °C throughout the year, are widespread in the terrestrial biosphere (Zakhia et al. 2008). These ecosystems are common in high mountain ranges, the high Arctic and Antarctica. In these areas, cold temperatures are often accompanied by freeze-thaw cycles, seasonally high solar radiation exposure, low nutrient supply, limited water availability, and high salinity. As a result of these conditions, such environments are mostly devoid of higher plants and are instead dominated by microbial communities. These areas have been referred to as the subnival zone, cold deserts, the allobiosphere, or the aeolian zone among other names (Bahl et al. 2011; Edwards 1988; King et al. 2008; Mayilraj et al. 2005; Swan 1992). Due to the absence of plants and large animals, comprehensive studies of the diversity of these ecosystems have been limited. However, recent advances in molecular techniques have allowed for more extensive study of the microorganisms that are abundant in these seemingly barren areas. High mountain cold desert areas are also receiving recent attention because they are in watersheds that are the source of water (from snow and ice melt) for a large proportion of the world's population, especially in areas downstream from the Andes, Rockies, and Himalayan Ranges. For example, the Himalayan-Karakorum-Hindu Kush Mountain complex provides water for approximately 1.3 billion people (Hua 2009). In addition, the global extent of cold, plant-free ecosystems is presently expanding rapidly as a result of glacier and ice cap melting due to global warming (Bradley et al. 2006; Byers 2007; Racoviteanu et al. 2008).

In this chapter, we review and synthesize information about bacterial and archaeal communities from high-latitude and high-elevation ecosystems across the globe. The relationship between latitude and elevation in demarcating the plant-free zone is illustrated in ❯ *Fig. 3.1*, which shows the approximate relationship of the upper and lower boundaries of the cold deserts with regard to latitude. The highest elevation plant-free ecosystems occur in the horse latitudes (approximately 30° north or south latitude), which are high-pressure zones that also contain the highest mountains on Earth, e.g., parts of the Andes, Karakorum, and Himalayan ranges (❯ *Fig. 3.1*). Despite the overall simplicity of ❯ *Fig. 3.1*, cold, plant-free ecosystems can have different microclimates and, therefore, the ecological barriers to plant establishment can vary depending on wind speeds, slope and aspect among other environmental variables (Ley et al. 2004). There are three main ecosystem types at extreme latitudes and elevations (Schmidt et al. 2012): (1) Exposed soils (usually windblown) that are devoid of snow cover for most of the year and are too cold or dry for plant growth. (2) Long duration snow packs ("snow-bank soils") that are covered with snow for more than 10 months each year making it impossible for plants to complete their life cycles. (3) Recently deglaciated (early successional), undeveloped soils that may eventually be colonized by plants, at least in regions with milder climates. It is useful to distinguish between these ecosystem types because the microbial communities that are adapted to cold, dry soils are very different from those found in snow bank soils or early successional soils (Schmidt et al. 2012). Despite the inability of plant life to colonize these areas, evidence suggests that all three ecosystem types described above have intermittently active microbial communities.

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_37,

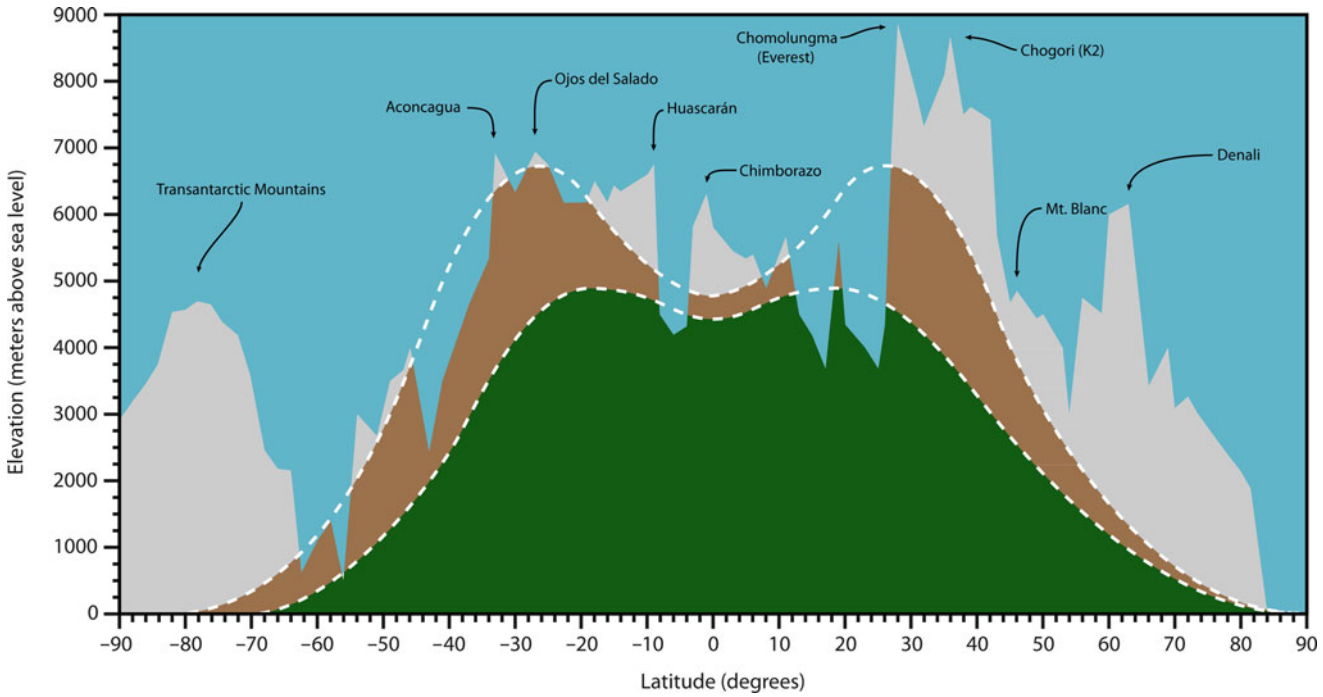


Fig. 3.1

The figure shows the relationship between latitude and elevation in determining the elevation limits for the mostly plant-free "cold deserts" (*brown*) discussed in this chapter. Vegetated regions are shown in *green* and permanently ice-covered regions in *light gray*. Negative latitudes represent the Southern Hemisphere. Boundaries between zones are approximate because they can vary dramatically even in one mountain range due to slope and aspect and distance from large bodies of water. The highest mountains in each region are marked for reference. The elevation estimates were obtained by searching latitudinal transects for mountain ranges and identifying the highest peaks in those ranges using the Google Maps Elevation API, and estimates for the latitudinal limits of plant distribution were obtained from Smith and Poncet (1985)

Although alpine and polar habitats share many common attributes (see above) there are also important differences. For example, the angle of solar insolation, partial pressure of gases, the extent of permafrost, and the duration of the growing season all vary markedly between alpine and polar environments (Nemergut et al. 2005; Richardson et al. 2003). Photoperiods are also very different in polar and alpine environments, as very high-latitude polar regions can receive up to 24 h of daylight in the summer, while mid-latitude alpine areas receive less than 15 h (Richardson et al. 2003). These differences result in greater fluctuations in temperature in alpine regions, especially in the summer when soils can freeze at night and warm up to above 55 °C during the day (King et al. 2010a; Lynch et al. 2012; Schmidt et al. 2009). In addition, alpine regions are often characterized by low relative humidity and steep slopes that enhance runoff, both of which increase moisture stress (Richardson et al. 2003). Other similarities and differences between polar and alpine regions are reviewed in Nemergut et al. (2005).

We focus here on describing the prokaryotic diversity of cold desert systems with emphasis on areas that are mostly devoid of plants due to extreme dryness (category 1, described above). A review of fungi in extreme cold environments has been recently published (Schmidt et al. 2012). Interest in these environments has exploded in recent years because they represent some of the most extreme terrestrial environments on Earth and are seen by some as the best analogs for Martian soils (Lee et al. 2011; Lynch et al. 2012; Schmidt et al. 2011b).

Site Descriptions

High Alpine

High-elevation cold deserts can be found in all of the major mountain ranges on Earth (*Fig. 3.1*), but are especially prevalent in the vast ranges of the Andes and Himalayan-Karakorum-Hindu Kush complex. Due to topographic heterogeneity and climatic variability the reasons for the lack of plants in high-elevation environments can vary dramatically as described above. In wetter regions such as the Alps and parts of the Rocky Mountains, the long duration of snow cover can limit plant establishment ("snow bed" communities) even when there is enough moisture to support plant growth. However, true high-elevation cold deserts mostly exist in drier regions

where the duration of snow cover is reduced and soils are exposed to drying winds, temperature fluctuations, and high levels of solar radiation for most of the year. The reality of climate change can further confound our understanding of high-elevation ecosystems, in that recent rapid retreat of glaciers has exposed large areas of new soils that may or may not eventually be colonized by plants, depending on long-term patterns of moisture availability (Nemergut et al. 2007).

Much of our current understanding of high-elevation cold soils comes from studies done at the Niwot Ridge Long-Term Ecological Research (LTER) site in the Colorado Rocky Mountains. The range of microenvironments at this site is wide and encompasses a continuum of snow bed to wind scoured, low snow habitats (King et al. 2010b; Ley et al. 2004). Most work at this site has been focused on the wetter end of this spectrum where soils are covered with snow for up to 10 months of the year and are only snow-free during the driest parts of the year (late summer and early fall). These sites contain complex microbial communities including multiple trophic levels of eukaryotes as described elsewhere (Freeman et al. 2009a, b). Early successional, high-elevation systems have been mostly studied in the European Alps, where plant colonization can be fairly rapid (Edwards et al. 2006; Miniaci et al. 2007). However, recent work on early successional soils in dry regions of the Andes is providing new insights into the earliest microbial stages of succession in the absence of plant invasion (Nemergut et al. 2007; Schmidt et al. 2008). Finally, the most extreme high-altitude environments studied to date are soils on the flanks of the largest volcanoes on Earth, in the Atacama region on the border between Chile and Argentina (Costello et al. 2009). The combination of the elevation (>6, 000 m.a.s.l.) and the aridity of the region make these some of the most oligotrophic and lowest diversity unglaciated, surface environments on Earth (Lynch et al. 2012). The present chapter focuses mostly on cold deserts of high alpine ecosystems with comparisons to polar deserts whenever possible.

Antarctica

Only about 0.4% or roughly 56,000 km^2 of the Antarctic continent is ice free. Of the 56,000 km^2, the McMurdo Dry Valleys account for more than 15% of the ice-free ground representing both the largest contiguous expanse of ice-free landscape and the best studied in terms of microbiology (Bockheim and McLeod 2008; Cary et al. 2010). The Dry Valleys are located in Southern Victoria Land along the western coast of McMurdo Sound and they remain ice free largely due to the presence of the Trans-Antarctic mountain range that blocks the flow of the East Antarctic Ice Sheet (Bockheim and McLeod 2008). Much of the remaining ice-free portions of Antarctica occur either along or near the coast or at high elevations in the Ellsworth, Transatlantic, and North Victoria Land mountains.

As with the other cold deserts reviewed in this chapter, inhabitants of the Dry Valleys are challenged with frigid temperatures (mean annual temperatures range from −15 °C to −30 °C), fluctuating temperatures (temperature fluctuations exceeding 20 °C are common), high UV radiation, low precipitation (less than 10 cm a year) and limited water availability (<2% soil water content) (Marchant and Head 2007; Smith et al. 1992; Vincent 1988). In addition these challenges are compounded by the often gale force katabatic winds that further desiccate the landscape and the high salt content of the soils (Claridge and Campbell 1976). It is for these reasons that the Dry Valleys represent one of the least hospitable environments on Earth and are often considered to be one of the coldest and driest deserts on the planet (Hopkins et al. 2006; Onofri 2004). Nevertheless, the Dry Valleys are home to a diverse assemblage of bacterial taxa and a limited number of Eukaryotes (see below and Cary et al. 2010).

The Arctic

Although tundra has received the most attention in past research, polar deserts constitute a major component of the Arctic comprising 44% of the Canadian high Arctic (Bliss and Gold 1999). Compared to other Arctic habitats, floral and faunal diversity and abundance are markedly lower in the Arctic deserts where growing seasons are about 2 months long (Bliss 1981). Like other cold deserts, Arctic deserts are characterized by limited moisture, extremes of temperature, UV radiation, nutrient limitations, and cryogenic disturbance (Cockell and Stokes 2006; Dickson 2000). Sorted and non-sorted polygons and soil stripes are common formations of patterned ground in these systems (Bliss 1981). While mosses predominate over lichens and vascular plants in total cover, both vascular plant and cryptogamic cover typically remain at <5% (Bliss 1981; Bliss et al. 1984; Bliss and Gold 1999). As a result, Arctic deserts display levels of soil organic matter, vegetation biomass, and net primary production one to three orders of magnitude lower than other already highly constrained Arctic ecosystem types (Callaghan et al. 2004). Nonetheless, the presence of sparse vascular plant cover in addition to lichens and moss stands in contrast to the Dry Valleys of Antarctica and the extreme high-elevation sites reviewed in this chapter. However, there are completely plant-free areas in the high Arctic, but it is not clear if these areas are just early successional stages that will eventually be colonized by plants.

Microbial Biomass and Activity

The first published description of the Dry Valleys of Antarctica by Captain Robert Falcon Scott in 1903 captured the seemingly lifeless nature of this and other cold desert landscapes where he noted the virtual absence of vascular plants, mosses, and even lichens (Scott 1905). The earliest studies of cold deserts in alpine

and polar systems focused primarily on the visible accumulations of organic matter including seasonal streams, endoliths, and ice-covered lakes (Friedmann 1982; Friedmann et al. 1993; Green et al. 1989; McKnight 1989; Swan 1963). When focus shifted to the barren soils, the largely culture-based surveys revealed an exceptionally low abundance and diversity of bacteria (e.g., 10^2–10^4 g^{-1}, reviewed in Cowan and Tow 2004). Conversely, more sensitive ATP-based assays suggest cellular densities several orders of magnitude higher at 10^6–10^8 prokaryotic cells g^{-1} (Cowan et al. 2002). Other recent efforts to quantify microbial biomass using the chloroform fumigation method have shown that dry sites in Antarctica and the high Himalayas (>5,500 m.a.s.l.) have biomass levels averaging about 20 μg microbial carbon per gram of soil (Ball et al. 2009; Schmidt et al. 2011b). At extreme high-elevation sites (>6,000 m.a.s.l.) in the Atacama region, Lynch et al. (2012) found microbial biomass levels of zero to 60 μg C g^{-1} using the same technique. For comparison, the biomass levels measured at these three extreme sites are two to three orders of magnitude lower than levels found in forest or tundra soils, and an order of magnitude lower than biomass levels in wetter (but plant-free) high-altitude soils of Colorado and Perú (King et al. 2008).

Despite the recent studies reporting measurable levels of microbial biomass in the most extreme cold desert soils on the planet (Ball et al. 2009; Lynch et al. 2012; Schmidt et al. 2011), it is still not clear how active these microbes are. In addition, the origin of the soil organic matter in these soils is unclear. Initial speculation centered on the Aeolian deposition of material from the more organic rich areas in the vicinity (Horowitz et al. 1972; Swan 1992). However, more recent isotopic analysis of the Antarctic Dry Valley organic matter suggests a limited role for present day aeolian redistribution of lacustrine carbon. Instead the data suggest that legacy carbon from late Wisconsin paleoenvironments and accumulation of autotrophic carbon fixation though the subsequent millennia account for the majority of current soil carbon stocks (Burkins et al. 2000; Moorhead et al. 1999). Furthermore, many cold desert soils harbor cyanobacteria, which can ostensibly power in situ photoautotrophic primary productivity (Freeman et al. 2009b; Novis et al. 2007; Starkenburg et al. 2011). Finally, the possibility of chemolithotrophic carbon fixation has not been adequately addressed in these systems, it is hinted at by the presence of putatively chemoautotrophic Thaumarchaeal and mixotrophic Actinobacterial sequences (Lynch et al. 2012; Tiao et al. 2012; Yergeau et al. 2007) and by limited studies in Arctic deserts when iron and sulfur compounds are prevalent (Borin et al. 2010).

Even in some of the most arid high alpine and Dry Valley sites, soil CO_2 flux is in fact measurable and estimates of mean residence time of C in Taylor Valley soils were on the order of 20 years indicating some in situ carbon fixation (Burkins et al. 2001; Parsons et al. 2004). Furthermore, although limited, recent culture-independent diversity assays have also shown that cold desert soils harbor previously underestimated diversity and unique prokaryotic community structures (see below and Lynch et al. 2012; Niederberger et al. 2008; Smith et al. 2006; Schmidt et al. 2011; Yergeau et al. 2007). It therefore appears likely that cold desert microbial communities are more complex and active than previously thought. Nevertheless, it remains to be seen which components of these systems are active and to what extent the heterotrophic community is dependent on endogenous autotrophy.

Microbial Diversity of Cold Desert Soils

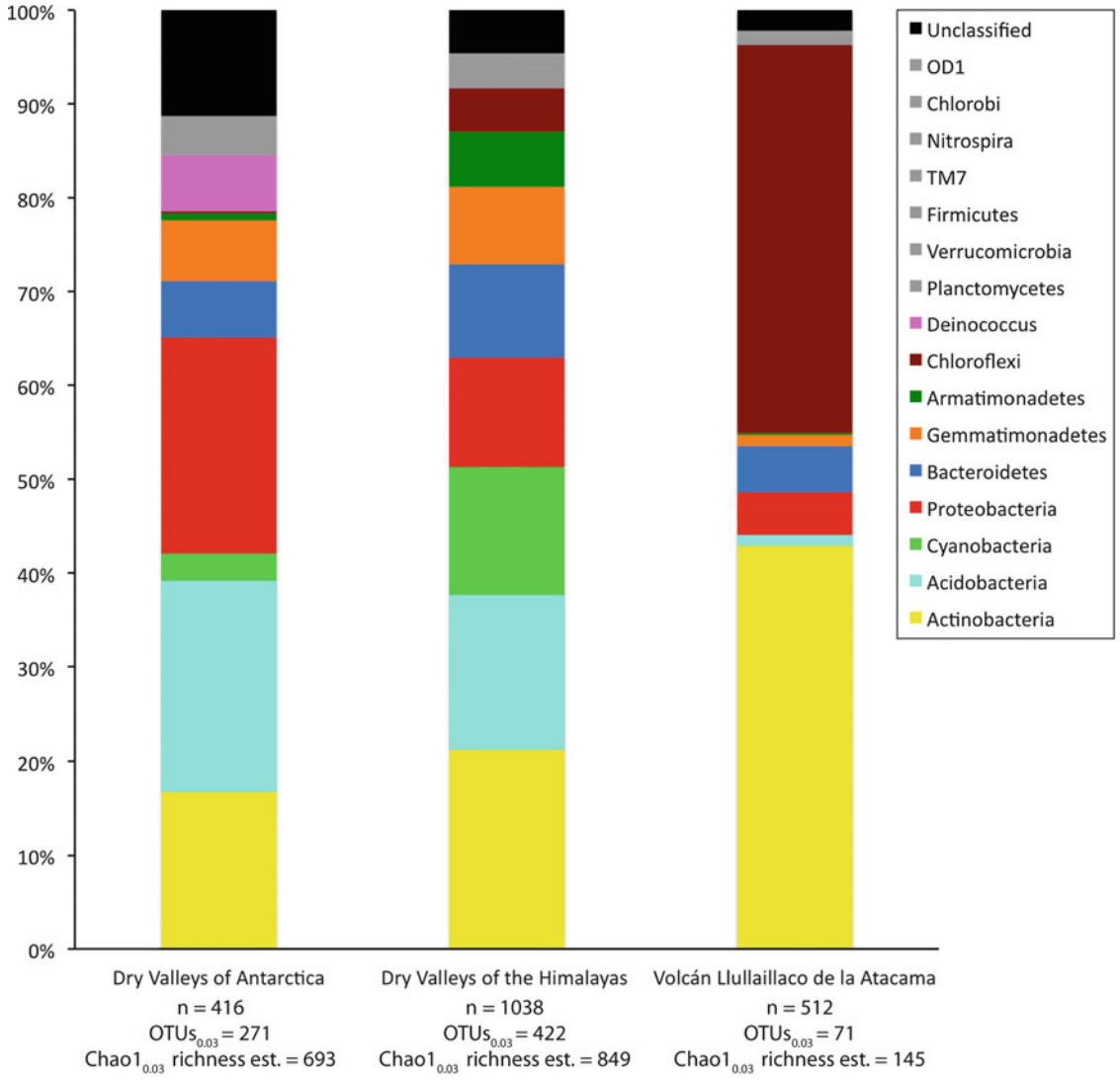
In this section, we give a brief overview of recent research on the microbial diversity of cold desert soils and then compare diversity across these environmentally similar, but geographically distant habitats.

Alpine

Culture-independent surveys of plant-free, high-elevation soils of the Rocky Mountains indicate that these heavily snowpack-impacted soils have a very high level of bacterial diversity approaching that of soils from temperate vegetated areas (Costello and Schmidt 2006; Freeman et al. 2009b; King et al. 2010b). However, drier high-elevation habitats have much lower bacterial diversity. For example, culture-independent surveys of high-elevation mineral soils from the dry valleys of the Himalayas, Vólcan Llullaillaco, and Vólcan Socompa show much lower levels of diversity (Costello et al. 2009; King et al. 2010b; Lynch et al. 2012)—very similar to the diversity of bacteria in the Dry Valleys of Antarctica (*Fig. 3.2*). The dominant groups of bacteria in cold deserts of the high Himalayas are Actinobacteria, Acidobacteria, and Cyanobacteria; whereas the dominant bacteria in the high Andes (Vólcan Llullaillaco) are Actinobacteria and Chloroflexi (*Fig. 3.2*). The apparent absence of Cyanobacteria on Vólcan Llullaillaco (Lynch et al. 2012) and the driest sites on Vólcan Socompa (Costello et al. 2009) is unique for a plant-free soil and Lynch et al. (2012) speculate that the dryness, high elevation, and extreme UV flux on Llullaillaco make it unsuitable for photosynthetic life. Another intriguing finding from the high Andes is the unusually high percentage of Chloroflexi in 16S libraries (Costello et al. 2009; Lynch et al. 2012). Most of the phylotypes in this group have no close relatives in low-elevation habitats but some are related to undescribed phylotypes that inhabit cold, plant-dominated high-elevation ecosystems (Costello and Schmidt 2006; Freeman et al. 2009b).

Antarctica

Like the alpine, the community structures of the Antarctic Dry Valleys are fairly unique compared to more temperate soils in that representative sequences of only 14 bacterial phyla have been recovered, with the Actinobacteria, Acidobacteria, and Gammaproteobacteria dominating (*Fig. 3.2*), and significantly fewer Alphaproteobacteria than most temperate soils (Cary et al. 2010; Niederberger et al. 2008;



Pairwise Comparison	Unifrac Distance	P-value	Jackknife support	Shared $OTUs_{0.03}$
Antarctica--Himalayas	0.89	0.0000	%100	22
Antarctica--Llullaillaco	0.93	0.0000	%100	3
Himalayas--Llullaillaco	0.93	0.0000	%100	4

Fig. 3.2

Proportional phylum-level representation of select bacterial 16S rDNA libraries from Antarctica and alpine barren mineral soils classified using the approach of Wang et al. (2007). Clustering analysis and Chao1 richness estimates (Schloss et al. 2009) highlight the low overall alpha diversity, particularly from the high-elevation sites (>6,000 m.a.s.l.) on Vólcan Llullaillaco. Despite the broad level similarities between these communities, Unifrac analysis (Lozupone and Knight 2005) shows how little total branch length is actually shared between communities, which is much less than would be expected by chance. Data are from (Aislabie et al. 2006; King et al. 2010a, b; Lynch et al. 2012; Niederberger et al. 2008; Schmidt et al. 2011a; Smith et al. 2006)

Smith et al. 2006). Culturing efforts also confirm the importance of the Actinobacteria with *Corynebacterium*, *Achromobacter*, and *Arthrobacter* spp. making up the majority of isolates, and *Micrococcus*, *Planococcus*, *Streptomyces*, and *Nocardia* also present (Cameron et al. 1972). Interestingly, most bacterial mineral soil isolates are psychrotrophic, although psychrophiles and

mesophiles are also readily isolated (Cowan and Tow 2004). This makes sense since psychrotrophs are more likely able to cope with the temperature cycles experienced in the mineral soils, while true psychrophiles are better suited to the more stable permafrost.

The prokaryotic communities seemingly have only one or two trophic levels, depending on the degree of water stress (Smith et al. 2006; Niederberger et al. 2008). In the more productive soils, the prokaryotes also support a third level of bacteriovorous protists and nematodes. The milder and wetter Dry Valley soils clearly harbor cyanobacteria, which can ostensibly power in situ photoautotrophic primary productivity (Novis et al. 2007; Vincent 2000). In the driest of Antarctic soils where cyanobacteria are less abundant, local carbon fixation may seem less likely; however, the possibility of chemolithotrophic carbon fixation has never been addressed. The presence of putatively mixotrophic Thaumarchaeal and Actinobacterial sequences certainly hints at this possibility (Bates et al. 2011; Tiao et al. 2012; Yergeau et al. 2007).

Arctic

Unexpectedly, recent culture-independent 16S rRNA studies have demonstrated that the degree of diversity displayed by high-Arctic tundra microbial communities is similar to that of soils from temperate latitudes (Chu et al. 2010; Neufeld and Mohn 2005). Evidence from some of the drier areas of the Arctic confirm this trend by revealing high diversity and species turnover (Schütte et al. 2010); by rough estimates Arctic desert microbial diversity in some cases appears to be as high as that of other Arctic ecosystems (Torsvik et al. 2002). It is presently unclear why Arctic polar deserts seem to have higher levels of microbial biodiversity than the driest areas of Alpine and Antarctic deserts, but overall the more maritime-influenced climate of most of the high Arctic may be partially responsible (Omelon et al. 2006). However, some inland sites of the high Arctic show somewhat decreased levels of diversity and a predominance of Actinobacteria and Acidobacteria (Cockell et al. 2001; Schütte et al. 2010) as has been observed in some of the driest cold deserts of Antarctica and the Andes (compare to ❯ *Fig. 3.2*).

Microscopy-based studies of Arctic desert soils have revealed an abundance of cyanobacteria, including members of the Nostocales, Chroococcales (*Gloeocapsa*), and Oscillatoriales (*Lyngbya, Phormidium*) (Cockell and Stokes 2006; Dickson 2000). Therefore, cyanobacteria are likely responsible for a dominant portion of primary production in Arctic deserts (Cockell and Stokes 2006). In addition, cyanobacteria, in particular *Nostoc commune*, predominate in Arctic desert soil crusts where N fixation rates can be as high as those of vegetated systems (Dickson 2000). Thus, N-fixing cyanobacteria are likely the primary source of biological N-inputs in Arctic deserts (Dickson 2000; Zielke et al. 2005). Overall, while molecular techniques in evaluating bacterial community diversity are being applied to Arctic ecosystems, little molecular research has specifically focused on cyanobacteria in Arctic deserts. The biogeography of cyanobacteria in Arctic cold deserts is discussed below in comparison to alpine and Antarctic sites.

Biogeography and Limiting Factors for Cold Desert Prokaryotes

The continued accumulation of culture-independent sequences from cold desert soils across the globe is providing some preliminary glimpses into the biogeography and factors that limit microbial life in these systems. In this section, we will briefly review some of this accumulating data and give specific examples of new insights being gained from these studies. Specifically, we will focus on two of the dominant groups found in cold desert soils, the Actinobacteria and Cyanobacteria; and a group that is extremely understudied in these systems, the Archaea.

Biogeography and Limiting Factors for Cyanobacteria

In recent studies of high alpine ecosystems throughout the world, cyanobacteria were found to dominate many less xeric and later successional seres of cold desert soils (Freeman et al. 2009b; Nemergut et al. 2007; Schmidt et al. 2009, 2011b). In addition, comparative studies have illustrated genetic similarities among Arctic and Antarctic aquatic cyanobacteria (Jungblut et al. 2010) as well as habitat distributions of cyanobacteria specific to cold desert soils compared to warm deserts (Bahl et al. 2011). Furthermore, detailed phylogeographic studies are revealing that Arctic, Alpine, and Antarctic cold deserts soils share many of the same cyanobacterial and algal phylotypes (Schmidt et al. 2011b). For example, the cyanobacterial species *Microcoleus vaginatus* is common to most cold desert sites in Antarctica, the Andes, the Himalayas, and the high Arctic, yet this species complex shows significant genetic divergence patterns in analyses of all pair-wise comparisons of sites at the intercontinental scale (Schmidt et al. 2011b). In other words, this and other groups of cyanobacteria are globally distributed, yet have specific genotypes in each region. Whether these patterns are driven mostly by geographic isolation or environmental selection remains to be fully elucidated.

In the Dry Valleys of Antarctica, the presence of cyanobacteria does not follow any strict latitudinal gradient; cyanobacteria have been detected as far south as ice-free environments persist (87°S; Broady and Weinsteinz 1998). However, Namsarev et al. (2010) found that cyanobacterial diversity is apparently highest between latitudes 70°S and 80°S and they speculate that low cyanobacterial biodiversity above 70°S may be explained by the presence of vegetation cover, since plants and mosses can competitively exclude cyanobacteria. Below 80°S, however, cyanobacteria are thought to be excluded from many sites by harsher environmental conditions. More favorable conditions may exist on northern facing slopes due to an increase in soil temperature and the availability of melt water (Elster and Benson 2004). In a similar vein, although cyanobacteria have

been observed in some of the highest ice-free regions of the Himalayas and the Peruvian Andes (Schmidt et al. 2009, 2011b), the driest sites yet studied in the Andes are apparently devoid of cynanobacteria. In the hostile ice-free areas atop Volcán Socompa and Volcán Llullaillaco in the Atacama Desert, recent culture-independent surveys, HPLC pigment assays, fluorometry, and culturing efforts have found no evidence of cyanobacteria and little or no evidence of phototrophy except in areas receiving supplemental moisture (Costello et al. 2009; Lynch et al. 2012). The soil in these environments is exposed to especially high levels of solar radiation, extreme daily temperature fluctuations (up to 70 °C) and very limited water availability. It therefore appears that some terrestrial environments are simply too extreme for cyanobacteria. However, very little work has focused on potentially more resistant phototrophs such as some Chloroflexi and Rhodospirillales that are common in these systems (King et al. 2010b; Lynch et al. 2012).

Other environmental variables (besides water availability) may also influence the distribution of cyanobacteria in cold desert soils. While Niederberger et al. (2008) identified cyanobacteria exclusively in sites within the Dry Valleys with significant soil water availability; Smith et al. (2006) found cyanobacteria in Dry Valley soils with as little as 0.7% water causing them to hypothesize that atmospheric water content was a more important factor than soil water content. Likewise, Wood et al. (2008) did not observe increased cyanobacterial diversity in Dry Valley soils with higher percent water contents, perhaps indicating that other variables may help shape the Antarctic soil cyanobacterial communities. Wood et al. (2008) further showed that magnesium and manganese correlated with cyanobacterial diversity. They also note that their site with the lowest cyanobacterial diversity was high in copper in accordance with the results of Lee et al. (2011). However, Lee et al. (2011) also reported that the most important factors affecting bacterial communities were elevation (which is highly correlated with temperature) and soil conductivity (which is a proxy for soil salinity and highly correlated with water availability). Finally Wood et al. (2008) concluded that cyanobacterial distributions are partly affected by their proximity to lakes and seasonal streams suggesting that aeolian distribution affects at least cyanobacterial diversity if not the entire microbial community.

Recent manipulative work at high-elevation sites in Colorado and Perú is also shedding some light on the factors that limit phototrophic life in cold deserts. So far this work indicates that phosphorus (P) availability is the second most limiting factor (after water availability) based on nutrient addition experiments (Schmidt et al. 2011b).

Biogeography and Limiting Factors for Actinobacteria

Actinobacteria have long been known as one of the most important groups of soil bacteria and have been studied intensively in the past because of their ability to produce numerous antibiotics (Waksman 1950). They are well adapted to the soil environment due to their hyphal growth (which allows them to grow through or around air gaps in soil) and their ability to form spores (which allows them to be easily dispersed and survive long periods of desiccation). Both of these traits are especially important in dry soils. Therefore, it is not too surprising that they dominate some of the driest soil environments yet studied (*Fig. 3.2*). However, the ecological role of Actinobacteria in these dry soils has received very little study and it is possible that they are only present in extremely dry habitats because their spores are so easily dispersed. In other words, they may just exist in these environments as spores that were produced elsewhere.

If Actinobacteria exist merely as dormant spores in cold desert soils, then one would expect random patterns of distribution and the same phylotypes in different soil types. However, this is not what has been found in several recent studies of cold deserts. Lee et al. (2011) examined bacterial diversity across multiple Antarctic Dry Valleys and showed that while on a phylum level the four sites were highly similar, with Actinobacteria dominating with 62–73% of all sequences, they differed considerably at lower taxonomic levels. Of the 81 species level (3% difference) OTUs belonging to the Actinobacteria only 9 were shared between more than two of the valleys. The heterogeneity of the microbial communities within the respective valleys is an indication that localized selection is occurring and that the communities do not represent solely aeolian distributions of dormant spores (Lee et al. 2011; Smith et al. 2010).

In addition, recent work by Lynch et al. (2012) indicates that some groups of Actinobacteria may be playing a pivotal role in cold desert soils. One line of evidence is the high level of diversity of the large subunit (*coxL*) of the carbon monoxide (CO) dehydrogenase gene (CODH) found in the extremely oligotrophic soils on Vólcan Llullaillaco. The nearest relatives (78–95% identity) to these sequences are uncultured sequences from globally distant (Hawaii) volcanic deposits and cultured CO-oxidizing Actinobacteria (as opposed to common CO-oxidizing Proteobacteria). The hypothesis that some Actinobacteria may be oxidizing CO in these soils needs more testing but is further reinforced by the presence of 16S genes from the genus *Pseudonocardia* in the same soils. The closest relatives (GQ495403, HM445437) to these sequences are from Icelandic and Azorean volcanic deposits (Lynch et al. 2012). In addition, the dominant 16S OTU from the highest sites on Llullaillaco (>6,300 m.a.s.l.) is most closely related to *Pseudonocardia asaccharolytica* (Y08536), which can oxidize dimethyl sulfide for energy (Reichert et al. 1998).

More evidence for the importance of *Pseudonocardia*-like organisms in cold desert soils comes from a phylogenetic analysis of the *Pseudonocardia* sequences from Vólcan Llullaillaco and their closest database matches (*Fig. 3.3*). Among the closest relatives to the Llullaillaco sequences are *Pseudonocardia antarctica* (Prabahar et al. 2004), a CO-oxidizing species (*Pseudonocardia carboxydivorans*) (Park et al. 2008) and environmental sequences from the Dry Valleys of Antarctica and the high Himalayas. Furthermore, the environmental sequences phylogenetically cluster predominantly by geographic region indicating that genetic divergence has occurred at each site,

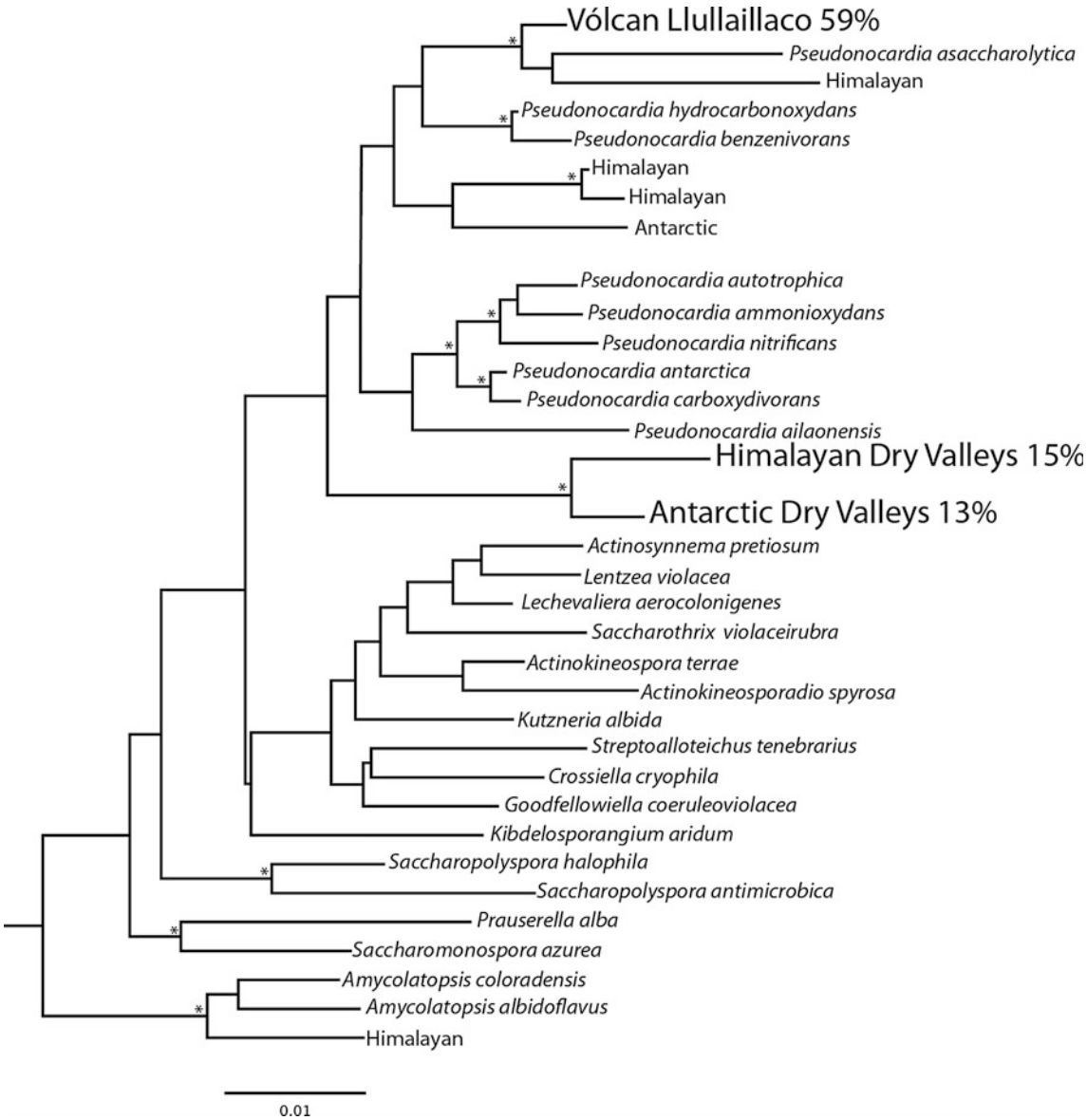


Fig. 3.3

Inferred relatedness between cultured representatives of the family Pseudonocardiaceae (Actinobacteria) and environmental Antarctic and alpine 16S rDNA sequences. Percentages indicate the proportion of Pseudonocardiaceae in the actinobacterial libraries from each region. Asterisks at nodes indicate bootstrap consistency in ≥75 % of 1,000 replicates. The tree was inferred using the SILVA database (Pruesse et al. 2007) and the neighbor-joining algorithm of MEGA5 (Tamura et al. 2011)

which implies growth and activity at each site (*Fig. 3.3*). Obviously, much more work is needed to establish the ecological importance of *Pseudonocardia*-like and other Actinobacteria in cold deserts, but our preliminary work indicates that they may be important players in these extreme environments.

Archaea in Cold Deserts

Compared to Bacteria, the diversity of Archaea in soils remains relatively unexplored, especially in plant-free ecosystems (Lynch et al. 2012; Zhang et al. 2009). However, Archaea may be particularly important in cold environments. The Crenarchaeota constitute 40% of the prokaryotic biomass in cold waters of the deep oceans (DeLong et al. 1994) and may dominate large portions of the cold ocean subsurface (Biddle et al. 2006). In addition, they are consistently found in molecular surveys of soil microbial communities (Brochier-Armanet et al. 2008) including high elevation, cold soils (Oline et al. 2006; Zhang et al. 2009). Likewise, ubiquitous soil Archaea have been detected in Antarctic Dry Valley soils (Aislabie et al. 2006; Hogg et al. 2010), and a recent survey conducted by Bates et al. (2011) of Dry Valley soils consistently reported a small yet measurable presence of archaeal DNA belonging to the

Thaumarchaeota soil groups. However, the techniques employed fail to differentiate between active organisms and cryopreserved organisms and DNA. Thus it is possible that the archaeal sequences detected represent the global dispersal of soil archaea via aeolian and other processes and do not actually reflect a functioning archaeal community. Other culture-independent surveys of the Dry Valley microbial communities either opted not to target Archaea (Lee et al. 2011) or failed to detect any archaeal signal (Pointing et al. 2009). Archaeal communities have been described in Arctic permafrost environments, yet their diversity and function remain poorly studied (Steven et al. 2008; Yergeau et al. 2010) and almost no work has been done in cold Arctic deserts.

However, there have been several recent attempts to investigate the function of Archaea in high-elevation cold deserts. The presence of archaeal ammonia monoxygenase subunit A (*amo*A) genes have been studied in the high Himalayas and many plant-free areas of the Rocky Mountains (Freeman 2010; Zhang et al. 2009) and archaeal *amo*A has been found even in the highest soils examined on Mt. Everest (Zhang et al. 2009). However, the abundance of putative archaeal *amo*A decreased with altitude relative to the abundance of bacterial *amo*A perhaps indicating that bacterial ammonia oxidizers are more active at colder temperature (Zhang et al. 2009). Likewise, a majority of the archaeal *amo*A sequences found in cold desert areas of the Andes and Rocky Mountains were in the clades A4 and A6 (Freeman 2010) that are most closely related to sequences found in soils of lower elevation ecosystems (Hansel et al. 2008; Tourna et al. 2008). Obviously, much more work is needed to understand the roles of soil Archaea in cold deserts and other ecosystems.

Oases

Within all of the cold desert soil environments discussed above there exist oases that offer respite from the hostile environment. These oases include volcanic fumaroles, dead animals, seasonal streams, fractured and porous rocks, and translucent minerals. Each respective sanctuary helps mitigate specific environmental challenges and greatly alters the local microbial community. We discuss some of these briefly below because they provide unique natural experiments that may help us to understand some of the factors limiting life in cold desert soils.

Dead Animals

The remains of mummified seals have been confounding scientists and explorers since Scott's *Discovery* expedition in the early 1900s. Carcasses in varying stages of decay and preservation have been observed more than 60 km from the shoreline and 1,800 m above sea level (Banks et al. 2009; Barwick and Balham 1967; Péwè et al. 1959). The cause of these suicidal migrations remains undetermined, but their effect on the local microbial community is profound. In addition to providing a comparatively high input of organic carbon and nutrients, the carcasses serve to retain soil humidity, stabilize soil temperature, and reduce UV radiation (Cary et al. 2010). Not surprisingly, Tiao et al. (2012) observed significant population differences (within 3 years) between a control, exposed Dry Valley site and a similar site beneath a mummified seal carcass. The seal-covered site displayed less phylogenetic diversity and maintained significant populations of only two of the seven most abundant phyla that were found at the control site. Cell densities increased at sites in direct contact with the carcass. However, the specific physiochemical changes that prompted these changes are unclear. Upon removal of the seal carcass, the previously sheltered community demonstrated a rapid decline despite the elevated levels of soil carbon and nitrogen (Tiao et al. 2012). Thus, it appears the primary benefit derived by the local microbial community from the seal carcass was the alleviation of the physical challenges and not the reservoir of nutrients.

In a similar vein, the activity of collared lemmings produces micro-oases within Arctic deserts that alter environmental conditions and microbial communities. Rotting lemming nests and scat are sources of organic matter that alter soil attributes and result in a series of feedbacks including vegetation growth (Cockell et al. 2001). Cockell et al. (2001) revealed that such micro-oases associated with lemming activity result in higher bacterial abundances, likely sustained by direct carbon inputs and resulting vegetation, as compared to the environment outside these micro-oases. The sites include cryptogamic crusts that contain N-fixing cyanobacteria, and distinct bacterial phylotypes have also been noted, such as the presence of *Enterococcus*, likely introduced from the lemming intestinal tract (Cockell et al. 2001).

Hypoliths and Cryptoendoliths

Hypoliths and cryptoendoliths are microorganisms that colonize the underside of translucent minerals and microsites within porous rocks, respectively. These rock matrices serve to shelter the community from wind and intense solar radiation (Broady 1981; Cockell et al. 2008; Cockell and Stokes 2004; Warren-Rhodes et al. 2007), increase water availability due to condensation (Warren-Rhodes et al. 2006), and buffer both temperature and humidity fluctuations (Cowan et al. 2010). The underlying geology, glacial processes, and freeze-thaw cycles have resulted in there being copious quantities of translucent rocks throughout the Dry Valleys (Broady 2005). Appropriately sized stones are, almost as a rule, colonized by hypolithic microbial communities (Cowan et al. 2010, 2011) suggesting that the generally phototrophic hypolithic communities are an important source of carbon to the region. Cowan et al. (2010) further classified the hypolithic communities into three categories, cyanobacterial dominated, fungal dominated, and moss dominated. The cyanobacterial hypolithic consortia were comprised primarily of the orders Nostocales and Oscillatoriales which accounted for approximately 45% of the cyanobacterial community. Cyanobacterial phylotypes have also been identified in the fungal-dominated hypoliths (Cowan et al. 2010).

Hypolithic communities are integral to ecosystem function in Arctic deserts where the habitat provides moisture retention, protection from UV, and temperature fluctuation. In a study of high Arctic deserts, Cockell and Stokes (2006) revealed that rocks on polygon edges were ubiquitously colonized. Dominant bacteria in these communities included microscopically identified *Gloecapsa*, *Chroococcidiopsis*, *Leptolyngbya*, and *Scytonema*. Cockell and Stokes (2006) also suggest that hypolithic bacterial communities contribute heavily to Arctic desert primary productivity. Using radiolabeled carbon, they calculated productivity values of hypolithic cyanobacteria that were comparable to plant primary productivity in the high Arctic desert study site, suggesting that hypolithic bacterial communities actually double previous primary productivity estimates in Arctic deserts (Cockell and Stokes 2004).

Hypolithons have recently been identified in high altitude tundra of central Tibet underneath of quartz rocks (Wong et al. 2010). As with many of the hypolithons of the polar deserts, the communities were dominated by cyanobacteria and provide significant carbon input to the surrounding environs. Unlike other hypolithons, however, archaea in the form of unidentified crenarchaeota comprised a small yet significant (4%) fraction of the Tibetan community (Wong et al. 2010). No work has yet been done on hypolithons in the extreme deserts of plant-free areas like of high Andes or Himalayas.

The cryptoendolithic communities of the Antarctic Dry Valleys are primarily found in the Beacon sandstones which dominate the northern end of the Dry Valleys (Cary et al. 2010; Nienow and Friedmann 1993). As with the hypoliths, the cryptoendolithic environments contain a range of microbial communities (Torre et al. 2003); some are dominated by cyanobacteria and others are predominantly composed of algae and lichens. The crypoendolithic inhabitants colonize preexisting pores in the rock at depths starting at 1 mm below the rock surface and extending up to 10 mm (Sun et al. 2010). The depth of colonization is largely determined by the degree of light penetration (Hughes and Lawley 2003; Matthes et al. 2001).

As sufficient radiation for photosynthesis and rock temperatures exceeding 0 °C are present for only a small fraction of the year, the opportunity for primary production is fleeting at best (Johnston and Vestal 1991; Sun et al. 2010). Consequently the generally warmer, north-facing exposures show increased colonization (Friedmann 1982; Kappen et al. 1981; McKay and Friedmann 1985). A further consideration is the availability of water. Unlike soil and hypolithic autotrophs, the cryptoendolithic communities do not have access to melting permafrost. The sole source of water for these communities is snow melt. Thus, the colonization sites generally occur on slopes and surfaces that lend themselves to snow catchment (Sun et al. 2010). In environments that are in immediate proximity to the cryptoendolithic communities, such as the higher elevation regions of the Taylor Valley, exfoliated cryptoendoliths can represent a significant input of organic matter and nutrients to the system (Burkins et al. 2000; Friedmann et al. 1980).

Fumaroles

From the slopes of Mt. Erebus in Antarctica, to Beerenberg in the far north, to the towering peak of Vólcan Socompa in the Andes, warm hydrothermal environments interpose themselves into some of the coldest and most arid environments on Earth. When hydrothermal systems interact with cold deserts, the effects on the surrounding environs are profound. These hydrothermal features, such as fumaroles, geysers, and hot springs, provide copious quantities of moisture, warmth, CO, CH_4, CO_2, and other potentially beneficial gases to an otherwise oligotrophic, desiccated, and frigid world. There has been some research on life inhabiting volcanically impacted soils in high alpine deserts. Costello et al. (2009) investigated the community structure of the microbial assemblages associated with high-elevation fumaroles on Vólcan Socompa. The Socompa fumaroles are relatively mild in that water vapor and gases seep through the porous soils creating patches of microbial mats and mosses in an otherwise barren landscape. Costello et al. (2009) investigated two categories of fumaroles on Socompa, warm fumaroles and cold fumaroles. The warm fumaroles raised the temperature of their surrounding environs to 25 °C and elevated the concentrations of CO_2 and CH_4 and in some areas were covered by mats of moss but were very oligotrophic where the moss had been removed by disturbance. Meanwhile, the cold fumarole soils remained at −5 °C but had increased levels of soil moisture and soil C and N and had high levels of microbial diversity with representatives from 19 phyla and subphyla dominated by the Acidobacteria, Alphaproteobacteria, Cyanobacteria, and Chloroflexi. Among these groups were microbes that could potentially participate in both nitrogen fixation and photosynthesis, suggesting that the cold fumarole environments could function as important islands of productivity within these extreme high alpine deserts. This assertion is further supported by the presence of chlorophyll *a* and an elevated soil ^{13}C signature potentially indicative of the fixation of magmatic CO_2 (Costello et al. 2009).

Future Directions

While cold deserts represent uniquely extreme environments that span a geographically substantial area of the globe (*Fig. 3.1*), we are only beginning to understand the dynamics of these microbial ecosystems. Recently, molecular techniques have allowed for new, higher resolution insights in microbial ecology; however, gaps in our understanding of cold desert microbiology are great compared to other biomes. Further assessing microbial community composition of these environments will provide a basis to understand patterns of community structure and function that control ecosystem process rates and ecosystem productivity in the absence of plant life. Given their relative simplicity, cold desert ecosystems have already facilitated studies of factors influencing microbial community assembly and distribution (Caruso et al. 2011; King et al. 2010b; Newsham et al. 2010; Stomeo et al. 2012), but more

comprehensive studies of these processes are needed. In addition, enhanced sequencing efforts across cold deserts may serve to help us better understand the physiology and ecology of novel taxa (especially understudied groups like the soil Chloroflexi and Archaea) and begin to sort out the biogeography of cold desert microbial distributions (Darcy et al. 2011; Schmidt et al. 2011b). Finally, cold desert environments present extreme environmental constraints that may select for microbes that best reflect life beyond our planet. Characterization of microbes, particularly those endemic to these extreme environments, will also advance perspectives in astrobiology and may uncover novel physiologies that could have application in biotechnology.

References

Aislabie J, Chhour K, Saul D, Miyauchi S, Ayton J, Paetzold R, Balks M (2006) Dominant bacteria in soils of Marble Point and Wright valley, Victoria Land, Antarctica. Soil Biol Biochem 38:3041–3056

Bahl J, Lau MCY, Smith GJD, Vijaykrishna D, Cary SC, Lacap DC, Lee CK, Papke RT, Warren-Rhodes KA, Wong FKY, McKay CP, Pointing SB (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. Nat Commun 2:163

Ball BA, Virginia RA, Barrett JE, Parsons AN, Wall WH (2009) Interactions between physical and biotic factors influence CO_2 flux in Antarctic Dry Valley soils. Soil Biol Biochem 41:1510–1517

Banks JC, Ross PM, Smith TE (2009) Report of a mummified leopard seal carcass in the southern Dry Valleys, McMurdo sound, Antartica. Antarct Sci 22:43

Barwick RE, Balham RW (1967) Mummified seal carcasses in a deglaciated region of South Victoria Land, Antarctic. Tautara 15:165–180

Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R, Fierer N (2011) Examining the global distribution of dominant archaeal populations in soil. ISME J 5:908–917

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sørensen KB, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Perú. Proc Natl Acad Sci USA 103:3846–3851

Bliss LC (1981) North American and Scandinavian tundra's and polar deserts. In: Bliss LC, Heal OW, Moore JJ (eds) Tundra Ecosystems: a comparative analysis. Cambridge University Press, Cambridge, UK, pp 8–24

Bliss LC, Gold WG (1999) Vascular plant reproduction, establishment, and growth and the effects of cryptogamic crusts within a polar desert ecosystem, Devon Island, NWT, Canada. Can J Botany 77:623–636

Bliss LC, Svoboda J, Bliss DI (1984) Polar deserts, their plant cover and plant production in the Canadian High Arctic. Holarctic Ecol 7:305–324

Bockheim JG, McLeod M (2008) Soil distribution in the McMurdo Dry Valleys, Antarctica. Geoderma 144:43–49

Borin S, Ventura S, Tambone F, Mapelli F, Schubotz F, Brusetti L, Scaglia B, D'Acqui LP, Solheim B, Turicchia S, Marasco R, Hinrichs K-U, Baldi F, Adani F, Daffonchio D (2010) Rock weathering creates oases of life in a high Arctic desert. Environ Microbiol 12:293–303

Bradley RS, Vuille M, Diaz HF, Vergara W (2006) Threats to water supplies in the tropical Andes. Science 312:1755–1756

Broady P (1981) The ecology of sublithic terrestrial algae at the Vestfold Hills, Antartica. Eur J Phycol 16:231–240

Broady PA (2005) The distribution of terrestrial and hydro-terrestrial algal associations at three contrasting locations in southern Victoria Land, Antarctica. Algal Stud 118:95–112

Broady PA, Weinsteinz RN (1998) Algae, lichens and fungi in La Gorce Mountains, Antartica. Antarct Sci 10:376–385

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat Rev Microbiol 6:245–252

Burkins MB, Virginia RA, Chamberlain CP, Wall DH (2000) Origin and distribution of soil organic matter in Taylor Valley, Antarctica. Ecology 81:2377–2391

Burkins MB, Virginia RA, Wall DH (2001) Organic carbon cycling in Taylor Valley Antarctica: quantifying soil reservoirs and soil respiration. Glob Change Biol 7:113–125

Byers AC (2007) An assessment of contemporary glacier fluctuations in Nepal's Khumbu Himal using repeat photography. Himal J Sci 4:21–26

Callaghan T, Björn, LO, Chernov Y, Chapin FS, Christensen T, Huntley B, Ims R, Johansson M, et al. (2004) Effects on the function of Arctic ecosystems in the short- and long-term perspectives. Ambio 33:448–458

Cameron R, Morelli F, Johnson R (1972) Bacterial species in soil and air of the Antarctic continent. Antarct J US 7:187–189

Caruso T, Chan Y, Lacap DC, Lau MCY, McKay CP, Pointing SB (2011) Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. ISME J 5:1406–1413

Cary SC, McDonald IR, Barrett JE, Cowan DA (2010) On the rocks: the microbiology of Antarctic dry valley soils. Nat Rev Micro 8:129–138

Chu H, Fierer N, Lauber CL, Caporaso JG, Knight R, Grogan P (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. Environ Microbiol 12:2998–3006

Claridge GGC, Campbell IB (1976) The salts in Antartic soils, their distribution and relationship to soil processes. Soil Sci 123:377–384

Cockell CS, Lee PC, Schuerger AC, Hidalgo L, Jones JA, Stokes MD (2001) Microbiology and vegetation of micro-oases and polar desert, Haughton impact crater, Devon Island, Nunavut, Canada. Arct Antarct Alp Res 33:306–318

Cockell CS, Stokes MD (2004) Widespread colonization by polar hypoliths. Nature 431:414

Cockell CS, Stokes MD (2006) Hypolithic colonization of opaque rocks in the Arctic and Antarctic Polar Desert. Arct Antarct Alp Res 38:335–342

Cockell CS, McKay CP, Warren-Rhodes K, Horneck G (2008) Ultraviolet radiation-induced limitation to epilithic microbial growth in arid deserts–dosimetric experiments in the hyperarid core of the Atacama Desert. J Photochem Photobiol B 90:79–87

Costello EK, Schmidt SK (2006) Microbial diversity in alpine tundra wet meadow soil: novel Chloroflexi from a cold, water-saturated environment. Environ Microbiol 8:1471–1486

Costello EK, Halloy SRP, Reed SC, Sowell P, Schmidt SK (2009) Fumarole-supported islands of biodiversity within a hyperarid, high-elevation landscape on Socompa Volcano, Puna de Atacama, Andes. Appl Environ Microbiol 75:735–747

Cowan DA, Russell NJ, Mamais A, Sheppard DM (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. Extremophiles 6:431–436

Cowan DA, Tow LA (2004) Endangered Antarctic environments. Annu Rev Microbiol 58:649–690

Cowan DA, Khan N, Pointing SB, Cary SC (2010) Diverse hypolithic refuge communities in the McMurdo Dry Valleys. Antarct Sci 22:714–720

Cowan DA, Pointing SB, Stevens MI, Craig Cary S, Stomeo F, Tuffin IM (2011) Distribution and abiotic influences on hypolithic microbial communities in an Antarctic Dry Valley. Polar Biol 34:307–311

Darcy JL, Lynch RC, King AJ, Robeson MS, Schmidt SK (2011) Global distribution of *Polaromonas* phylotypes - evidence for a highly successful dispersal capacity. PLoS One 6(8):e23742. doi:10.1371/journal.pone.0023742

DeLong EF, Wu KY, Prézelin BB, Jovine RV (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 371:695–697

Dickson LG (2000) Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, NWT, Canada. Arct Antarct Alp Res 32:40–45

Edwards IP, Bürgmann H, Miniaci C, Zeyer J (2006) Variation in microbial community composition and culturability in the rhizosphere of *Leucanthemopsis alpina* (L.) Heywood and adjacent bare soil along an alpine chronosequence. Microb Ecol 52:679–692

Edwards JS (1988) Life in the allobiosphere. Trends Ecol Evol 3:111–114

Elster J, Benson EE (2004) Life in the Polar Terrestrial Environment with a Focus on Algae and Cyanobacteria. In: Fuller BJ, Lane N, Benson EE (eds) Life in the Frozen State Libro. CRC Press, Boca Raton, pp 111–150

Freeman KR (2010) Toward an understanding of microbial communities and biogeochemistry in high-elevation, un-vegetated soils. Ph.D. dissertation, University of Colorado, Boulder

Freeman KR, Martin AP, Karki D, Lynch RC, Mitter MS, Meyer AF, Longcore JE, Simmons DR, Schmidt SK (2009a) Evidence that chytrids dominate fungal communities in high-elevation soils. Proc Natl Acad Sci USA 106:18315–18320

Freeman KR, Pescador MY, Reed SC, Costello EK, Robeson MS, Schmidt SK (2009b) Soil CO_2 flux and photoautotrophic community composition in high-elevation, "barren" soil. Environ Microbiol 11:674–686

Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053

Friedmann EI, Garty J, Kappen L (1980) Fertile stages of cryptoendolithic lichens in the dry valleys of southern Victoria Land. Antarct J US 12:6–30

Friedmann EI, Kappen L, Meyer MA, Nienow JA (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. Microb Ecol 25:51–69

Green WJ, Gardner TJ, Ferdelman TG, Angle MP, Varner LC, Nixon P (1989) Geochemical processes in the Lake Fryxell Basin (Victoria Land, Antarctica). Hydrobiologia 172:129–148

Hansel CM, Fendorf S, Jardine PM, Francis CA (2008) Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. Appl Environ Microbiol 74:1620–1633

Hogg ID, Cary SC, Convey P, Newsham KK, O'Donnell AG, Adams BJ, Aislabie J, Frati F, Stevens MI, Wall DH (2010) Biotic interactions in Antarctic terrestrial ecosystems: are they a factor? Soil Biol Biochem 38:3035–3040

Hopkins DW, Sparrow AD, Novis PM, Gregorich EG, Elberling B, Greenfield LG (2006) Controls on the distribution of productivity and organic resources in Antarctic Dry Valley soils. Proc R Soc B 273:2687–2695

Horowitz NH, Cameron RE, Hubbard JS (1972) Microbiology of the dry valleys of Antarctica. Science 176:242–245

Hua O (2009) The Himalayas - water storage under threat. Sustain Mountain Develop 56:3–5

Hughes KA, Lawley B (2003) A novel Antarctic microbial endolithic community within gypsum crusts. Environ Microbiol 5:555–565

Johnston CG, Vestal JR (1991) Photosynthetic carbon incorporation and turnover in Antarctic cryptoendolithic microbial communities: are they the slowest-growing communities on Earth? Appl Environ Microbiol 57:2308–2311

Jungblut AD, Lovejoy C, Vincent WF (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. ISME J 4:191–202

Kappen L, Friedmann EI, Garty J (1981) Ecophysiology of lichens in the dry valleys of Southern Victoria-Land, Antarctica. I. Microclimate of the cryptoendolithic-lichen habitat. Flora 171:216–235

King AJ, Freeman KR, McCormick KF, Lynch RC, Lozupone C, Knight R, Schmidt SK (2010b) Biogeography and habitat modelling of high-alpine bacteria. Nat Commun 1:53

King AJ, Karki D, Nagy L, Racoviteanu AE, Schmidt SK (2010a) Microbial biomass and activity in high elevation (>5100 meters) soils from the Annapurna and Sagarmatha regions of the Nepalese Himalayas. Himal J Sci 6:11–18

King AJ, Meyer AF, Schmidt SK (2008) High levels of microbial biomass and activity in unvegetated tropical and temperate alpine soils. Soil Biol Biochem 40:2605–2610

Lee CK, Barbier BA, Bottos EM, McDonald IR, Cary SC (2011) The inter-valley soil comparative survey: the ecology of Dry Valley edaphic microbial communities. ISME J. doi:10.1038/ismej.2011.170

Ley RE, Williams MW, Schmidt SK (2004) Microbial population dynamics in an extreme environment: controlling factors in talus soils at 3750 m in the Colorado Rocky Mountains. Biogeochemistry 68:297–311

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235

Lynch RC, King AJ, Farias ME, Sowell P, Vitry C, Schmidt SK (2012) The potential for microbial life in the highest elevation (>6000 m.) mineral soils of the Atacama region. J Geophys Res Biogeosci 117: G02028 doi:10.1029/2012JG001961

Marchant DR, Head JW (2007) Antarctic Dry Valleys: microclimate zonation, variable geomorphic processes, and implications for assessing climate change on Mars. Icarus 192:187–222

Matthes U, Turner SJ, Larson DW (2001) Light attenuation by limestone rock and its constraint on the depth distribution of endolithic algae and cyanobacteria. Int J Plant Sci 162:263–270

Mayilraj S, Prasad GS, Suresh K, Saini HS, Shivaji S, Chakrabarti T (2005) *Planococcus stackebrandtii* sp. nov., isolated from a cold desert of the Himalayas, India. Int J Syst Evol Microbiol 55:91–94

McKay CP, Friedmann EI (1985) The cryptoendolithic microbial environment in the Antarctic cold desert: temperature variations in nature. Polar Biol 4:19–25

McKnight DM (1989) Antarctic lakes: biogeochemistry of dissovled organic material in lakes in the dry valleys. In: USGS Yearbook, Fiscal Year 1988. U.S. Geological Survey (USGS), Denver, CO, pp 80–81

Miniaci C, Bunge M, Duc L, Edwards I, Bürgmann H, Zeyer J (2007) Effects of pioneering plants on microbial structures and functions in a glacier forefield. Biol Fert Soils 44:289–297

Moorhead DL, Doran PT, Fountain AG, Lyons WB, McKnight DM, Priscu JC, Virginia RA, Wall DH (1999) Ecological legacies: impacts on ecosystems of the McMurdo Dry Valleys. Bioscience 49:1009–1019

Namsarev Z, Mano M-J, Fernandez R, Wilmotte A (2010) Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas. Ann Glaciol 51:171–177

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK (2007) Microbial community succession in unvegetated, recently-deglaciated soils. Microb Ecol 53:110–122

Nemergut DR, Costello EK, Meyer AF, Pescador MY, Weintraub MN, Schmidt SK (2005) Structure and function of alpine and arctic soil microbial communities. Res Microbiol 156:775–784

Neufeld JD, Mohn WW (2005) Unexpectedly high bacterial diversity in arctic tundra relative to boreal forest soils, revealed by serial analysis of ribosomal sequence tags. Appl Environ Microbiol 71:5710–5718

Newsham KK, Pearce DA, Bridge PD (2010) Minimal influence of water and nutrient content on the bacterial community composition of a maritime Antarctic soil. Res Microbiol 165:523–530

Niederberger TD, McDonald IR, Hacker AL, Soo RM, Barrett JE, Wall DH, Cary SC (2008) Microbial community composition in soils of Northern Victoria Land, Antarctica. Environ Microbiol 10:1713–1724

Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI, Thistle AB (eds) Antarctic microbiology. Wiley-Liss, New York, NY., pp 343–412

Novis PM, Whitehead D, Gregorich EG, Hunt JE, Sparrow AD, Hopkins DW, Elberling B, Greenfield LG (2007) Annual carbon fixation in terrestrial populations of Nostoc commune (Cyanobacteria) from an Antarctic dry valley is driven by temperature regime. Global Change Biol 13:1224–1237

Oline DK, Schmidt SK, Grant MC (2006) Biogeography and landscape-scale diversity of the dominant Crenarchaeota of soil. Microb Ecol 52:480–490

Omelon C, Pollard W, Ferris FG (2006) Environmental controls on microbial colonization of high Arctic cryptoendolithic habitats. Polar Biol 30:19–29

Onofri S (2004) Antarctic microfungi as models for exobiology. Planet Space Sci 52:229–237

Park SW, Park ST, Lee JE, Kim YM (2008) *Pseudonocardia carboxydivorans* sp. nov., a carbon monoxide-oxidizing actinomycete, and an emended description of the genus *Pseudonocardia.* Int J Syst Evol Microbiol 58:2475–2478

Parsons AN, Barrett JE, Wall DH, Virginia RA (2004) Soil carbon dioxide flux in Antarctic Dry Valley ecosystems. Ecosystems 7:286–295

Péwè TL, Rivard NR, Llano GA (1959) Mummified seal carcasses in the McMurdo Sound region, Antarctica. Science 130:716

Pointing SB, Chan Y, Lacap DC, Lau MCY, Jurgens JA, Farrell RL (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proc Natl Acad Sci USA 106:19964–19969

Prabahar V, Dube S, Reddy GSN, Shivaji S (2004) *Pseudonocardia antarctica* sp. nov. an Actinomycete from McMurdo Dry Valleys, Antarctica. Syst Appl Microbiol 27:66–71

Pruesse E, Quast C, Knittel K, Fuchs B, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucl Acids Res 35:7188–7196

Racoviteanu AE, Arnaud Y, Williams MW, Ordoñez J (2008) Decadal changes in glacier parameters in the Cordillera Blanca, Peru, derived from remote sensing. J Glaciol 54:499–510

Reichert K, Lipski A, Pradella S, Stackebrandt E, Altendorf K (1998) *Pseudonocardia asaccharolytica* sp. nov. and *Pseudonocardia sulfidoxydans* sp. nov., two new dimethyl disulfide-degrading actinomycetes and emended description of the genus *Pseudonocardia*. Internat. J Syst Evol Microbiol 48:441–449

Richardson MI, Moore IT, Soma KK, Lei F-M, Wingfield JC (2003) How similar are high latitude and high altitude habitats? A review and a preliminary study of the drenocortical response to stress in birds of the Qinghai-Tibetan Plateu. Acta Zool Sinica 49:1–19

Schloss PD et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7544

Schmidt SK, Cleveland CC, Nemergut DR, Reed SC, King AJ, Sowell P (2011a) Estimating phosphorus availability for microbial growth in an emerging landscape. Geoderma 163:135–140

Schmidt SK, Lynch RC, King AJ, Karki D, Robeson MS, Nagy L, Williams MW, Mitter MS, Freeman KR (2011b) Phylogeography of microbial phototrophs in the dry valleys of the high Himalayas and Antarctica. Proc R Soc B Sci 278:702–708

Schmidt SK, Naff CS, Lynch RC (2012) Fungal communities at the edge: ecological lessons from high alpine fungi. Fungal Ecol 5:443–452

Schmidt SK, Nemergut DR, Miller AE, Freeman KR, King AJ, Seimon A (2009) Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. Extremophiles 13:807–816

Schmidt SK, Reed SC, Nemergut DR, Stuart Grandy A, Cleveland CC, Weintraub MN, Hill AW, Costello EK (2008) The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. Proc R Soc B Sci 275:2793–2802

Schütte UME, Abdo Z, Foster J, Ravel J, Bunge J, Solheim B, Forney LJ (2010) Bacterial diversity in a glacier foreland of the high Arctic. Mol Ecol 19:54–66

Scott RF (1905) The voyage of the discovery, vol 1. Smith, Elder and Co, London, UK

Smith JJ, Tow LA, Stafford W, Cary C, Cowan DA (2006) Bacterial diversity in three different Antarctic Cold Desert mineral soils. Microb Ecol 51:413–421

Smith JL, Barrett JE, Tusnády G, Rejtö L, Cary SC (2010) Resolving environmental drivers of microbial community structure in Antarctic soils. Antarct Sci 22:673–680

Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D (1992) Ozone depletion – ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959

Smith RIL, Poncet S (1985) New southernmost record for Antarctic flowering plants. Polar Record 22:425–427

Starkenburg SR, Reitenga KG, Freitas T, Johnson S, Chain PSG, Garcia-Pichel F, Kuske CR (2011) Genome of the cyanobacterium *Microcoleus vaginatus* FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. J Bacteriol 193:4569–4570

Steven B, Pollard WH, Greer CW, Whyte LG (2008) Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. Environ Microbiol 10:3388–3403

Stomeo F, Makhalanyane TP, Valverde A, Pointing SB, Stevens MI, Cary CS, Tuffin MI, Cowan DA (2012) Abiotic factors influence microbial diversity in permanently cold soil horizons of a maritime-associated Antarctic Dry Valley. FEMS Microbiol Ecol. doi:10.1111/j.1574-6941.2012.01360.x

Sun HJ, Nienow JA, McKay CP (2010) The antarctic cryptoendolithic microbial ecosystem. In: Doran PT, Lyons WB, McKnight DM (eds) Life in Antarctic deserts and other cold dry environments: astrobiological analogs. Cambridge University Press, Cambridge, UK, pp 110–138

Swan LW (1963) Aeolian zone. Science 140:77–78

Swan LW (1992) The Aeolian biome. Bioscience 42:262–270

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tiao G, Lee CK, McDonald IR, Cowan DA, Cary SC (2012) Rapid microbial response to the presence of an ancient relic in the Antarctic Dry Valleys. Nat Commun 3:660

Torre JR, Goebel BM, Friedmann EI, Pace NR (2003) Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. Appl Environ Microbiol 69:3858–3867

Torsvik V, Øvreås L, Thingstad TF (2002) Prokaryotic diversity–magnitude, dynamics, and controlling factors. Science 296:1064–1066

Tourna M, Freitag TE, Nicol GW, Prosser JI (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environ Microbiol 10:1357–1364

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, New York, NY

Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 321–340

Waksman SA (1950) The Actinomycetes, their nature, occurrence, activities and importance. The Chronica Botanica Co., Waltham MA

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Warren-Rhodes KA, Rhodes KL, Pointing SB, Ewing SA, Lacap DC, Gómez-Silva B, Amundson R, Friedmann EI, McKay CP (2006) Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert. Microb Ecol 52:389–398

Warren-Rhodes KA, Rhodes KL, Boyle LN, Pointing SB, Chen Y, Liu S, Zhuo P, McKay CP (2007) Cyanobacterial ecology across environmental gradients and spatial scales in China's hot and cold deserts. FEMS Microbiol Ecol 61:470–482

Wong FKY, Lacap DC, Lau MCY, Aitchison JC, Cowan DA, Pointing SB (2010) Hypolithic microbial community of quartz pavement in the high-altitude tundra of central Tibet. Microb Ecol 60:730–739

Wood SA, Rueckert A, Cowan DA, Cary SC (2008) Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME J 2:308–320

Yergeau E, Hogues H, Whyte LG, Greer CW (2010) The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J 4:1206–1214

Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environ Microbiol 9:2670–2682

Zakhia F, Jungblut A-D, Taton A, Vincent WF, Wilmotte A (2008) Cyanobacteria in cold ecosystems. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, pp 121–135

Zhang L-M, Wang M, Prosser JI, Zheng Y-M, He J-Z (2009) Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest. FEMS Microbiol Ecol 70:208–217

Zielke M, Solheim B, Spjelkavik S, Olsen RA (2005) Nitrogen fixation in the high arctic: role of vegetation and environmental conditions. Arct Antarct Alp Res 37:372–378

4 Plant Rhizosphere Microbial Communities

Dror Minz[1] · *Maya Ofek*[1] · *Yitzhak Hadar*[2]
[1]Institute for Soil, Water and Environmental Sciences, ARO, Volcani Research Center, Bet-Dagan, Israel
[2]Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food & Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Introduction

Plants have evolved in a microbial world. Thus, plant-microbe interactions may be inherent to plants' adaptation to their environment. On the other hand, plants are the major source of organic nutrients in the soil, the driving force for microbial activity. The soil microflora interacts with plant roots and can even modulate the plant's response to both biotic and abiotic stresses. Here, we describe the rhizosphere as an organized unit, composed of the root and its associated microbiome. This interaction occurs in the limited soil region directly influenced by the living plant root. The presence and activities of the root affect the surrounding soil chemically, physically, and biologically. Thus, numerous processes occur in parallel in the rhizosphere, creating a unique and active niche. The chemical processes involve passive and active deposition of a multitude of compounds, mostly labile organic matter from the plant root and sloughed-off plant cells and tissues. The deposits discharged from the roots into the surrounding soil include different amino acids and proteins, organic acids, carbohydrates and sugars, vitamins, and the mucilage, accounting for a large proportion of the plant's fixed carbon. These, of course, are the driving force for alterations in the activity, function, abundance, composition and structure of the soil microbial community. The rhizosphere community will, in turn, affect root health and development.

Is it possible to consider the plant-rhizobacteria complex as a "holobiont" composed of the plant and its accompanying microbiome, acting as a consortium, a unit of selection in evolution (Rosenberg et al. 2007; Zilber-Rosenberg and Rosenberg 2008; Rosenberg and Zilberg-Rosenberg 2011)? Rosenberg and Zilberg-Rosenberg (2011) suggested four criteria for the hologenome theory. These criteria can be examined with regard to the rhizosphere: (1) the rhizosphere contains abundant and diverse microorganisms acquiring a nutrient-rich environment from the plant, (2) the rhizobacteria affect the plant's fitness, and (3) variation in the hologenome can be brought about by changes in either the plant genome or the microbial population genomes. The fourth criterion of the hologenome theory suggests the ability to transmit genetic variation from one generation to the next. In the case of the rhizosphere, this is not straightforward. However, the genetic variation in the soil microbiota is enormous: 1 g of soil can contain millions of bacterial cells belonging to more than 10,000 unique taxa (Fierer et al. 2007). It may be suggested that roots grown in such a soil will enrich the required functional, rather than phylogenetic group, to support its development under the given conditions. The high degree of coadaptation between plants and soil microorganisms is manifested by the high diversity of root-associated and endophytic species (Manter et al. 2010; Uroz et al. 2010) and

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_38,

the concomitant high frequency of plant-growth-promotion-related traits in soil and rhizosphere bacteria (De Brito Alvarez et al. 1995; Cattelan et al. 1999; Berg et al. 2002, 2006; Ahmad et al. 2008; Garbeva et al. 2008; Zachow et al. 2008; Sato et al. 2009; Fürnkranz et al. 2009; Çakmakçi et al. 2010).

In this chapter, we describe the rhizosphere and its microbiome, focusing on data and theories describing general natural rhizospheric microbial ecology in health and disease. We discuss the anthropogenic and global warming impacts on rhizosphere microbiome, and the effects of mycorrhiza. We describe the structure and function of the microbial community at the rhizosphere, and the great impact recent developments in molecular techniques has and will continue to have in the near future in this field. We do not, however, discuss specific symbiotic/pathogenic interactions and mechanisms.

The Rhizosphere: Definitions, Compartments, and Spatial and Temporal Scales

Plant roots are linear units that can be divided into compartments that differ in their degree of development and differentiation, as well as in their functional, physiological, and biochemical characteristics. Plant root systems also exhibit high physiological and biochemical plasticity (Waisel and Eshel 2002), which is manifested by changes in root properties and activities (Neumann and Römheld 2002). Moreover, within a single root system, different types of roots are formed, even when grown under homogeneous aeroponic conditions (Waisel and Eshel 2002). These root types may differ in their structure; rates of water and nutrient uptake; growth and accumulation of ions; responses to salinity, hypoxia, and nutrient deprivation; and expression and activity of important enzymes. The life span of roots ranges from days to over a year, depending on the plant species and root type, as influenced by abiotic and biotic factors. Roots elongate continuously. The different compartments formed along the growing root axis include the root cap, root tip, elongation zone, root-hair zone, and mature zone. Each compartment represents a different level of differentiation and performs distinctive functions. Roots also produce lateral roots, whose sites of emergence constitute yet another root compartment. Finally, wounds caused by friction with soil particles, as well as by grazers and pathogens, and mycorrhiza also contribute to the array of compartments within the root system.

A rhizosphere is created around each root as it grows and the root's activity changes the chemical, physical, and biological properties of the soil in its immediate vicinity. Thus, the rhizosphere is defined by its function rather than its "geometry" and can vary greatly in its spatial and temporal dimensions, even under transient or minute modulation of any one of its components. The radial dimensions of the rhizosphere may span several millimeters in diameter for soluble nutrients (such as nitrate) or volatiles, but is much more restricted (<1 mm) for sparingly soluble minerals (such as P and Fe) (Neumann and Römheld 2002). Root compounds released into the soil may directly facilitate the plant's acquisition of mineral nutrients. These include excreted and secreted compounds (carbon dioxide, bicarbonates, protons, electrons, etc.) that affect the soil pH and redox potentials. Other secreted compounds, such as phytosiderophores, target specific nutrients and directly increase their availability to the plant. Rates of release of these compounds are highly affected by nutrient limitations. Although inorganic compounds can directly modify the biogeochemistry of the surrounding soil (Hinsinger 2001; Cheng et al. 2004; Vetterlein and Reinhold 2004; Hinsinger et al. 2009), the dramatic rhizosphere effect is mainly attributed to the release of large amounts of organic compounds.

Many factors affect the quantity and composition of root-released organic carbon: plant species (Hütsch et al. 2002; Jones et al. 2009), environmental factors (light, temperature), nutritional balance, stresses (including herbivores), and biological interactions, including mycorrhiza and prokaryotes, which act as strong sinks (Neumann and Römheld 2002; Jones et al. 2004). Concentrations of organic root depositions are inversely related to the distance from the root surface (Cheng et al. 1996; Kuzyakov et al. 2003; Gao et al. 2011). The main components of organic root depositions are thought to be root debris, which includes cell lysates, sloughed-off root cap cells (border cells), and senescent tissue (Uren 2001). Therefore, the composition of root depositions includes the entire array of root products. Root exudates, defined as compounds released from intact root cells by either diffusion or secretion, account for a smaller fraction of root depositions, but can have a direct and immediate function in rhizospheric processes (Neumann and Römheld 2002). Many types of low-molecular-weight organic compounds diffuse from intact cells into the soil. The most abundant diffusates are the principal cytoplasm compounds (e.g., sugars, organic acids, and amino acids) that move out of the cells due to the dramatic gradient in their concentration between the root and its environment. These sharp gradients are maintained by the rapid consumption of such compounds by soil microorganisms.

Carbon Flow in the Rhizosphere and Microbial Responses

Root deposition of carbon (C) in the soil is of major importance in regulating ecosystem functioning. However, it is clear that C flow in the rhizosphere is an extremely complex process, varying spatially and temporally along the root and affected by myriad interactions between the plant root and biotic and abiotic environmental factors (Jones et al. 2004). Experiments conducted using pulse-labeling with ^{14}C and ^{13}C isotopes have enabled a description of the flow of plant-assimilated C into the soil microbial biomass. Roughly, half of the biological activity in soils is supported by recent (hours to a few days) photosynthesis-assimilated C (Högberg and Read 2006). On average, 17% of the total C assimilated by photosynthesis is released into the soil (Nguyen 2003). However, the actual percentage may vary greatly among plant species, and usually decreases with plant age (Gransee and Wittenmayer 2000; Nguyen 2003). While most of the released C is rapidly respired by the root and soil

microorganisms, about a third resides in the soil incorporated in the microbial biomass or in the soil organic matter (Kuzyakov and Domanski 2000; Nguyen 2003; Jones et al. 2009). Assimilation of newly photosynthesized organic compounds into soil microbial biomass occurs rapidly—within hours for different grass species (Rattray et al. 1995; Domanski et al. 2001; Kuzyakov and Domanski 2002; Rangel-Castro et al. 2005a) and after 2 days for Scots pine trees (Högberg et al. 2008). Rapid incorporation of assimilates into bacterial RNA (Rangel-Castro et al. 2005b; Vandenkoornhuyse et al. 2007) and membrane fatty acids (Treonis et al. 2004) has also been confirmed. In grassland soil, microbial RNA turnover was estimated to be 5 days with a mean residence time of 15–20 days (Ostle et al. 2003). RNA stable isotope probing, combined with community profiling methods, revealed that the most active bacterial populations residing in the rhizosphere utilize recently fixed C (Rangel-Castro et al. 2005b; Vandenkoornhuyse et al. 2007). However, the degree of labeling of different populations was uneven, indicating differences in rates of assimilation and C turnover, as well as reliance on other sources of organic C, including soil organic matter or remnant dead roots (Rangel-Castro 2005b; Vandenkoornhuyse et al. 2007).

Several studies have demonstrated interrelations between plant deposits and the microbial community. In rice rhizosphere, following a labeling period of 7 days, the assimilation of root-derived compounds by microorganisms was inversely related to distance from the root (Lu et al. 2007). This is consistent with rhizosphere dogma. Rhizosphere bacteria respond to changes in root exudation rates and composition. For example, Liljeroth et al. (1990) used ^{14}C labeling of wheat to demonstrate that at higher N, exudation, as well as bacterial numbers, increase. A mutation in an ABC transporter of *Arabidopsis thaliana* involved in the secretion of phytochemicals resulted in a shift in composition of root exudates and a concomitant shift in the rhizosphere-associated bacterial community (Bardi et al. 2009). It was also confirmed that plant root exudation is influenced by association with bacteria. For *Lolium perenne* plants grown under sterile conditions, metabolites produced by *Pseudomonas aeruginosa* significantly increased root exudation (Meharg and Killham 1995). In contrast, inoculation of sterile-grown maize plants with P-solubilizing, growth-promoting *Pantoea agglomerans* led to a significant decrease in root exudation (Laheurte and Berthelin 1988).

It is important to note here that C flow in the rhizosphere is bidirectional: roots take up organic compounds from the soil, which can be later transferred to the shoot (Jones et al. 2009). Of high importance is the uptake of sugars and amino acids, that is mediated by membrane transporters. However, a growing body of evidence indicates that uptake of large molecules, including proteins and DNA which can sustain plant growth as sole sources of N and P, respectively, probably occurs via endocytosis (Paungfoo-Lonhienne et al. 2008, 2010a). Furthermore, recent evidence has shown that intact *Escherichia coli* as well as *Saccharomyces cerevisiae* cells are taken up and consumed by roots of *Arabidopsis thaliana* and tomato plants, respectively, and that the consumed microbial-derived N is incorporated in the leaves (Paungfoo-Lonhienne et al. 2010b).

Spatial Distribution of Root-Associated Microbial Communities

As we have seen, the spatial-temporal heterogeneity of the rhizosphere is enormous. Although well-recognized, a relatively small proportion of rhizosphere studies have addressed the issue of spatial distribution of bacterial populations on roots and in the rhizosphere. Naturally, such topological studies require in situ visualization of root-adhering bacteria with minimal physical disruption of the samples. Accordingly, the main technical arsenal includes different microscopy technologies (light and fluorescence microscopy, confocal laser-scanning microscopy, transmission and/or scanning electron microscopy) coupled (or not) with suitable reporting systems (such as fluorescence-labeled probes or antibodies, general stains, and reporter genes). As a result, the studies are laborious and the number of samples that can be thoroughly processed is limited. Furthermore, most of the knowledge obtained is related to studies examining the root- or seed-colonization pattern of a specific inoculated bacterial species, many times under gnotobiotic conditions. Nevertheless, the basic and applied knowledge culled has been very valuable.

Several studies have demonstrated colonization of roots by either indigenous soil communities or inoculated strains. These studies have outlined several basic aspects of root colonization topology.

1. The major part of the root surface is bacteria-free. Early scanning electron microscopy (SEM) observations of wheat, ryegrass, and clover roots revealed that only a small fraction of the root surface is occupied by bacteria (Campbell and Rovira 1973; Rovira and Campbell 1974). Using light microscopy (LM) and cell staining, rhizoplane coverage by indigenous bacteria was estimated to be between 5% and 10% for eight different grasses and perennials grown in soil (Rovira et al. 1974). In the rhizoplane of pine (*Pinus radiata*) inoculated with *Pseudomonas* sp. or *Bacillus* sp. isolates, microbial coverage ranged between 10% and 20% of the surface area (Bowen and Theodorou 1979). SEM and LM examination of rice seedlings gave estimates of 1–9% coverage (Asanuma et al. 1979). More recent studies have also shown low relative coverage of the root surface (Hansen et al. 1997) or seed surface (Hood et al. 1998), but without providing numerical estimates. However, Watt et al. (2006) estimated that bacteria attached to wheat roots grown in natural soil cover between 12% and 15% of the root surface area.
2. Microorganisms are not randomly distributed on roots: they tend to aggregate. The relative scarcity of root-surface colonization by microorganisms has led to the hypothesis that root colonization is not random and that a few sites on the root are favorable. Newman and Bowen (1974) used a statistical approach to pattern analysis of bacterial rhizoplane colonization in different plant species. They confirmed variance in bacterial densities not only on a small scale (fields 100 μM apart), but also on larger scales, that is, along a single root and between different roots of the same root system.

Nonrandom aggregation of bacteria on root surfaces was again demonstrated for tomato roots inoculated with *Pseudomonas fluorescens* under gnotobiotic conditions using a geostatistical model (Dandurand et al. 1997) and for wheat roots grown in natural soil (Watt et al. 2006). While in both cases, nonrandom distribution was conclusive, the different authors pointed out the difficulty in establishing the causes underlying the pattern of root colonization, due to high variance between samples. Patchy distribution was also confirmed for Euryarchaeota colonizing rice roots (Grosskopf et al. 1998).

An explanation for the large variance may relate to the mode of root infection. Although the number of bacteria in a grain of soil may be huge, they occupy only a minute fraction of the grain's surface area (Young and Crawford 2004; Young et al. 2008) and are preferentially associated with organic debris (including particular organic matter and plant residues). Therefore, spatial variance in root colonization may, in part, stem from the low probability of a physical encounter. Indeed, sites of contact between dead root remnants and live roots have been shown to be bacterial colonization hot spots in wheat roots (Watt et al. 2006).

One important aspect is the distance between neighboring bacterial microcolonies on the root surface, and between microcolonies in the rhizosphere soil. Since much of microbe-microbe communication relies on volatile and diffusible chemical compounds, the distance between microcolonies will determine the degree of interaction between populations. For wheat roots grown in natural soil, the average distance between bacterial microcolonies was 84 μm (Watt et al. 2006). Quorum-sensing signals of *Pseudomonas putida* were efficient at eliciting a response in populations as far apart as 37 μm in the root tip/elongation zone and 78 μm in the root-hair zone (Gantner et al. 2006).

Bacteria are thought to colonize favorable microsites, including junctions between adjacent cells, cells and regions of increased rates of root exudation (root cap, root hair, sites of lateral root emergence), and sites of lysed rhizodermal cells. Aggregation of bacteria at such sites was corroborated in a series of experiments tracking root colonization by inoculated beneficial bacterial strains. In general, the colonization pattern of inoculants showed a preference for different root features. Most studies found aggregation of bacteria at junctions between rhizodermal cells, in agreement with early (Rovira and Campbell 1974; Asanuma et al. 1979) and more recent (Lübeck et al. 2000; Watt et al. 2006; Ofek et al. 2011) observations of native rhizosphere communities. Foster and Bowen (1982) proposed that this pattern results from higher rates of exudation at the junctions. Surface roughness, which often affects microbial aggregation on surfaces (Riedewald 2006), was suggested as an alternative explanation (Dandurand et al. 1997). Surface properties, rather than shifts in exudation, were also suggested as an explanation for abrupt changes in root-colonization patterns observed on cucumber seedling roots between the root-hair zone and the tips of emerging lateral roots in that same zone (Ofek et al. 2011).

Preferential colonization of root segments at different developmental stages has also been frequently observed in inoculation studies. For example, *Azospirillum brasilense* could be found attached to all types of root surfaces of wheat and several non-cereal crops, but was most abundant in the root-hair zone, on root-hair cells, in the elongation zone, and at sites of lateral root emergence (Bashan et al. 1991; Assmus et al. 1995; Guerrero-Molina et al. 2011). *Bacillus megaterium* colonizing *Morus alba* (Ji et al. 2010), and *Burkholderia cepacia* colonizing maize and rice (Liu et al. 2006), were also found preferentially in the root-hair zone and sites of lateral root emergence, through which these bacteria had penetrated the root cortex to reside as endophytes. Favored colonization of sites of lateral root emergence and the root-elongation zone en route to endophytic colonization appears to be common for root endophytes (Senthilkumar et al. 2011). Root colonization by many plant-growth-promoting *Pseudomonas* spp. was highest at the root base and markedly decreased toward the root tip (Hansen et al. 1997; Dekkers et al. 2000; Lübeck et al. 2000). This pattern of colonization was suggested to be related to the method of inoculation (seed or young axenic seedling inoculation, rather than soil inoculation) (Benizri et al. 2001). However, investigation of wheat-root colonization by indigenous soil *Pseudomonas* populations revealed that the pattern of distribution is affected by mechanical impedance of the soil, which dictates the rate of root elongation (Watt et al. 2003): in loose soil, wheat roots grew rapidly, and accumulation of native *Pseudomonas* was positively related to the distance from the root tip. In compact soil, root growth was slow and *Pseudomonas* accumulation was similar along the entire length of the root. Nevertheless, heterogeneity in the composition of bacterial colonization of different root compartments has been demonstrated in community-level studies (Schallmach et al. 2000; Marschner et al. 2001b; Baudoin et al. 2002; Marschner et al. 2004).

Examination of differences in community-level densities between different root compartments has produced contradictory results. Rovira and Campbel (1974) and Asanuma et al. (1979) concluded that microbial colonization initiates in the root-hair zone. In contrast, bacterial numbers were highest in the root cap zone of wheat plants grown in soil, and the elongation zone was the least colonized (Watt et al. 2006). Bacterial densities were high on the root tip and in mature root compartments of young cucumber seedlings, while the root-hair zone was sparingly colonized, if at all (Ofek et al. 2011). These discrepancies most probably result from differences between the plant-soil systems examined. Altogether, accumulated evidence suggests that nonrandom distribution of bacteria on the root is the outcome of variations in the soil, root, and microbiome characteristics and their interactions.

3. A significant proportion of the root is coated by gels of root or microbial origin, collectively termed mucilage (Foster 1986). Typically, the mucilaginous material will cover the root cap and extend from the root tip to the region of root-hair senescence. In more mature root parts, the mucilage is usually absent due to microbial degradation (Foster 1986).

Bacteria have been shown to have an effect on the mucilage, increasing its amount on the root surface (Bashan et al. 1991). Bacteria have been found embedded in the mucilage and attached to the roots below it (Rovira and Campbell 1975; Werker and Kislev 1978; Chin-A-Woeng et al. 1997; Bacilio-Jiménez et al. 2001; Puente et al. 2004; Poonguzhali et al. 2008). Beyond its role as a nutrient source for the microorganisms (Mary et al. 1993; Knee et al. 2001; Puente et al. 2004), the mucilage has been suggested to have protective value against stressors such as desiccation (Watt et al. 1994). Additionally, it was demonstrated that bacteria embedded in wheat root mucilage could even resist chloroform fumigation (Martin and Foster 1985).

Complexity of the Rhizosphere Microbial Community

The complexity of biological communities is described by their taxonomic richness and the relative abundance distribution of these taxa, collectively termed diversity. Several factors determine the successful estimation of community diversity: the adequacy of the sampling effort, the technique used, and the estimation model. Determination of adequate sample size is deemed a critical stage in ecological surveys. This is particularly true for the determination of prokaryotic diversity in soil habitats, as both the numbers of individuals and the numbers of distinct taxa are exceptionally high (Torsvik et al. 1996; Øvreås and Torsvik 1998; Whitman et al. 1998; Gans et al. 2005; Roesch et al. 2007; Fierer et al. 2007 AEM).

Based on major inconsistencies between plate counts and direct microscopy quantifications, our ability to describe the prokaryotic diversity of the rhizosphere using culture media was acknowledged to be poor long before molecular tools were introduced into microbial ecology studies (Rovira 1965; Rovira et al. 1974). For nearly 30 years now, analysis of rhizosphere bacterial community composition has been based primarily on analyses of molecular markers, mostly ribosomal (r) RNA gene sequences and their transcripts, amplified directly from DNA/RNA extracted from intact samples (Kowalchuk et al. 2010). Methods such as PCR-denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene-clone libraries, ribosomal intergenic spacer analysis (RISA), and terminal restriction length polymorphism (T-RFLP) have allowed us to make a giant leap in understanding rhizosphere microbial ecology. However, these techniques have not provided us with anything more than a better characterization of the numerically dominant populations. Indeed, the percentage of shared taxa detected simultaneously in, for example, clone libraries compared to cultivation is very low for samples of rhizospheric soil communities (Dunbar et al. 1999), or rhizoplane communities (Kaiser et al. 2001), indicating that we are still far from a census of rhizospheric bacterial diversity (Donachie et al. 2007; Dunbar et al. 2002). Novel high-throughput sequencing technologies have, in essence, lifted the barrier to adequately sampling complex microbial communities (Schloss and Handelsman 2006), at least with respect to molecular markers such as rRNA genes. Although still relatively few in number, published studies utilizing high-throughput sequencing for description of root-associated bacterial communities have vastly improved estimates of diversity (Bardi et al. 2009; Lauber et al. 2009; Manter et al. 2010; Navarro-Noya et al. 2010; Teixeira et al. 2010; Uroz et al. 2010; Gardner et al. 2011; Gomes et al. 2010; Gottel et al. 2011; Inceoğlu et al. 2011; Kolton et al. 2011; Ofek et al. 2011; Somenahally et al. 2011). *Table 4.1* provides examples of different diversity estimates derived from studies employing isolation, clone libraries, and high-throughput sequencing strategies.

Root-associated populations represent a subset of the bulk soil community (Normander and Prosser 2000; Weinert et al. 2008). Increasing selective pressure with proximity to the root, due to the root's presence and activity, is therefore expected to result in a gradual decrease in species richness, and a shift in composition and in relative abundance distribution patterns (expressed by rank-abundance patterns or evenness/dominance indices). Reductions in complexity from bulk to rhizosphere soil, rhizoplane, and endorhiza have been reported for different wild and cultivated plant species (Germida et al. 1998; Marilley et al. 1998; Dunbar et al. 1999; Kielak et al. 2008; Ofek et al. 2009). Reduced complexity in rhizosphere soil compared to bulk soil can also be manifested by an increased level of dominance, without reduction in species richness (Navarro-Noya et al. 2010). Uroz et al. (2010) reported a 15% decrease in species richness between the bulk and rhizosphere soils of oak trees, from 7,070 to 6,018 operational taxonomic units (OTUs) classified at 97% sequence similarity threshold. Being a soil compartment, it is not surprising that species richness in the rhizosphere soil was of the same order of magnitude as that in the bulk soil. Similarly, PhyloChip analysis of the rhizosphere of wild oats (*Avena fatua*) revealed a significant change in relative abundance for only 7% of the rhizosphere microbial community members (DeAngelis et al. 2009). The rhizosphere effect on bacterial community complexity is much more pronounced in the rhizoplane and endorhiza (Marilley et al. 1998; Normander and Prosser 2000; Green et al. 2006; Belcom and Crowley 2009; Ofek et al. 2009; Han et al. 2011), where species richness may be one to two orders of magnitude lower than that of the bulk soil or rhizosphere soil communities (Gottel et al. 2011; Ofek et al. 2011). Still, hundreds to thousands of species may coexist in these niches.

The complexity of the rhizosphere microbial community may increase with plant age for some plant species (Gomes et al. 2001; Ibekwe and Grieve 2004), and may vary between cultivars within species, as has been demonstrated for potato (Inceoğlu et al. 2011). However, the opposite trend has also been reported (Ibekwe et al. 2010). Selective enrichment of different microbial consortia at different root locations (Schallmach et al. 2000; Marschner et al. 2001b; Baudoin et al. 2002; Marschner et al. 2004), by different root types (Marschnner et al. 2002; Ofek et al. 2007; Weisskopf et al. 2008) or different states of mycorrhization (Marschner and Baumann 2003; Söderberg et al. 2002) also contribute to the overall complexity of the rhizosphere's microbial community.

Table 4.1
Examples of published diversity estimates of root associated and soil bacterial communities

Sample	Method	Sample size[a]	Diversity estimates		
			OTUs[b]	Chao1[c]	H′[d]
Endorhiza					
Saccharum officinarum[e,1]	Isolation	44	23		
Oryza sativa[e,2]	Clone library	192	52		
Populus deltoides[f,3]	Pyrosequencing	1,170	86		
Solanum tuberosum[e,4]	Pyrosequencing	12,000	477	1,265	
Rhizoplane					
Trifolium repens[e,5]	Clone library	29	15		0.99
Lycopersicon esculentum[e,6]	Isolation	316	96		
Hordeum vulgare[e,7]	Clone library	466	152		
Cucumis sativus[g,8]	Pyrosequencing	2,379	472	689	
Rhizosphere soil					
Trifolium repens[e,5]	Clone library	29	23		1.31
Pinyon pine[e,9]	Isolation	37	14		3.25
Saccharum officinarum [e,1]	Isolation	61	25		
Saccharum officinarum [g,10]	Clone library	78	64	217	4.09
Pinyon pine[e,9]	Clone library	212	161		7.09
Colobanthus quitensis[f,11]	Pyrosequencing	2,709	649	1,363	4.15
Populus deltoides[f,3]	Pyrosequencing	4,778	1,319		
Sweet pepper[e,12]	Pyrosequencing	5,035	1,660		
Quercus sp.[f,13]	Pyrosequencing	37,000	6,018	12,308	
Solanum tuberosum[e,14]	PhyloChip		2,432		
Bulk soil					
Trifolium repens[e,5]	Clone library	29	27		1.42
Pinyon pine[e,9]	Isolation	46	8		2.41
Pinyon pine[e,9]	Clone library	196	154		7.07
Quercus sp.[f,13]	pyrosequencing	37,000	7,070	16,272	
80 different soils[f,15]	Pyrosequencing	1,501	1,017		

[a]Number of individual isolates/clones/amplicons examined
[b]Operational taxonomic units
[c]Chao1 nonparametric estimate of species richness
[d]Shannon-Weiner index of diversity
[e]Based on the sum of all individuals examined
[f]Averages across all samples examined are presented
[g]One example from the presented data is presented
[1]Mendes et al. 2007; [2] Sun et al. 2008; [3]Gottel et al. 2011; [4]Manter et al. 2010; [5]Marilley et al. 1998; [6]Shiomi et al. 1999; [7]Buddrus-Schiemann et al. 2010; [8]Ofek et al. 2011; [9]Dunbar et al. 1999; [10] Pisa et al. 2011; [11]Teixeira et al. 2010; [12]Kolton et al. 2011; [13]Uroz et al. 2010; [14]Weinert et al. 2011; [15]Lauber et al. 2009

Rhizosphere Microbial Community Composition

Cultivable Root-Associates

The many limitation of cultivation strategies in microbial ecology were repeatedly acknowledged and emphasized by many authors (Torsvik et al. 1996; Rondon et al. 1999; Amann and Lodwig 2000; Van Elsas and Bersma 2011). Biased as it may be, this fraction includes some of the most important plant symbionts, pathogens, and plant-growth-promoting species. In many respects, cultivation is irreplaceable even today as the advantages of high-throughput sequencing technologies become available to a growing part of the scientific community. Most importantly, physiology and function of populations can be inferred from genetic data only in cases where homology to genetic data obtained from cultivated species or strains (Giovannoni and Stingl 2007; Nichols 2007).

Over a century of investigation on rhizosphere prokaryotic communities has relied mostly on cultivation of the

microorganisms on defined media, thus primarily described the cultivable aerobic (as well as facultative anaerobic) heterotrophic fraction of root-associated prokaryotes, but also specific groups of autotrophs (e.g., denitrifying bacteria and archaea) and anaerobes (methanogenic bacteria and archaea). One basic hypothesis of rhizosphere microbial ecology states that the activity and numbers of fast-growing opportunistic species (r-strategists or copiotrophs, as opposed to k-strategists or oligotrophs) and symbionts will increase with proximity to the root, due to availability of labile organic carbon or specific signaling molecules. Where representatives of these specific groups were targeted, specifically *Pseudomonas*, *Burkholderia*, and *Rhizobium*, the hypothesis was readily supported by cultivation assays (Thies et al. 1995; Schortemeyer et al. 1996; Grayston et al. 1998b; Miller et al. 2002; Van Elsas et al. 2002; Berg et al. 2006; Garbeva et al. 2008). However, with respect to general enrichment of copiotrophs, the picture was more complex. De Leij et al. (1993) have proposed that the concept of copiotrophs to oligotrophs (C:O) ratios could be examined by cultivation using defined media, and recording of colony appearance over long incubation periods. In that study, C:O distribution that characterized the bulk soil and mature washed roots of wheat was even, but in roots of young plants copiotrophs dominated. Using the same method, a shift from copiotrophs domination to more even C:O distribution during plant maturation was also reported for maize rhizosphere (Chiarini et al. 1998; Kozdrój et al. 2004) and wheat roots (De Leij et al. 1995). In another study, even C:O distribution in the bulk soil and in maize rhizosphere soil was found regardless of plant age (Brusetti et al. 2004). Along roots of lettuce (Maloney et al. 1997) and cucumber (Folman et al. 2001), the C:O ratio decreased toward the root base. However, in tomato, the ratio was constant among all root locations (Maloney et al. 1997). Decrease in C:O ratio tip to base characterized young but not mature chrysanthemum plants (Duineveld and van Veen 1999). Although concentrations of labile organic carbon increase with proximity to the root, Sarathchandra et al. (1997) found that the proportion of copiotrophs was lower in the rhizoplane compared to rhizosphere soil for *Lolium perenne* and *Trifolium repens* growing in pasture soil. Furthermore, these authors reported a significant difference in C:O ratios between the two plant species. Differences in C:O proportions were also reported for the rhizosphere of a single maize cultivar planted in different soils (Chiarini et al. 1998).

Cultivation strategies have been used in order to assess the composition or rhizosphere and root colonizing bacteria with defined plant-growth-promoting and pathogens antagonistic properties. Those include phosphorous solubilization, nitrogen fixation, siderophores production, plant hormones production, chitinases, and antibiotic substances. The relative abundance of bacteria showing (in vitro) plant-growth-promotion-related traits is frequently higher in the rhizosphere compared to bulk soil. For instance, in the rhizosphere of strawberry and oilseed rape, the relative abundance of *Verticillium* antagonists was two to three times higher compared to the bulk soil (Berg et al. 2002, 2006). Among those *Verticillium* antagonistic isolates, the incidence of concomitant antagonism toward other phytopathogenic fungi, as well as production of secondary metabolites and indole-acetic acid was highly frequent (Berg et al. 2002). Similarly, relative abundance of *Rhizoctonia solani* bacterial antagonists was higher in the rhizosphere of maize, oat, barley, and *Lolium* spp. compared to the bulk soil (Garbeva et al. 2008). In some studies, conducted in temperate European agricultural soils, Gram-negative bacteria and particularly *Pseudomonas* spp. were most dominant among antagonists of fungal plant pathogens (Berg et al. 2002, 2006; Krechel et al. 2002; van Overbeek and van Elsas 2008; Zachow et al. 2008). Furthermore, the occurrence and frequency of antibiotics-producing pseudomonads was related to the natural development of take-all suppressive soils (Raaijmakers et al. 1997; de Souza et al. 2003). This has motivated studies dedicated to exploration of the diversity of antagonistic rhizosphere pseudomonads in different crops and sites (Picard et al. 2000; Mazzola and Gu 2002; Garbeva et al. 2004; Bergsma-Vlami et al. 2005; Costa et al. 2006). However, the diversity of cultivated fungal antagonists includes representatives of many other Gram-negative as well as Gram-positive genera. Interestingly, in studies performed under warm climate conditions, Gram-positive bacteria and particularly *Bacillus* dominated the fungal-antagonistic cultivated population (Yang et al. 2008; Köberl et al. 2011). Examples of the dominant taxonomic groups isolated in surveys for single or multiple plant-growth-promoting traits are listed in *Table 4.2*.

The Contribution of Cultivation-Independent Methods

The dominant rhizosphere bacterial community generally includes members of Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, and Acidobacteria. The same groups are dominant in soils (Roesch et al. 2007; He et al. 2010; Uroz et al. 2010; Will et al. 2010). Therefore, at such gross level of resolution, the transition from culture-dependent to high-throughput culture-independent strategy has little revolutionized our view of taxonomy of rhizosphere bacterial community (*Table 4.3*). One striking exception is predominance of Acidobacteria in the rhizosphere. Members of this group were recognized as a novel division rather recently (Kuske et al. 1997) and are poorly represented in standard culture media used for cultivation and isolation of soil and rhizosphere bacteria. Cultivation-independent analyses revealed dominance of Acidobacteria in the rhizosphere of Lodgepole pine (Chow et al. 2002), *Thlaspi caerulescens* (Gremion et al. 2003), and Oak (Uroz et al. 2010). Remarkable dominance of Acidobacteria (>50%) was described in the rhizosphere of chestnut tree (*Castanea crenata*) in both DNA- and RNA- derived 16S rRNA clone libraries (Lee et al. 2008). Singh et al. (2007) had reported a strong rhizosphere effect on Acidobacteria for different grass species, with high relative abundance in the rhizosphere soil (29–55%), while in the respective bulk soils relative abundance was 10% on average. Similar trend was previously observed for

Table 4.2

Examples of dominant bacterial genera retrieved in cultivation-based surveys of plant-growth-promoting bacteria

Functional group	Dominant taxa
Fungal/bacterial pathogens antagonists	[5]*Arthrobacter*, [1]*Azotobacter*, [1,3,5,13,21]*Bacillus*, [11]*Brevundimonas*, [21]*Burkholderia*, [13]*Chryseobacterium*, [13]*Enterobacter*, [20]*Flavobacterium*, [13,20]*Lysobacter*, [5]*Micrococcus*, [13]*Paenibacillus*, [13]*Pantoea*, [1,3,4,5,6,9,13,20]*Pseudomonas*, [4,5,13]*Serratia*, [5,13,16,20]*Streptomyces*
Chitin/glucan degrading enzymes	[5]*Arthrobacter*, [5,7]*Bacillus*, [5]*Micrococcus*, [28]*Micromonospora*, [6]*Pantoea*, [5,7,27]*Pseudomonas*, [6,28]*Serratia*, [27]*Stenotrophomonas*; [5,28]*Streptomyces*
Nematocidal activity	[26]*Agrobacterium*, [26,27]*Bacillus*, [24]*Burkholderia*, [24]*Corybacterium*, [22,23,25,27]*Pseudomonas*, [22]*Rhizobium*, [27]*Stenotrophomonas*, [25]*Streptomyces*
Siderophores production	[8]*Achromobacter*, [10]*Agrobacteirum*, [5]*Arthrobacter*, [1]*Azotobacter*, [1,5,8,15]*Bacillus*, [2]*Bradyrhizobium*, [8]*Brevundimonas*, [15]*Chryseomonas*, [8]*Ensifer*, [7]*Flavobacterium*, [14]*Methylobacterium*, [8,10]*Microbacterium*, [5]*Micrococcus*, [8]*Ochrobacterium*, [14]*Okibacterium*, [1,5,7,10]*Pseudomonas*, [10]*Ralstonia*, [2]*Rhizobium*, [14]*Rhodococcus*, [8, 10,15]*Serratia*, [8]*Sinorhizobium*, [5,16]*Streptomyces*
Phytohormones production	[8]*Achromobacter*, [12]*Acinetobacter*, [10]*Agrobacterium*, [10,12]*Alcaligenes*, [10]*Arthrobacter*, [1]*Azotobacter*, [1,8,12,15]*Bacillus*, [2]*Bradyrhizobium*, [8,11]*Brevundimonas*, [11]*Burkholderia*, [1,6,10,11,12]*Pseudomonas*, [1]*Mesorhizobium*, [2]*Rhizobium*, [8,10]*Microbacterium*, [11,15]*Chryseomonas*, [12]*Enterobacter*, [8]*Ochrobacterium*, [12]*Pantoea*, [10]*Ralstonia*, [10,15]*Serratia*, [8,10]*Sinorhizobium*, [11]*Sphingomonas*, [11,15]*Stenotrophomonas*, [16]*Streptomyces*
Associative nitrogen fixation	[19]*Alcaligenes*, [17,19] *Azospirillum*, [17]*Azoarcus*, [7]*Bacillus*, [7,11]*Burkholderia*, [11,15]*Chryseomonas*, [19]*Enterobacter*, [7]*Flavobacterium*, [19]*Klebsiella*, [19]*Pantoea*, [11]*Pseudomonas*, [11]*Sphingomonas*, [19]*Xanthobacter*, [17]*Zoogloea*
Phosphate solubilization	[7]*Acinetobacter*, [1]*Azotobacter*, [1,15,18]*Bacillus*, [2]*Bradyrhizobium*, [18]*Burkholderia*, [1]*Mesorhizobium*, [18]*Pantoea*, [1,7]*Pseudomonas*, [2]*Rhizobium*, [15]*Serratia*, [15]*Stenotrophomonas*, [18]*Streptomyces*
1-Aminocyclopropane-1-carboxylic acid degradation	[8]*Achromobacter*, [10]*Alcaligenes*, [7,8]*Bacillus*, [8]*Ensifer*, [14]*Methylobacterium*, [8]*Microbacterium*, [8]*Ochrobacterium*, [14]*Okibacterium*, [7,10]*Pseudomonas*, [8]*Sinorhizobium*

[1]Ahmad et al. 2008; [2]Antoun et al. 1998; [3]Aranda et al. 2011; [4]Berg et al. 2002; [5]Berg et al. 2005; [6]Berg et al. 2006; [7]Cattelan et al. 1999; [8] Cavalca et al. 2010; [9]de Souza et al. 2003; [10]Dell'Amico et al. 2005; [11]Donate-Correa et al. 2004; [12]Egamberdleva et al. 2008; [13]Garbeva et al. 2008; [14]Idris et al. 2004; [15]Idris et al. 2009; [16]Khamna et al. 2009; [17]Malik et al. 1997; [18]Oliveira et al. 2009; [19]Oyaizy-Masuchi and Komagata 1988; [20]van Overbeek and van Elsas 2008; [21]Yang et al. 2008; [22]Ashoub and Amara 2010; [23]Kluepfel et al. 1993; Kloepper et al. 1992; [25]Krechel et al. 2002; [26]Racke and Sikora 1992; [27]Insunza et al. 2002; [28]El-Tarabily et al. 2000

Lolium perenne (Mariley and Aragno 1999), but, for *Trifolium repens* grown in the same soil, Acidobacteria relative abundance in all rhizosphere compartments was lower than in the bulk soil. For the class Holophagae, within Acidobacteria, a complex response to root proximity was described (da Rocha et al. 2010). The abundance of Holophagae increased between the bulk soil and outer rhizosphere of leek (*Allium porrum*), but at the inner rhizosphere abundance had dropped below the bulk soil levels. With a rare exception (Zhang et al. 2011b), a high level of Acidobacterial dominance appears to be more common among trees in native habitats and wild plant species relative to agricultural crops.

The advantage of culture-independent strategies is highly evident in fine resolution description of microbial communities. Cultivation-independent methods facilitate the discovery and investigation of novel important lineages (at the genera and species level), even within the most common root-associated ones (Kowalchuk et al. 2010). We focus on the genus *Massilia* (Oxalobacteraceae, β-proteobacteria) as an example. Members of *Massilia* were first isolated from clinical samples and were defined as a novel genus less than 15 years ago (La Scola et al. 1998; Lindquist et al. 2003). In recent years, *Massilia* were described in environmental samples of many sources (including air, dust, and soil samples) over a wide geographic distribution, using culture-independent techniques (Nagy et al. 2005; Pakarinen et al. 2008; Blatny et al. 2011). Such techniques have also placed *Massilia* among dominant and important root-colonizing bacteria of many plant species (Dohrmann and Tebbe 2005; Abou-Shanab et al. 2007; Green et al. 2007; Weinert et al. 2010; Brooks et al. 2011; Weisskopf et al. 2011), indicating high underestimation of this group's prevalence using cultivation strategies (Weisskopf et al. 2011). Particularly high dominance of *Massilia* was found in the spermosphere and roots of young seedlings of cucumber (Green et al. 2007; Ofek et al. 2009; Ofek et al. 2011). Similarly, root age-related decline in *Massilia* dominance was reported in cluster roots of white lupin (Weisskopf et al. 2011). Like numerous other "novel" root-associated bacterial lineages, the ecological significance and role of this group in the rhizosphere niche remains to be elucidated.

Role of Archaea

Since their discovery in the late 1970s (Woese and Fox 1977), Archaea were traditionally associated with extreme

Table 4.3

Examples of composition of rhizosphere and rhizoplane bacterial communities in different plant species

Plant species	Method[u]	Sample	Proteobacteria				Firm	Actin	Bact	Acid	Planc	Verru	Cyan	Chlo	Uncl
			α	β	γ	δ									
Cucumis sativus[a]	IS (1,790)	RP	2–11	0.5–26	19–63		9–20	4–10	7–18						3–9
Medicago sativa [b]	IS (452)	RS/RP	1–30	9–17	16–74			15–32	0.4–5						
Chenopodium album[b]	IS (407)	RS/RP	15–17	61–62	5–18			5–9	2–8						
Brassica napus[c]	IS(111)	RP	15	28	37		5	14	0.9						
	CL (103)	RP	52	9	6		3	0.97	30						
Solanum tuberosum[d]	IS(283)	RS/RP	4–6	10–25	28–45		4–20	10–12	5–14						1–8
Pinus contorta[e]	CL (709)	RS	24	19	9	3		3	3	19		3			15
Thlaspi caerulescens[f]	CL (142)	RS	20	12	2	0.7	0.0	33	0.7	15	9	7			
Lasiurus sindicus[g]	CL (58)	RS/RP	5	5–7	0–2	0–2	16–33	45–49	0–3	0–2					10–12
Hordeum vulgare[h]	CL (466)	RP	11–13	35–41	11–12	7–10	1–3	3–5	11–15	0–3	0–1	3–6		1–7	
Citrus sinensis[i]	CL (528)	RP	31–72	0–19	3–17	0–2	3–11	0–10	0–9	0–2					8–13
Zea mays[j]	CL (274)	RS	48				1.8	10.3	9.9	5		7	0.4	2	13
Gossypium hirsutum[k]	CL (600)	RS	0–38	0–30	0–30	0–19	0–4	0–3.7	0–10	7–45	0–15	0–7	0–4		0–10
Oryza sativa[l]	CL (731)	RP	4–12	11–24	12–16	0–0.6	0.3–4	3–28	9–12	6–8	0–0.3	1–2	0.3	2–5	7–13
Picea mariana[m]	CL (300)	RS	0–3	0–18	7–21	0–5	0–9	9–22		10–36		0–6			0–8
Solanum tuberosum[n]	TeP	RE	20	13	21	0.5	0.9	5	30	2	0.1	0.2	0.47	0.2	5
Capsicum annuum[o]	TeP (20,142)	RS	47–72				5–6	7–10	12–30						
Cucumis sativus[p]	TeP (55,105)	RP	0.1–21	8–72	13–86		0.2–23		0.03–3		0–0.7	0–0.1			
Quercus sp.[q]	TeP (133,231)	RS	38–41				0.3–0.8	11–12	2	20–27	0.5–2	1–2			18–20

Table 4.3 (continued)

Plant species	Method[u]	Sample	Proteobacteria				Firm	Actin	Bact	Acid	Planc	Verru	Cyan	Chlo	Uncl
			α	β	γ	δ									
Beta vulgaris[r]	PhyloChip	RS	39				20	9	4	2	2	2	1	1	16
Lolium arundinaceum[s]	FISH	RS	3–8	3–8	3–8			10–40	4–32		32–79				
Cirsium arvense[t]	FISH	RS/RP	13–14	24	33–56		0.1–1	0.1–0.9	0.3–0.6						

Abbreviations: *Firm* Firmicutes, *Act* Actinobacteria, *Bact* Bacteroidetes, *Planc* Planctomyces, *Verru* Verrucomicrobia, *Cyan* Cyanobacteria, *Chlo* Chloroflexi, *Uncl* Unclassified, *IS* Isolates obtained by cultivation, *CL* Clone libraries, *TeP* Tag-encoded pyrosequencing of 16S rRNA gene fragments, PhyloChip, *FISH* Fluorescence in situ hybridization, *RS* rhizosphere soil, *RP* rhizoplane, *RE* root endophytes

[a]Mahaffee and Kloepper 1997
[b]Schwieger and Tebbe 2000
[c]Kaiser et al. 2001
[d]Heuer et al. 2002
[e]Chow et al. 2002
[f]Gremion et al. 2003
[g]Chowdhury et al. 2009
[h]Buddrus-Schiemann et al. 2010
[i]Trivedi et al. 2010
[j]Chauhan et al. 2011
[k]Zhang et al. 2011a
[l]Ikeda et al. 2011
[m]Filion et al. 2004
[n]Manter et al. 2010
[o]Kolton et al. 2011
[p]Ofek et al. 2011
[q]Uroz et al. 2010
[r]Mendes et al. 2011
[s]Jenkins et al. 2006
[t]Cavalca et al. 2010
[u]The numbers in parentheses indicate the total (cumulative) size of the samples analyzed

environments and therefore rarely studied in soils, and even less in association with plant roots. Rhizosphere colonizing Archaea first received attention due to observation of methane production by rice roots placed under anoxic conditions (Frenzel and Bosse 1996). Soon after, it was reported that Archaea may appear in substantial relative abundance in the rhizoplane of mature rice plants, as indicated by the Archaeal signature compounds—diether lipids (Richardt et al. 1997). It was then confirmed by analysis of Archaea-specific clone libraries that the rhizoplane of rice was inhabited by Archaea including both Crenarchaeota and Euryarchaeota (Grosskopf et al. 1998). The composition of Archaea associated with rice and other waterlogged plant roots and rhizosphere is dominated by Euryarchaeota (Conrad et al. 2008; Cadillo-Quiroz et al. 2010; Kao-Kniffin et al. 2010), including important families of known methanogens such as Methanosarcinaceae, Methanosaetaceae, Methanomicrobiaceae, and Methanobacteriaceae. Novel Euryarchaeal lineages discovered in the rice rhizosphere (Grosskopf et al. 1998; Ramakrishnan et al. 2001), mainly rice cluster I, were later classified as methanogens with wide global distribution (Conrad et al. 2006) and may substantially contribute to methane emission from rice fields into the atmosphere.

In moderate dry oxic soils, rhizosphere-associated Crenarchaeota were first reported for young and senescent roots of tomato grown in field soil (Simon et al. 2000). Crenarchaeota were consistently detected in various plant species grown in a native temperate environment (Sliwinski and Goodman 2004). In contrast, in rhizosphere samples of plants grown in high altitude, detection of Archaea was rare and inconsistent (Ferrero et al. 2010). Furthermore, no Archaea were detected in the rhizosphere of different proteaceae species (Stanford et al. 2005). In contrast to plants with waterlogged root systems, Crenarchaea dominate the Archaeal community associated with rhizosphere, roots, and mycorrhiza of plants growing in such soils (Nelson et al. 2010; Bomberg et al. 2011).

Another archaeal group of functional importance is ammonia oxidizing Archaea (AOA). AOA were found in the rhizosphere and on roots of several plant species, including *Zea mays*, *Vicia faba*, *Brassica oleracea*, and the macrophyte *Littorella uniflora* (Herrmann et al. 2008; Fan et al. 2011; Kleineidam et al. 2011; Nelson et al. 2010). Similarly to soils (Leninger et al. 2006), AOA appear to predominate the ammonia-oxidizing consortium in the rhizosphere (Kleineidam et al. 2011; Nelson et al. 2010). However, the diversity of rhizosphere colonizing AOA may be lower than that of ammonia oxidizing bacteria (Fan et al. 2011). *Table 4.4* describes the composition of Archaea associated with different plant species.

Effect of Mycorrhizal Association

Arbuscular mycorrhizal and ectomycorrhizal fungi (AMF and EMF, respectively) create a new structure and function for the rhizosphere, also termed "mycorrhizosphere." The unique relationships in the mycorrhizosphere, compared to the non-mycorrhizal rhizosphere, change the allocation of plant resources between the rhizosphere bacteria and the symbiotic partner. On the other hand, the contribution of the mycorrhizal fungi affects the plant's physiology and root environment with respect to mineral nutrition and water availability (Bending et al. 2006).

The effect of AMF on rhizosphere bacterial communities has been investigated mostly by inoculation studies. Such experiments have revealed consistent differences between bacterial consortia associated with mycorrhitic and non-mycorrhitic roots and the activity of selected microbial enzymes (Vázquez et al. 2000; Marschner et al. 2001a; Söderberg et al. 2002; Wamberg et al. 2003; Marschner and Timonen 2005; Roesti et al. 2006; Vestergard et al. 2008; Solís-Domínguez et al. 2011). Offre et al. (2007) compared and distinguished bacterial communities colonizing mycorrhiza of *Medicago truncatula* and roots of a mutant plant that does not form mycorrhiza. EMF effects have been studied by inoculation and by analysis of mycorrhizal and non-mycorrhizal roots sampled in the field (Olsson and Wallander 1998; Timonen et al. 1998; Probanza et al. 2001; Faye et al. 2009). These reports present mycorrhiza-related shifts in the bacterial community assemblages.

Infection with mycorrhizal fungi results in systemic changes in the plant. Therefore, changes in the rhizosphere bacterial community structure and/or function could be an indirect response to the mycorrhiza. Such an indirect effect was demonstrated by Marschner and Baumann (2003) in maize using a split-root system. The bacterial communities in the non-mycorrhitic half of the root system were different from respective controls where neither half of the root system was inoculated. Unfortunately, however, this exciting topic has not yet been further explored.

Changes observed in the mycorrhizosphere bacterial community can be attributed to local and direct effects of the fungi or indirect effects mediated by systemic changes in the infected plant. A direct effect might simply be attachment of soil bacteria to the hyphae of the mycorrhizal fungus. For example, Scheublin et al. (2010) showed rapid colonization of bacteria from the family of Oxalobacteraceae and *Pseudomonas*. Based on the high frequency of Oxalobacteraceae, those authors suggested the existence of a specific interaction (Scheublin et al. 2010). Offre et al. (2007) also identified bacterial groups belonging to the Oxalobacteraceae preferentially associated with mycorrhizal roots of *Medicago truncatula*. FISH analysis of the ectomycorrhizosphere of beech (*Fagus sylvatica*) growing in a natural forest revealed bacteria of the α-, β, and γ subclasses of the Proteobacteria attached in high numbers to the mantle surfaces (Mogge et al. 2000). One mechanism suggested for the direct effect is the influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community composition. Toljander et al. (2007) used a split Petri dish system to produce mycelial exudates. Following amendment of soil with these exudates, a significant shift in soil bacterial community composition occurred, marked by significant enrichment of specific Enterobacteriaceae members. Frey-Klett et al. (2005) argued that ectomycorrhizal symbiosis also has an indirect positive effect on the plant via its selective pressure on bacterial communities.

Table 4.4

Composition of Archaea associated with plant roots (R), rhizosphere soil (RS) and mycorrhiza (Myc)

Taxonomy	Rice [a]	Acid bog plants[b]	Wetland[c]	Scot pine[d]	Silver brich[d]	Norway sprouce[d]	Maize[e]	Soybean[f]	Macrophyte [g]	Tomato[h]	Various grasses[i]	Barley[j]
Euryarchaeota							RS	RS				
Methanosarcinaceae	R, RS	R	RS	R, Myc	R, Myc	Myc						
Methanomicrobiaceae	R, RS	R	RS									
Methanobacteriaceae	R, RS	R	RS									
Methanosaetaceae	R, RS	R	RS									
Rice cluster I	R, RS	R	RS									
Rice cluster II	R											
Rice cluster III	R, RS	R										
Rice cluster V	R											
LDS cluster	R											
Halobacteriales				Myc	R							
Crenarchaeota												
Rice cluster IV	R, RS											
1.1a							R, RS	RS	RS			
1.1b				Myc			RS	RS	R	RS	RS	RS
1.1 C		R		R, Myc	R, Myc	R	RS	RS			RS	
1.2	R											
1.3b		R										

[a] *Oryza sativa* L. (Grosskopf et al. 1998; Lehmann-Richter et al. 1999; Scheid et al. 2003; Lu et al. 2005; Krüger et al. 2005; Lu and Conrad 2005; Conrad et al. 2008; Wu et al. 2009b)
[b] *Dulichium arundinaceum, Sarracenia purpurea* (Cadillo-Quiroz et al. 2010)
[c] Forbs and graminoids (Kao-Kniffin et al. 2010)
[d] *Pinus sylvestris, Betula pendula, Picea abies* (Bomberg and Timonen 2007; Bomberg et al. 2010; Bomberg et al. 2011)
[e] *Zea mays* (Chelius and triplett 2001; Nelson et al. 2010)
[f] *Glycine max* (Nelson et al. 2010)
[g] *Littorella uniflora* (Herrmann et al. 2008)
[h] *Lycopersicum esculentum* L. (Simon et al. 2000)
[i] Pasture grasses (Nicol et al. 2003)
[j] *Hordeum vulgare* L. (Poplawski et al. 2007)

They showed that ectomycorrhizal symbiosis determines the structure of *Pseudomonas fluorescens* populations in the soil and selects for potentially beneficial bacteria. Soil bacteria can promote mycorrhizal formation by means of a variety of mechanisms (Poole et al. 2001; Rigamonte et al. 2010), and such communities have been termed "mycorrhization helper bacteria" (MHB). Among the identified lineages of MHB are bacteria belonging to diverse groups and genera, such as Gram-negative Proteobacteria (*Agrobacterium, Azospirillum, Azotobacter, Burkholderia, Bradyrhizobium, Enterobacter, Pseudomonas*, *Klebsiella*, and *Rhizobium*), Gram-positive Firmicutes (*Bacillus, Brevibacillus*, and *Paenibacillus*), and Gram-positive actinomycetes (*Rhodococcus, Streptomyces*, and *Arthrobacter*) (Bending et al. 2002; Artursson et al. 2006; Frey-Klett et al. 2007).

Bacterial Communities Associated with Roots of Pathogen-Infected Plants

Pathogen infection and disease propagation affect the host plant's physiology in many ways which, in turn, can locally (at the site of infection) or systemically affect plant-bacteria interactions. This issue is relatively unexplored. However, several studies have compared the composition and structure of microbial communities associated with healthy and diseased plants.

Yang et al. (2001) compared rhizosphere bacterial communities associated with healthy and *Phytophthora*-infected avocado roots using 16S rRNA gene fingerprinting. In that study, bacterial communities from healthy roots were represented by a few predominant species, and were approximately 80% similar in structure among replicates. In contrast, roots that were infected with *Phytophthora*, but which did not yet show visible symptoms of disease, were colonized by much more variable bacterial communities with significantly different structures from those of healthy roots. The effect of oomycete pathogens, including *Phytophthora cryptogea*, *Pythium aphanidermatum*, and *Pythium* group F, was also examined in a soilless growth system with tomatoes (Cavalo-Bado et al. 2006). There, an increase in bacterial abundance was found associated with oomycete-infected roots, but the community composition was unaltered. In another study, infection of tomato plants with *Phytophthora nicotianae* did not significantly affect the bacterial community structure (Lioussanne et al. 2010). Comparison to infection with the AM fungi *Glomus intraradices* or *Glomus mosseae* suggested that rhizospheric bacteria are less sensitive to pathogen invasion than to mycorrhizal colonization. Tomato rhizosphere bacterial communities were also examined in response to infection with *Fusarium oxysporum* f. sp. *radicis lycopersici* and its biocontrol antagonistic *Fusarium* strain (*F. solani* strain FsK) (Karpouzas et al. 2011). These introductions also resulted in only marginal response of the bacterial community.

In a *Pythium aphanidermatum*-cucumber experimental pathosystem, multiple aspects of the spermosphere bacterial communities significantly differed between inoculated and control germinating seeds (Ofek et al. 2011). Microscopic examination of germinating seeds revealed bacterial crowding at sites of seed infection by *Pythium* hyphae and heavily colonizing the hyphae themselves (Fig. 4.1). Furthermore, the spermosphere of infected seeds had significantly lower diversity and was dominated (66% of the total bacteria) by members of the genus *Massilia* (Oxalobacteraceae).

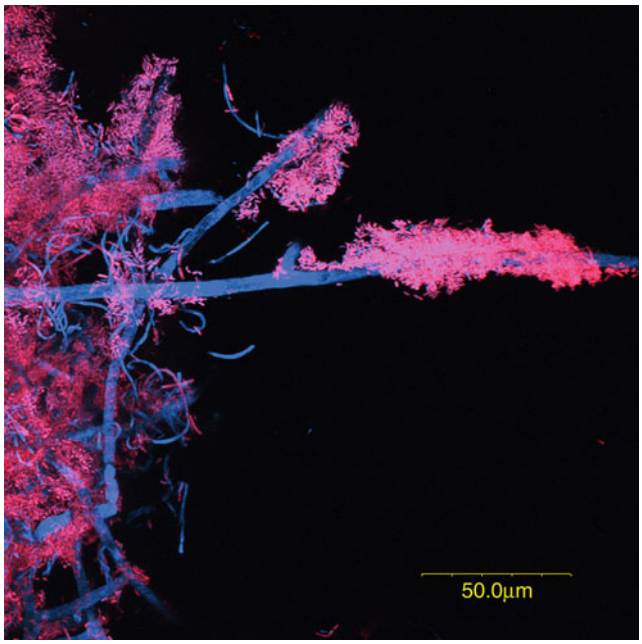


Fig. 4.1
***Pythium aphanidermatum* hyphae (*white arrows*) infecting the seed coat of cucumber seed following 24 h of germination in *P. aphanidermatum*-inoculated perlite. Seed samples were stained with DAPI (*blue*) and hybridized with fluorescently labeled probe EUB338, targeting the domain Bacteria (*red*) (Image was taken by confocal laser-scanning microscopy)**

Two interesting studies examined the rhizosphere of healthy plants and plants with natural incidence of disease. Filion et al. (2004) selected healthy and diseased root rot-symptomatic samples of roots from black spruce (*Picea mariana*) seedlings growing in a nursery. The rhizosphere-associated bacterial and fungal communities of healthy and diseased *P. mariana* seedlings differed: the main differences described at the community level were a higher proportion of Acidobacteria, Gammaproteobacteria, and Homobasidiomycetes clones associated with healthy seedlings, while the diseased-seedling rhizosphere showed a higher proportion of Actinobacteria, Sordariomycetes, and environmental clones. The authors debated on whether the communities associated with healthy roots might be responsible for disease suppression or whether their presence is simply a direct consequence of the absence of the pathogen. In a recent study, the rhizosphere of scab-diseased apple trees was compared to that of disease-free ones (Shanmugam et al. 2011). There, while the rhizosphere bacterial community composition and structure were similar, the activities of chitinase and β-1,3 glucanase were higher in rhizosphere samples from disease-free plants.

Contradictory observations could be the result of variation between the studied systems or of higher complexity of the rhizosphere of diseased plants and its multiple effects. Microbial communities in the rhizosphere of healthy and diseased plants may promote suppression via antagonism, induce resistance, or modify patterns of root-exudate release. Thus, the effects on the community could be either direct or indirect. Nevertheless, additional studies may shed more light on the interactions occurring in the rhizosphere of infected plants and may assist in developing ecologically based control methods. It is assumed that specific populations provide protection and that these will eventually be developed for biological control.

Effects of Agrosystem Management on Rhizosphere Bacterial Communities

Agricultural practices designed to improve plant performance and yield may result in nontargeted rhizosphere modulation. For example, the composition of rhizosphere bacterial communities of cucumber and sudan grass seedlings shifted following nitrogen or phosphorus fertilization of nitrogen- and phosphorus-deficient soils (Marschner et al. 2004). In iron-limited soil, foliar application of iron shifted the composition of the root-associated bacterial community (Yang and Crowley 2000). Changes in rhizosphere bacterial community composition and activity have been observed to result from crop rotation practices and land-use history (van Elsas et al. 2002; Alvey et al. 2003; Salles et al. 2004; Garbeva et al. 2008). Shifts in bacterial community composition in response to herbicides (Sessitsch et al. 2004) or pesticides (Lin et al. 2007) and a strong effect of tillage practice (Griffiths et al. 2007) have also been reported.

Other soil treatments aim to modify or manipulate specific or general rhizosphere components. These include organic soil amendments, introduction of plant-beneficial organisms via inoculation (i.e., *Rhizobium*, mycorrhiza, associative plant-growth-promoting rhizobacteria), application of chemical or biocontrol agents, and genetically engineered plants.

Effect of Organic Soil Amendment

Long-term experiments (16–50 years) have confirmed that different fertilization regimes, both organic and inorganic, affect soil bacterial communities to varying degrees, in terms of biomass, activity, and composition (Enwell et al. 2005; Ros et al. 2006; Widmar et al. 2006; Chu et al. 2007; Esperschütz et al. 2007; Toljander et al. 2008). Generally, organic amendments (manures, green and dry plant residues, sewage sludge, and compost) have more pronounced effects on the soil microbial communities than mineral fertilization. Organic soil amendments and compost improve soil structure, elevate soil content of organic matter, and supply macro- and micronutrients. Moreover, compost application to soil results in the introduction of a rich and diverse microbial community. Thus, the effects on the rhizosphere community can be either direct or indirect, by changing the abiotic root environment. Several studies have provided evidence for the persistence of amendment-derived microbes in association with the rhizosphere. Germinating seeds were colonized by amendment-derived microbes and this community changed during the transition from spermosphere to rhizosphere (Green et al. 2006; Ofek et al. 2011). The rhizosphere bacterial community is distinct in compost-amended soil compared to non-amended soil (Benitez et al. 2007; Tiquia et al. 2002). Root-associated communities of cucumber seedlings grown in perlite medium were more diverse but less abundant in the presence of disease-suppressive compost than in the non-amended controls (Ofek et al. 2009). Rhizosphere colonization by *Streptomyces* was affected by compost amendment (Inbar et al. 2005). This impact was strongly affected by proximity to the root and compost concentration. While the compost's effect on the community was mitigated with increasing proximity to the root, high levels of compost amendment resulted in the detection of compost-derived species, even on the root surface. On the other hand, in both rhizosphere and non-rhizosphere soils, the community composition of *Streptomyces* was strongly affected by even modest compost amendment (Inbar et al. 2005).

Jack et al. (2011) tested the effect of organic amendments on growth, field performance, and rhizosphere bacterial communities of tomato plants. They showed that different amendments significantly affect rhizosphere bacterial communities. These differences persisted for at least 1 month after seedlings were transplanted to the field, then diminished over the course of the field season (Jack et al. 2011). In cucumbers, compost had qualitative and quantitative effects on bacterial communities colonizing roots of young cucumber plants. These effects were dynamic in nature and strongly related to plant age (Ofek et al. 2009, 2011). Soil amendment with chitin resulted in shifts in both soil and rhizosphere bacterial community size and composition (Hallmann et al. 1999). On the other hand, Scott and Knudsen (1999) found that residues of rape as green manure had no effect on heterotrophic bacteria colonizing the rhizosphere of pea.

Inoculation with Plant-Growth-Promoting Rhizobacteria (PGPR) and Biocontrol Agents

Introduction of microorganisms by inoculation represents a technically simple approach to directly modifying the rhizosphere. Indeed, this approach has high appeal as it proposes a targeted solution for the purposes of sustainable agriculture and is considered inexpensive and environmentally benign. The objectives of inoculation are diverse and include enhancement of symbiotic and associative nitrogen fixation, plant-growth promotion, improvement of plant nutrition, control of plant-pathogenic microorganisms, and degradation of contaminating xenobiotic compounds (Vessey 2003; Lugtenberg and Kamilova 2009). PGPR and biocontrol species of bacteria are primarily rhizosphere, rhizoplane, and endophytic microorganisms; however, their natural quorum is relatively low and insufficient to

induce the desired positive effect. Enrichment through inoculation can potentially increase their abundance so that their phytoeffective potential can be expressed. Several recent reviews have described the high and still growing number of formulated and tested inoculants, and their plant-growth-promoting and biocontrol mechanisms and activities (Rodríguez-Díaz et al. 2008; Lugtenberg and Kamilova 2009; Compant et al. 2010a; Dutta and Podile 2010; Hayat et al. 2010). Here, we focus on the successful establishment of inoculated bacteria and their effect on the indigenous resident community.

Integration and nontarget effects on bacterial communities were studied for different plant-growth-promoting and biocontrol agents. Apparently, a prominent effect of crop plant inoculation with PGPR or biocontrol bacterial agents on resident bacterial communities is rare, transient, and spatially limited. This was the case with associative nitrogen-fixing *Azospirillum* (Bashan et al. 1995; Herschkovitz et al. 2005a, b; Lerner et al. 2006; Felici et al. 2008; Pedraza et al. 2009), antibiotic-producing *Pseudomonas* (rev. in Castro-Sowinski et al. 2007), siderophore-producing *Pseudomonas* (Buddrus-Schiemann et al. 2010), phytohormone-producing *Pseudomonas*, *Serratia*, and *Pantoea* (Lottmann et al. 2000; Mishra et al. 2011), among others.

One clear exception to the rule is the response of resident rhizospheric microbial communities to inoculation with host-compatible symbiotic nitrogen-fixing rhizobia. The establishment of *Sinorhizobium meliloti* in the rhizoplane of its host plant, *Medicago sativa*, and in the rhizoplane of a non-host plant, *Chenopodium album*, was examined in a field experiment (Schwieger and Tebbe 2000). Following inoculation of the soil by spraying and 12 weeks of growth, the abundance of *S. meliloti* had increased in the rhizoplane of both plants. However, the numbers of *S. meliloti* were two orders of magnitude higher for the host compared to the non-host plant. Marked changes in the composition of total and cultivable bacterial communities were found in the host plant, while communities of non-host plants were unaffected. A specific effect of *S. meliloti* on the indigenous rhizospheric bacterial community in the host but not non-host plants was also demonstrated by Miethling et al. (2000) in a mesocosm experiment. Similarly, significant shifts in bacterial community composition due to host-compatible rhizobial inoculation have been reported for common bean (Robleto et al. 1998), faba bean (Zhang et al. 2010), and soybean (Zhang et al. 2011a).

Another emerging exception is the effect of PGPR inoculation in forest trees. Inoculation with two phytohormone-producing *Bacillus* PGPRs resulted in shifts in total and cultivable bacterial communities associated with roots of *Pinus pinea* seedlings (Probanza et al. 2001, 2002). This effect lasted months after a single inoculation. Lucas-García et al. (2004) inoculated pine and holm oak with PGPR strains belonging to *Enterobacter*, *Pseudomonas*, *Cryseobacterium*, and *Phosphoric bacillus*. There, perturbation of the tree seedlings' rhizospheric bacterial communities was robust, but varied in degree for the different specific bacteria-plant pair examined. In another survey of ten isolates selected for their plant-growth-promoting potential, inoculation of one strain (*Arthrobacter* sp.) resulted in a strong alteration of *P. pinea* seedlings' rhizospheric bacterial community, along with a strong positive effect on the seedlings' growth (Barriuso et al. 2008). Inoculation of European alder with auxin-producing *Bacillus pumilus* showed contrasting results for two different soils (Ramos et al. 2003): in the native soil from which the isolate was retrieved, the inoculation effect appeared early and was transient; in the second soil, the effect of inoculation on the resident bacterial community was most pronounced at late stages of the experiment (6 and 8 weeks).

Some inoculants, such as biocontrol agents, are selected for their ability to compete with other microorganisms. Nevertheless, it seems that their ability to change the bacterial balance in the rhizosphere is limited in most cases and transient in others.

Plant Genetic Manipulation Targeting Rhizosphere Associations

Relying on the concept that root deposits are the major selective factor in root-microbe associations, Ryan et al. (2009) reviewed the possibilities for rhizosphere engineering. One suggested route for manipulation was interference with central metabolic pathways. For example, a fourfold greater efflux of citrate from tobacco seedlings was achieved by transformation with a citrate synthase gene from *Pseudomonas aeruginosa* (de la Fuente et al. 1997). The effect of extensive citrate release on rhizosphere bacteria can be demonstrated by the case of white lupin (*Lupinus albus*) cluster roots. This specialized type of root is produced in response to low phosphorus availability. When the cluster root matures, large amounts of citrate are released for the purpose of phosphorus chelation. This event coincides with a significant decrease in the number of root-associated and rhizospheric bacteria and a dramatic shift in their composition (Weisskopf et al. 2005). Another possibility for the manipulation of root exudate composition or amount is through modification of transporter proteins. Recently, this was demonstrated in *Arabidopsis thaliana* (Bardi et al. 2009). A single mutation in an ABC transporter (abcg30) resulted in more phenolics and fewer sugars in the exudates, compared to the wild type. This shift in exudate profile resulted in a substantial shift in the root-associated bacterial community, including an increase in the relative abundance of operational taxonomic units (OTUs) related to known PGPR species. These examples may be limited, but clearly demonstrate the potential for designed rhizospheres. However, the magnitude and consequences of such modifications in root depositions will require a thorough determination of possible undesirable effects.

Plant Genetic Manipulation: Rhizosphere Bacteria as Nontarget Organisms

Genetic engineering has been applied to crop plants to address different agricultural traits, for example, resistance to chemical herbicides, insect pest resistance, stress tolerance, and food quality.

Herbicide-resistant transgenic crop lines are the most widespread transgenic crops in commercial use. Among these, glyphosate- or glufosinate-resistant lines have been most studied with respect to possible nontarget rhizosphere effects (Kremer and Means 2009). Studies of oilseed rape (*Brassica napus*) cultivars yielded variable results. Siciliano and Germida (1999) isolated heterotrophic rhizospheric bacteria and endorhiza of cv. Excel and its glyphosate-resistant derivative "Quest" and found that in the genetically modified line, bacteria of the genera *Bacillus, Micrococcus*, *Variovorax*, and *Arthrobacter* were negatively affected whereas *Flavobacterium* and *Pseudomonas* were enriched. Confirming this result, both fatty acid methyl ester and carbon-substrate utilization profiles of the total endorhizal communities were found to vary between these lines (Dunfield and Germida 2001). Conversely, comparative analysis using PCR-DGGE revealed only a minor and growth-stage-dependent effect of glufosinate-tolerance introduction on the total rhizosphere bacterial community and on the composition of *Pseudomonas* populations (Gyamfi et al. 2002). Similarly, Sessitsch et al. (2004) found that the effect of the transgenic modification on rhizosphere bacterial communities' DGGE profiles and selective activities was more apparent at early stages of oilseed rape growth. The total (Schmalengerger and Tebbe 2002) and denitrifying (Philippot et al. 2006; Hart et al. 2009) bacterial communities in the rhizosphere of maize were similar for conventional and glyfosate-resistant lines, whereas a study of sugar beet (*Beta vulgaris*) varieties revealed differences in the compositions of rhizosphere bacterial communities of conventional versus herbicide-resistant plants, as determined by genetic fingerprinting of 16S rRNA genes (Schmalenberger and Tebbe 2003). In contrast, a reduction in cultivable fluorescent *Pseudomonas* was found in a comparison of conventional and glyphosate-resistant soybean (Kremer and Means 2009).

Another example of a widely commercialized transgenic trait is insect resistance, conferred by genetic modification for expression of the crystal (Cry) protein from the bacterium *Bacillus thuringiensis* (Bt crops). The protein may be released from the roots into the rhizosphere through natural wounding of roots and from sloughed-off and senescent cells, resulting in nontarget effects. Brusetti et al.'s (2004) study indicated a significant effect on cultivable and total bacterial communities in Bt compared to nontransgenic maize. In addition, the numbers of some cultivable bacterial groups (nitrogen-fixing, phosphorus-solubilizing, potassium-solubilizing) were lower in Bt lines of cotton compared to the parental line during the early and mid-stages of growth (Rui et al. 2005). In contrast, reports from experiments with maize (Baumgrate and Tebbe 2005), rice (Liu et al. 2008; Wu et al. 2009a), and *Brassica rapa* (Jung et al. 2008) concluded that the nontarget effect of Bt transformation is marginal.

T4 lysozyme expression in transgenic crops is a strategy developed to overcome plant diseases for which the pathogenic agent is a bacterium (e.g., *Erwinia carotovora*). Indeed, in cell-free extracts of tubers from transgenic potatoes, the lytic activity against bacterial cultures was higher than that present in the nontransgenic lines (De Vries et al. 1999). Those bacteria included Gram-positive and Gram-negative plant-pathogenic species, but also plant-growth-promoting species, such as *Rhizobium leguminosarum*. In addition, Ahrenholtz et al. (2000) demonstrated increased killing of inoculated *Bacillus subtilis* at the root-hair zone of potato roots. It is therefore surprising that a set of subsequent experiments concluded that the effect of T4 lysozyme expression in potatoes on rhizosphere or root endophytic bacterial communities is minor compared to the effects of other factors, such as the soil or site, or plant growth stage (Lottmann et al. 1999, 2000; Heuer et al. 2002; Rasche et al. 2006 FEMS; van Overbeek and van Elsas 2008). This lack of effect was explained by accelerated degradation of the T4 lysozyme by proteases in the soil, and by inaccessibility of the residing bacterial population. Similar results were obtained with transgenic plants modified to produce other enzymes, including lytic peptides (Sessitsch et al. 2003; Rasche et al. 2006FEMS) and lectins (Griffiths et al. 2000), and with zeaxanthin- (Weinert et al. 2009) and amylopectin-accumulating (Gschwendtner et al. 2010a, b; Gschwendtner et al. 2011) transgenic plants.

Overall, the different genetic modifications of plants rarely result in an overhaul of the rhizosphere bacterial community. It is therefore likely that the fraction of affected populations is small, and high-resolution methods are required for their detection and identification. Nevertheless, such effects should be considered on a case-by-case basis.

Consequences of Climate Change

One of the most important challenges faced by the scientific community today is predicting the outcome of global climate change on ecosystem functioning. With respect to soil microbial communities, this challenge is deemed difficult to impossible, due to its complexity and the virtually infinite ways in which different climate drivers (CO_2 and O_3 concentrations, temperature, precipitation, UV-B radiation) and their interactions might affect soil microorganisms and their activities (Bardgett et al. 2008). As the "hotspot" of microbial activity in soil, the effects of changes in climate drivers may be most pronounced in the rhizosphere. Moreover, rhizosphere processes may be central to plant productivity responses to elevated atmospheric CO_2 and, consequently, important controllers of the ecosystem response (Phillips 2007). Among rhizospheric processes, those related to the status of mineral nutrients should be specifically considered, since their availability may determine the plant's response to climate changes (Lewis et al. 2010; Tobita et al. 2011).

The most explored changing climate driver in rhizosphere research is elevated atmospheric CO_2 (eCO_2) (Drigo et al. 2008). Generally, atmospheric enrichment in CO_2 increases productivity of both C3 and C4 plants through stimulation of photosynthesis and improved water-use efficiency (Wand et al. 1999; Morgan et al. 2004; Lopes and Foyer 2012). However, the effect of eCO_2 varies with plant species and in response to variations in abiotic conditions, including nutrient availability, temperature, soil moisture, and salinity, among others (Lopes and

Foyer 2012). With respect to below-ground plant responses, increased root biomass (Ferris and Taylor 1993; Rogers et al. 1994; Phillips et al. 2009), changes in root morphology (Pregitzer et al. 2000; Larigauderie et al. 1994), and increased mycorrhization (rev. by Drigo et al. 2008 and by Compant et al. 2010b) have all been reported. However, most important might be changes in the amount and composition of root depositions. Increases in rhizodeposition have been reported in several studies (de Graaff et al. 2009; Phillips et al. 2009, 2011), but the opposite situation has also been indicated (Augustine et al. 2011). All of the above are considered key factors affecting the root-associated prokaryotes. Therefore, shifts in rhizospheric microbial communities' biomass, composition, and activity are anticipated (Díaz et al. 1993; Paterson et al. 1997). However, in comparison to the fairly conclusive results regarding fungi, and in particular mycorrhiza (Compant et al. 2010b), the responses of rhizospheric prokaryotes to eCO_2 have been much more difficult to generalize.

A number of methods have been used to estimate the effect of eCO_2 on the size of rhizosphere microbial communities, including chloroform fumigation, determination of phospholipid fatty acids (PLFA), direct viable counts, cultivation on defined media, and quantitative real-time PCR, with mixed results (Zak et al. 2000; Drigo et al. 2008). Rattray et al. (1995), Paterson et al. (1996) and Griffiths et al. (1998) reported a significant reduction in the proportion of root-derived carbon assimilated by rhizospheric bacteria under eCO_2. This was hypothesized to be related to reduction in the availability of nutrients, including nitrogen, due to higher consumption by the plant. Levels of nutrient limitation could explain the variability in bacterial biomass response to eCO_2, as do other variables such as water limitation (Augustine et al. 2011). Indeed, microbial biomass was found to increase under eCO_2 following addition of mineral nutrients (Klironomos et al. 1996) or organic matter (Dorodnikov et al. 2009). However, in other experiments, viable rhizosphere bacterial count (Rillig et al. 1997) or number of cultivable heterotrophic bacteria (Grayston et al. 1998a) remained steady under eCO_2, regardless of fertilization.

Community-level examination of composition and structure, using PLFA profiles and PCR-DGGE, has also produced mixed results. Responses to eCO_2 manifested by shifts in composition have been reported for different plant-soil systems (Ringelberg et al. 1997; Jossi et al. 2006; Drigo et al. 2007; Kao-Kniffin and Balser 2007; Kohler et al. 2010), but reports of stability are equally common (Montealegre et al. 2002; Rønn et al. 2002; Wasaki et al. 2005; Haase et al. 2008; Paterson et al. 2008). No response in archaeal community composition was found in maize, but in soybean the relative abundance of Crenarchaeota was reduced (Nelson et al. 2010). Using PLFA-based stable isotope probing, a specific response of a group of Gram-positive bacteria was detected within the metabolically active subset of a mixed-grasses rhizosphere community (Denef et al. 2007). In addition, Jossi et al. (2006) showed better manifestation of the effect of eCO_2 in active rhizosphere populations compared to the total community, based on a comparison of RNA- and DNA-based community profiles. This suggests that the responsive population may not necessarily be numerically dominant.

The effect of eCO_2 may be more pronounced when specific microbial groups are targeted. For instance, significant effects of elevated eCO_2 on *Pseudomonas* and *Rhizobium* populations were reported (Marilley et al. 1999; Drigo et al. 2009; Schortemeyer et al. 1996; Montealegre et al. 2000). This response varied in trend among different plant hosts and among different soils. In the rhizosphere of *Larrea tridentate*, the response to changes in CO_2 level was restricted to Firmicutes (Nguyen et al. 2011). In the rhizosphere of a wetland plant, *Typha angustifolia*, relative abundance of acetate-consuming methanogenic Archaea increased in response to eCO_2 (Kao-Kniffin et al. 2011). Succession of root-inhabiting methanogenic Archaea in rice was slowed down under eCO_2 (Hashimoto-Yasuda et al. 2005). In contrast, where examined, the composition of nitrifying or denitrifying rhizosphere bacteria remained stable under eCO_2 (Deiglmayr et al. 2004; Bowatte et al. 2007; Nelson et al. 2010; Pereira et al. 2011).

The effects of additional climatic drivers on soil and rhizosphere microbial communities and their interactions have been much less studied. For the Antarctic plant *Deschampsia antarctica*, changes in UV-B irradiation level affected community-level physiological profiles (CLPP) but not the number of cultivable bacteria (Avery et al. 2003). Increased UV-B irradiation over *Eriophorum russeolum plants* resulted in shifts in PLFA profiles and CLPP, with no effect on total microbial biomass (Rinan et al. 2008). Ozone-stressed grasses showed remarkable similarity in the composition of their associated rhizosphere bacterial communities compared to respective controls (Dohrmann and Tebbe 2005). In contrast, following long-term exposure to ozone stress, composition of rhizosphere bacterial communities of *Fagus sylvatica* trees was significantly altered (Schloter et al. 2005; Esperschütz et al. 2009).

The data accumulated so far describe only the response of the community to relatively short-term changes. However, predictive hypotheses regarding adaptation of the rhizosphere prokaryotes and plants to climatic and atmospheric changes are, at this stage, somewhat premature. This results, in part, from the relative lack of long-term experimental data. In addition, all experiments, regardless of their excellent design, suffer from the bias of rather rapid changes in conditions. In reality, however, one would expect the shifts to be orders of magnitude slower, possibly allowing evolution and coevolution of the plants and associated microorganisms.

Genetic Traits Related to Rhizosphere Competence

Rhizosphere competence is a term that describes the specific ability of a microorganism to successfully colonize and survive in the rhizosphere. Several studies in recent years have demonstrated a number of bacterial functions, such as motility, attachment, growth, type III secretion, transport, stress resistance, and production of secondary metabolites, linked to rhizosphere

competence (for review see Kiely et al. 2006 and Barret et al. 2011). For example, Matilla et al. (2007) conducted a microarray-based experiment in which they studied *Pseudomonas putida* KT2440 (a known root-colonizing bacterium) genes, expressed during the interaction of the cells with corn (*Zea mays*) roots. They compared these to genes expressed under three other conditions: planktonic cells growing exponentially in rich medium, planktonic cells in stationary phase, and sessile populations established in sand microcosms. The expression level of a large number of genes was upregulated in the rhizosphere, many of which were highly induced relative to the other three control conditions (90 genes were upregulated at least twofold in the rhizosphere versus all three controls and of those, over 50 genes were induced more than sixfold!). One of the most impressive findings of their study was that amid the extensive rhizosphere-induced enhancement of gene expression, not one significantly downregulated gene could be found. This phenomenon might have been related to the mixed physiological status of individual cells within the rhizosphere population. The rhizosphere-activated genes included genes involved in amino acid uptake and metabolism of aromatic compounds, reflecting the availability of particular nutrients in this plant's root exudates. In addition, efflux pumps and enzymes for glutathione metabolism were upregulated, suggesting that adaptation to adverse conditions and stress (oxidative) response plays an important role in rhizosphere competence in this system. The finding of a GGDEF/EAL domain response regulator among the induced genes suggests a role for the secondary messenger c-diGMP in root colonization and survival of *P. putida* in this rhizosphere system (Matilla et al. 2007).

When *P. fluorescens* WCS365 was applied to tomato monoaxenic root system, several genes were identified as involved in competitive root colonization (Lugtenberg et al. 2001; Lugtenberg and Kamilova 2009). Among the genes and traits identified were those related to motility and chemotaxis toward, and utilization of, root exudates. Amino acids and dicarboxylic acids, but not sugars, were important root attractants in this *P. fluorescens* WCS365 and tomato system, while in *Arabidopsis*, malate was a major attractant of *Bacillus subtilis* FB17 (Rudrappa et al. 2008). Other competitive root-colonization-related genes and traits were involved in adhesion, synthesis of amino acids, uracil, and vitamin B1, lipopolysaccharide structure, the ColR/ColS sensory system, the putrescine-uptake system, site-specific recombinases, NADH:ubiquinone oxidoreductase, protein secretion, and the type III secretion system (Lugtenberg et al. 2001; Lugtenberg and Kamilova 2009).

When *Rhizobium leguminosarum* was grown in the rhizospheres of pea (its legume nodulation host), alfalfa (non-host legume), and sugar beet (non-legume), several host-specific traits were identified (Ramachandran et al. 2011). Many plasmid (pRL8)-encoded genes were specifically induced in the pea nodulation host. As expected, *nod* genes were induced only in the rhizospheres of the two legumes. In the pea rhizosphere, a specific transporter, possibly for monosaccharides, was also found to be important. In addition, increased expression of genes encoding enzymes of the glyoxylate cycle was found in the pea rhizosphere. The study also identified bacterial responses common to rhizospheres of all three host plants, such as organic acid, C1-C2 and aromatic amino acid metabolism, hypoosmotic regulation, detoxification and multidrug resistance (MDR) family efflux pump, genes involved in the response to stress (general and oxidative), and many genes encoding proteins of unknown function.

So far, studies with single strains, PGPR, or *Rhizobium* have provided valuable information on gene expression of these organisms and on symbiotic microbe-legume interactions (Becker et al. 2004; Ruffel et al. 2008). However, despite advances in metagenomic and metatranscriptomic techniques, due to the extreme complexity of the system, the functions of the complex natural rhizosphere community have not yet been described. As nicely put by Schenk et al. (2012) in a recent review, "It is likely that an unbiased multi-species approach such as metatranscriptomics will lead to the discovery of potentially interesting (yet unknown) plant-microbe relationships."

Conclusions

The importance of rhizosphere communities to plant health and development is clear.

This chapter attempted to illustrate and analyze the microbial ecology in the rhizosphere, as revealed by enormous body of literature, resulting from over a century of research. Nevertheless, our ability to draft a comprehensive and ubiquitous ecological theory on the behavior of microorganisms in the rhizosphere becomes impossible, due to the ambiguous and partial picture still arising from the current knowledge. This is in contrast to the currently established theories in macroecology, and results from the enormous complexity of the system, affected by multiple parameters such as plant species and its physiological state and age, soil characteristics and environmental conditions, as well as the microbial diversity. We are therefore limited in our current ability to draw general hypotheses regarding the rhizosphere prokaryotes and this area is therefore restricted to case-by-case studies.

Several future possibilities may be envisioned: One is that additional research and technological advances in in situ studies of microbial structure and function will lead to general understanding of the rhizosphere ecology. Alternatively, indeed due to the diversity and complex nature of the system, and of different rhizospheres, they are directed to individual unique paths. In either case, it is imperative to move forward rhizosphere research, taking advantage of the advanced molecular and imaging tools developed in recent years.

Many questions still remain open: What makes an organism rhizosphere competent? What makes one population dominant? Is there a cross-talk between the plant and the bacteria in nonspecific interactions? Is it possible to detect coevolution between plants and their rhizosphere community? If so, could different mechanisms occur in different plant species? How to make inoculation with beneficial microorganism a success?

References

Abou-Shanab RAI, van Berkum P, Angel JS (2007) Heavy metal resistance and genotypic analysis of metal resistance genes in Gram-positive and Gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. Chemospehre 68:360–367

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ahrenholtz I, Harms K, De Vries J, Wackernagel W (2000) Increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 lysozyme-producing potatoes. Appl Environ Microbiol 66:1862–1865

Alvey S, Yang CH, Buerkert A, Crowley DE (2003) Cereal/legume rotation effects on rhizosphere bacterial community structure in west African soils. Biol Fertil Soils 37:73–82

Amann R, Lodwig W (2000) Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiol Rev 24:555–565

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–67

Aranda S, Montes-Borrego M, Jiménez-Díaz RM, Landa BB (2011) Microbial communities associated with the root system of wild olives (*Olea europaea* L. subsp. *europaea* var. sylvestris) are good reservoirs of bacteria with antagonistic potential against *Verticillium dahlia*. Plant Soil 343:329–345

Artursson N, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Asanuma S, Tanaka H, Yatazawa M (1979) Rhizoplane microorganisms of rice seedlings as examined by scanning electron microscopy. Soil Sci Plant Nutr 25:539–551

Ashoub AH, Amara MT (2010) Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. J Am Sci 6:321–328

Assmus B, Hutzler P, Kirchhof G, Amann, R, Lawrence JR, Hartmann A (1995) In situ localization of *Azospirillum brasilense* in the rhizosphere of wheat with fluorescently labeled, rRNA-targeted oligonucleotide probes and scanning confocal laser microscopy. Appl Environ Microbiol 61:1013–1019

Augustine DJ, Dijkstra FA, Hamilton EW, Morgan JA (2011) Rhizosphere interactions, carbon allocation, and nitrogen acquisition of two perennial North American grasses in response to defoliation and elevated atmospheric CO_2. Oecologia 165:755–770

Avery LM, Lewis Smith RI, West HM (2003) Response of rhizosphere microbial communities associated with Antarctic hairgrass (*Deschampsia antarctica*) to UV radiation. Polar Biol 26:525–529

Bacilio-Jiménez M, Aguilar-Flores S, del Valle MV, Pérez A, Zepeda A, Zenteno E (2001) Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. Soil Biol Biochem 33:167–172

Bardgett RD, Freeman C, Ostle N (2008) Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2:805–814

Bardi DV, Quintana N, El Kassis EG, Kim KH, Choi YH, Sugiyama A, Verpoorte R, Martinoia E, Manter DK, Vivanco JM (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol 151:2006–2017

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. Biol Fertil Soils 47:729–743

Barriuso J, Ramos Solano B, Santamaría C, Gutiérez Mañero FJ (2008) Effect of inoculation with putative plant growth-promoting rhizobacteria isolated from *Pinus* spp. on *Pinus pinea* growth, mycorrhization and rhizosphere microbial communities. J Appl Microbiol 105:1289–1309

Bashan Y, Levanony H, Whitmoyer RE (1991) Root surface colonization of non-cereal crop plants by pleomorphic *Azospirillum brasilense* Cd. J Gen Microbiol 137:187–196

Bashan Y, Puente ME, Rodriguez-Mendoza MN, Toledo G, Holguin G, Ferrera-Cerrato R, Pedrin S (1995) Survival of *Azospirillum brasilense* in the bulk soil and rhizosphere of 23 soil types. Appl Environ Microbiol 61:1938–1945

Baudoin E, Benizri E, Guckert A (2002) Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145

Baumgrate S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol Ecol 14:2539–2551

Becker A, Berges H, Krol E, Bruand C, Ruberg S, Capela D, Lauber E, Meilhoc E, Ampe F, de Bruijn FJ, Fourment J, Francez-Charlot A, Kahn D, Kuster H, Liebe C, Puhler A, Weidner S, Batut J (2004) Global changes in gene expression in Sinorhizobium meliloti 1021 under microoxic and symbiotic conditions. Mol Plant-Microbe Interact 17:292–303

Belcom IN, Crowley DE (2009) Pyrene effects on rhizoplane bacterial communities. Int J Phytoremediat 11:609–622

Bending GD, Poole EJ, Whipps JM, Read DJ (2002) Characterisation of bacteria from *Pinus sylvestris-Suillus luteus* mycorrhizas and their effects on root-fungus interactions and plant growth. FEMS Microbiol Ecol 39:219–227

Bending GD, Aspray TJ, Whipps JM (2006) Significance of microbial interactions in the mycorrhizosphere. Adv Appl Microbiol 60:97–131

Benitez MS, Tustas FB, Rotenberg D, Kleinhenz MD, Cardina J, Stinner D, Miller SA, McSpadden Gardene BB (2007) Multiple statistical approaches of community fingerprint data reveal bacterial populations associated with general disease suppression arising from the application of different organic field management strategies. Soil Biol Biochem 39:2289–2301

Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci Techn 11:557–574

Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticullium* host plants. Appl Environ Microbiol 68:3328–3338

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol 51:215–229

Berg G, Opelt K, Zachow C, Lottmann J, Götz M, Costa R, Smalla K (2006) The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. FEMS Microbiol Ecol 56:250–261

Bergsma-Vlami M, Prins ME, Raaijmakers JM (2005) Influence of plant species on population dynamics, genotypic diversity and antibiotic production in the rhizosphere by indigenous *Pseudomonas* spp. FEMS Microbiol Ecol 52:59–69

Blatny JM, Ho J, Skogan G, Fykse EM, Aarskaug T, Waagen V (2011) Airborne Legionella bacteria from pulp waste treatment plant: aerosol particles characterized as aggregates and their potential hazard. Aerobiologia 27:147–162

Bomberg M, Timonen S (2007) Distribution of Cren- and Euryarchaeota in Scots pine mycorrhizospheres and boreal forest humus. Microb Ecol 54:406–416

Bomberg M, Montonen L, Timonen S (2010) Anaerobic Eury- and Crenarchaeota inhabit ectomycorrhizas of boreal forest Scots pine. Eur J Soil Biol 46:356–364

Bomberg M, Münster U, Pumpanen J, Ilvesniemi H, Heinonsalo J (2011) Archaeal communities in boreal forest tree rhizospheres respond to changing soil temperatures. Microb Ecol 62:205–217

Bowatte S, Asakawa S, Okada M, Kobayasi K, Kimura M (2007) Effect of elevated atmospheric CO_2 concentration on ammonia oxidizing bacteria communities inhabiting in rice roots. Soil Sci Plant Nutr 53:32–39

Bowen GD, Theodorou C (1979) Interactions between bacteria and ectomycorrhizal fungi. Soil Biol Biochem 11:119–126

Brooks DD, Chan R, Starks ER, Grayston SJ, Jones MD (2011) Ectomycorrhizal hyphae structure components of the soil bacterial community for decreased phosphate production. FEMS Microbiol Ecol 76:245–255

Brusetti L, Francia P, Bertolini C, Pagliuca A, Borin S, Sorlini C, Abruzzese A, Sacchi G, Viti C, Giovannetti L, Giuntini E, Bazzicalupo M, Daffonchio D (2004) Bacterial communities associated with the rhizosphere of transgenic

Bt 176 maize (*Zea mays*) and its non transgenic counterpart. Plant Soil 266:11–21

Buddrus-Schiemann K, Schmid M, Schreiner K, Welzl G, Hartmann A (2010) Root colonization by *Pseudomonas* sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. Microb Ecol 60:381–393

Cadillo-Quiroz H, Yavitt JB, Zinder SH, Thies JE (2010) Diversity and community structure of Archaea inhabiting the rhizoplane of two contrasting plants from and acidic bog. Microb Ecol 59:757–767

Çakmakçi R, Dönmez MF, Ertürk Y, Erat M, Haznedar A, Sekban R (2010) Diversity and metabolic potential of culturable bacteria from the rhizosphere of Turkish tea grown in acidic soils. Plant Soil 332:299–318

Campbell R, Rovira AD (1973) The study of the rhizosphere by scanning electron microscopy. Soil Biol Biochem 5:747–752

Castro-Sowinski S, Hersckovitz Y, Okon Y, Jurkevitch E (2007) Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiol Lett 276:1–11

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth–promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Cavalca L, Zanchi R, Corsini A, Colombo M, Romagnoli C, Canzi E, Andreoni V (2010) Arsenic-resistant bacteria associated with roots of the wild *Cirsium arvense* (L.) plant from an arsenic polluted soil, and screening of potential plant growth-promoting characteristics. Sys Appl Microbiol 33:154–164

Cavalo-Bado LA, Petch G, Parsons NR, Morgan JAW, Pettitt TR, Whipps JM (2006) Microbial community responses associated with the development of oomycete plant pathogens on tomato roots in soilless growing systems. J Appl Micrbiol 100:1194–1207

Chauhan PS, Chaudhry V, Mishra S, Nautiyal CS (2011) Uncultured bacterial diversity in tropical maize (*Zea mays* L.) rhizosphere. J Basic Microbiol 51:15–32

Chelius MK, Triplett EW (2001) The diversity of Archaea and Bacteria in association with the roots of *Zea mays* L. Microb Ecol 41:252–263

Cheng W, Zhang Q, Coleman DC, Carroll CR, Hoffman CA (1996) Is available carbon limiting microbial respiration in the rhizosphere? Soil Biol. Biochem 28:1283–1288

Cheng Y, Howieson JG, O'Hara GW, Watkin ELJ, Souche G, Jaillard B, Hinsinger P (2004) Proton release by roots of *Medicago murex* and *Medicago sativa* growing in acidic conditions, an implication for rhizosphere pH changes and nodulation at low pH. Soil Biol Biochem 36:1357–1365

Chiarini L, Bevivino A, Dalmastri C, Nacamulli C, Tabacchioni S (1998) Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Appl Soil Ecol 8:11–18

Chin-A-Woeng TFC, de Priester W, van der Bij AJ, Lugtenberg BJJ (1997) Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. Mol Plant Microbe Interact 1:79–86

Chow ML, Radomski CC, McDermott JM, Davis J, Axelrood PE (2002) Molecular characterization of bacterial diversity in Lodgepole pine (*Pinus contorta*) rhizosphere soils from British Columbia forest soils differing in disturbance and geographic source. FEMS Microbiol Ecol 42:347–357

Chowdhury SP, Schmid M, Hartmann A, Tripathi AK (2009) Diversity of 16S-rRNA and *nifH* genes derived from rhizosphere soil and roots of an endemic drought tolerant grass, *Lasiurus sindicus*. Eur J Soil Biol 45:114–122

Chu H, Lin X, Fujii T, Morimoto S, Yagi K, Hu J, Zhang J (2007) Soil bacterial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol Biochem 39:2971–2976

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

Compant S, van der Heijden MGA, Sessitsch A (2010b) Climate change effects on beneficial plant-microorganism interactions. FEMS Microbiol Ecol 73:197–214

Conrad R, Erkel C, Liesack W (2006) Rice cluster I methanogenes, an important group of Archaea producing greenhouse gas in soil. Curr Opin Biotech 17:262–267

Conrad R, Klose M, Noll M, Kemnitz D (2008) Soil type links microbial colonization of rice roots to methane emission. Glob Change Biol 14:657–669

Costa R, Gomes NCM, Peixoto RS, Rumjanek N, Berg G, Mendoça-Hagler LCS, Smalla K (2006) Diversity and antagonistic potential of *Pseudomonas* spp. associated to the rhizosphere of maize grown in a subtropical organic farm. Soil Biol. Biochem 38:2434–2447

Dandurand LM, Schotzko DJ, Knudsen GR (1997) Spatial patterns of rhizoplane population of *Pseudomonas fluorescens*. Appl Environ Microbiol 63:3211–3217

da Rocha UN, van Elsas JD, van Overbeek LS (2010) Real-time PCR detection of Holophagae (*Acidobacteria*) and *Verrucomicrobia* subdivisión 1 groups in bulk and leek (*Alium porrum*) rhizosphere soils. J Microbiol Meth 83:141–148

De Brito Alvarez MA, Gagné S, Antoun H (1995) Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Appl Environ Microbiol 61:194–199

De Graaff MA, Van Kessel C, Six J (2009) Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO_2. Soil Biol Biochem 41:1094–1103

de la Fuente JM, Ramírez-Rodríguez V, Cabrera-Ponce JL, Herrera-Estrella L (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1568

De Leij FAAM, Whipps JM, Lynch JM (1993) The use of colony development for the characterization of bacterial communities in soil and on roots. Microb Ecol 24:81–97

De Leij FAAM, Sutton EJ, Whipps JM, Fenlon JS, Lynch JM (1995) Impact of field release of genetically modified Pseudomonas fluorescens on indigenous microbial populations of what. Appl Environ Microbiol 61:3443–3453

de Souza JT, Weller DM, Raaijmakers JM (2003) Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. Phytopathology 93:54–63

De Vries J, Harms K, Broer I, Kriete G, Mahn A, Düring K, Wackernagel W (1999) The bacteriolytic activity in transgenic potatoes expressing a chimeric T4 lysozyme gene and the effect of T4 lysozyme on soil- and phytopathogenic bacteria. Sys Appl Microbiol 22:280–286

DeAngelis KM, Brodie EK, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3:168–1780

Deiglmayr K, Philippot L, Hartwig UA, Kandeler E (2004) Structure and activity of the nitrate-reducing community in the rhizosphere of *Lolium perenne* and *Trifolium repens* under long-term elevated atmospheric CO_2. FEMS Microbiol Ecol 49:445–454

Dekkers LC, Mulders IHM, Phoelich CC, Chin-A-Woeng TFC, Wijfjes AHM, Lugtenberg BJJ (2000) The sss colonization gene of the tomato-*Fusarium oxysporum* f. sp. *radicis-lycopersici* biocontrol strain *Pseudomonas fluorescens* WCS365 can improve root colonization of other wild-type *Pseudomonas* spp. bacteria. Mol. Plant-Microbe Interact 13:1177–1183

Dell'Amico E, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. FEMS Microbiol Ecol 52:153–162

Denef K, Bubenheim H, Lenhart K, Vermeulen J, van Cleemput O, Boeckx P, Müller C (2007) Community shifts and carbon translocation within metabolically-active rhizosphere microorganisms in grasslands under elevated CO_2. Biogeosciences 4:769–779

Díaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364:616–617

Dohrmann AB, Tebbe CC (2005) Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. Appl Environ Microbiol 71:7750–7758

Domanski G, Kuzyakov Y, Siniakina SV, Stahr K (2001) Carbon flows in the rhizosphere of rygrass (*Lolium perenne*). J Plant Nutr Soil Sci 164:381–387

Donachie SP, Foster JS, Brown MV (2007) Culture clash: challenging the dogma of microbial diversity. ISME J 1:97–102

Donate-Correa J, León-Barrios M, Pérez-Galdona R (2004) Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant Soil 266:261–272

Dorodnikov M, Blagodatskaya E, Blagodatsky S, Marhan S, Fangmeier A, Kuzyakov Y (2009) Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Glob Change Biol 15:1603–1614

Drigo B, Kowalchuk GA, Yergeau E, Bezemer TM, Boschker HS, van Veen JA (2007) Impact of elevated carbon dioxide on the rhizosphere communities of *Carex arenaria* and *Festuca rubra*. Glob Change Biol 13:2396–2410

Drigo B, Kowalchuk GA, van Veen JA (2008) Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere. Biol Fert Soils 44:667–679

Drigo B, van Veen JA, Kowalchuk GA (2009) Specific rhizosphere bacterial and fungal groups respond differently to elevated atmospheric CO_2. ISME J 3:1204–1217

Duineveld BM, Van Veen JA (1999) The number of bacteria in the rhizosphere during plant development: relating colony-forming units to different reference units. Biol Fert Soils 28:285–291

Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl Environ Microbiol 65:1662–1669

Dunbar J, Barns SM, Ticknor LO, Kuske CR (2002) Empirical and theoretical bacterial diversity in four Arizona soils. Appl Environ Microbiol 68:3035–3045

Dunfield KE, Germida JJ (2001) Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. FEMS Microbiol Ecol 38:1–9

Dutta S, Podile AR (2010) Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Crit Rev Microbiol 36:232–244

Egamberdleva D, Kamllova F, Valldov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

El-Tarabily KA, Soliman MH, Nassar AH, Al-Hassani HA, Sivasithamparam K, McKenna F, Hardi GESJ (2000) Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Plant Pathol 49:573–583

Enwell K, Philippot L, Hallin S (2005) Activity and composition of denitrifying bacterial community respond differentially to long-term fertilization. Appl Environ Microbiol 71:8335–8343

Esperschütz J, Gattinger A, Mäder P, Shloter M, Fließbach A (2007) Response of soil microbial biomass and community structure to conventional and organic farming systems under identical crop rotations. FEMS Microbiol Ecol 61:26–37

Esperschütz J, Pritsch K, Gattinger A, Welzl G, Haesler F, Buegger F, Winkler JB, Munch JC, Schloter M (2009) Influence of chronic ozone stress on carbon translocation pattern into rhizosphere microbial communities of beech trees (*Fagus sylvatica* L.) during a growing season. Plant Soil 323:85–95

Fan F, Zhang F, Lu Y (2011) Linking plant identity and interspecific competition to soil nitrogen cycling through ammonia oxidizer communities. Soil Biol Biochem 43:46–54

Faye A, Krasova-Wade T, Thiao M, Thioulouse J, Neyra M, Prin Y, Galiana A, Ndoye I, Dreyfus B, Duponnois R (2009) Controlled ectomycorrhization of an exotic legume tree species *Acacia holoseriacea* affects the structure of root nodule bacteria community and their symbiotic effectiveness on *Faidherbia albida*, a native Sahelian *Acacia*. Soil Biol Biochem 41:1245–1252

Felici C, Vettori L, Giraldi E, Forino LMC, Toffanin A, Tagliasacchi AM, Nuti M (2008) Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. Appl Soil Ecol 40:260–270

Ferrero MA, Menoyo E, Lugo MA, Negritto MA, Farías ME, Anton AM, Siñeriz F (2010) Molecular characterization and in situ detection of bacterial communities associated with rhizosphere soil of high altitude native Poaceae from the Andean Puna region. J Arid Environ 74:1177–1185

Ferris R, Taylor G (1993) Contrasting effects of elevated CO_2 on the root and shoot growth of four native herbs commonly found in chalk grassland. New Phytol 125:855–866

Fierer N, Breitbart M, Nulton J, Salomon P, Lozupone C, Jones R, Robeson M, Edwards RE, Felts B, Rayhawk S, Knight R, Rohwer F, Jackson RB (2007) Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of Bacteria, Archaea, Fungi and Viruses in soil. Appl Environ Microbiol 73:7059–7066

Filion M, Hamelin RC, Bernier L, St-Arnaud M (2004) Molecular profiling of rhizosphere microbial communities associated with healthy and diseased black spruce (*Picea mariana*) seedlings grown in a nursery. Appl Environ Microbiol 70:3541–3551

Folman LB, Postma J, Van Veen JA (2001) Ecophysiological characterization of rhizosphere bacterial communities at different root locations and plant developmental stages of cucumber grown on rockwool. Microb Ecol 42:586–597

Foster RC (1986) The ultrastructure of the rhizoplane and rhizosphere. Annu Rev Phytopathol 24:211–234

Foster RC, Bowen GD (1982) Plant surfaces and bacterial growth: the rhizosphere and rhizoplane. In: Mount MS, Lacy GH (eds) Phytopathogenic prokaryotes. Academic, New York, pp 159–185

Frenzel P, Bosse U (1996) Methyl fluoride, an inhibitor of methane oxidation and methane production. FEMS Microbiol Ecol 21:25–36

Frey-Klett P, Chavatte M, Clausse ML, Courrier S, Le Roux C, Raaijmakers JM, Martinotti MG, Pierrat JC, Garbaye J (2005) Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent Pseudomonads. New Phytol 165:317–328

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Fürnkranz M, Müller H, Berg G (2009) Characterization of plant growth promoting bacteria from crops in Bolivia. J Plant Dis Protect 4:149–155

Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309:1387–1390

Gantner S, Schmid M, Dürr C, Schuhegger R, Steidle A, Hutzler P, Langebartels C, Eberl L, Hartmann A, Dazzo FB (2006) *In situ* quantification of the spatial scale of calling distances and population density-independent N-acylhomoserine lactone-mediated communication by rhizobacteria colonized on plant roots. FEMS Microbiol Ecol 56:188–194

Gao Y, Yang Y, Ling W, Kong W, Zhu X (2011) Gradient distribution of root exudates and polycyclic aromatic hydrocarbons in rhizosphere soil. Soil Sci Soc Am J 75:1694–1703

Garbeva P, van Veen JA, van Elsas JD (2004) Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. FEMS Microbiol Ecol 47:51–64

Garbeva P, van Elsas JD, van Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. Plant Soil 302:19–32

Gardner T, Acosta-Martinez V, Senwo Z, Dowd SE (2011) Soil rhizosphere microbial communities and enzyme activities under organic farming in Alabama. Diversity 3:308–328

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

Giovannoni S, Stingl U (2007) The importance of culturing bacterioplancton in the 'omics' age. Nat Rev Microbiol 5:820–826

Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendoça-Halger L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232:167–180

Gomes NCM, Cleary DFR, Pinto NF, Egas C, Almeida A, Cunha A, Mendonça-Hagler LCS, Smalla K (2010) Taking root: Enduring effect of rhizosphere bacteiral colonization in mangroves. PLoS One 5:e14065

Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW (2011) Distinct Microbial Communities within the Endosphere and Rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl Environ Microbiol 77:5934–5944

Gransee A, Wittenmayer L (2000) Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J Plant Nutr Soil Sci 163:381–385

Grayston SJ, Campbel CD, Lutze JL, Gifford RM (1998a) Impact of elevated CO_2 on the metabolic diversity of microbial communities in N-limited grass swards. Plant Soil 203:289–300

Grayston SJ, Wang S, Campbell CD, Edwards AC (1998b) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378

Green SJ, Inbar E, Michel FC Jr, Hadar Y, Minz D (2006) Succession of bacterial communities during early plant development: transition from seed to root and effect of compost amendment. Appl Environ Microbiol 72:3975–3983

Green SJ, Michel FC Jr, Hadar Y, Minz D (2007) Contrasting patterns of seed and root colonization by bacteria from the genus Chrysobacterium and from the family Oxalobacteraceae. ISME J 1:291–299

Gremion F, Chatzinotas A, Harms H (2003) Comparative 16S rDNA and 16S rRNA sequence analysis indicates that *Actinobacteria* might be a dominant part of the metabolically active bacteria in heavy metal contaminated bulk and rhizosphere soil. Environ Microbiol 5:896–907

Griffiths BS, Ritz K, Ebblewhite N, Paterson E, Killham K (1998) Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with observations on wheat rhizosphere. Soil Biol Biochem 30:315–321

Griffiths BS, Geoghegan IE, Robertson WM (2000) Testing genetically engineered potato, producing lectins GNA and Con A, on non-target soil organisms and processes. J Appl Ecol 37:159–170

Griffiths BS, Caul S, Thompson J, Birch ANE, Cortet J, Andersen MN, Krogh PH (2007) Microbial and microfaunal community structure in cropping systems with genetically modified plants. Pedobiologia 51:195–206

Grosskopf R, Stubner S, Liesack W (1998) Novel Euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl Environ Microbiol 64:4983–4989

Gschwendtner S, Reichmann M, Müller M, Radl V, Munch JC, Schloter M (2010a) Abundance of bacterial genes encoding for proteases and chitinases in the rhizosphere of three different potato cultivars. Biol Fertil Soils 46:649–652

Gschwendtner S, Reichmann M, Müller M, Radl V, Munch JC, Schloter M (2010b) Effects of genetically modified amylopectin-accumulating potato plants on the abundance of beneficial and pathogenic microorganisms in the rhizosphere. Plant Soil 335:413–422

Gschwendtner S, Esperschütz J, Buegger F, Reichmann M, Müller M, Munch JC, Schloter M (2011) Effects of genetically modified starch metabolism in potato plants on photosynthate fluxes into the rhizosphere and on microbial degraders of root exudates. FEMS Microbiol Ecol 76:564–575

Guerrero-Molina MF, Einik BC, Pedraza RO (2011) More than rhizosphere colonization of strawberry plants by *Azospirillum brasilense*. Appl Soil Ecol. doi:10.1016/j.apsoil.2011.10.011

Gyamfi S, Pfeifer U, Stierschneider M, Sessitisch A (2002) Effects of transgenic glufosinate-tolerant oilseed rape (*Brassica napus*) and the associated herbicide application on eubacteiral and *Pseudomonas* communities in the rhizosphere. FEMS Microbiol Ecol 14:181–190

Haase S, Philippot L, Neumann G, Marhan S, Kandeler E (2008) Local response of bacterial densities and enzyme activities to elevated atmospheric CO_2 and different N supply in the rhizosphere of *Phaseolus vulgaris* L. Soil Biol Biochem 40:1225–1234

Hallmann J, Rodŕiguez-Kábana R, Kloepper JW (1999) Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biol Biochem 31:551–560

Han J, Song Y, Liu Z, Hu Y (2011) Culturable bacterial community analysis in the rot domains of two varieties of tree peony (*Paeonia ostii*). FEMS Microbiol Lett 322:15–24

Hansen M, Kragelund L, Nybroe O, Sørensen J (1997) Early colonization of barley roots by *Pseudomonas fluorescens* studies by immonofluorescence technique and confocal laser scanning microscopy. FEMS Microbiol Ecol 23:353–360

Hart MM, Powell JR, Gulden RH, Dunfield KE, Pauls KP, Swanton CJ, Klironomos JN, Antunes PM, Koch AM, Trevors JT (2009) Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn. Pedobiologia 52:253–262

Hashimoto-Yasuda T, Ikenaga M, Asakawa S, Kim HY, Okada M, Kobayashi K, Kimura M (2005) Effect of free-air CO_2 enrichment (FACE) on methanogenic Archaeal communities inhabiting rice roots in a Japanese rice field. Soil Sci Plant Nutr 51:91–100

Hayat R, Ali S, Amara U, Khalid R, Ahmad I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

He Z, Xu M, Deng Y, Kang S, Kellogg L, van Nostrand JD, Hobbie SE, Reich PB, Zhou J (2010) Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. Ecol Lett 13:564–575

Herrmann M, Saunders AM, Schramm A (2008) Archaea dominate the ammonia-oxidizing community in the rhizosphere of the freshwater macrophyte *Littorella uniflora*. Appl Environ Microbiol 74:3279–3283

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

Herschkovitz Y, Lerner A, Davidov Y, Rothballer M, Hartmann A, Okon Y, Jurkevitch E (2005b) Inoculation with the plant-growth-promoting rhizobacterium *Azospirillum brasilense* causes little disturbance in the rhizosphere and rhizoplane of maize (*Zea mays*). Microb Ecol 50:277–288

Heuer H, Kroppenstedt RM, Lottmann J, Berg G, Smalla K (2002) Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. Appl Environ Microbiol 68:1325–1335

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237:173–195

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152

Högberg P, Read DJ (2006) Towards a more plant physiological perspective on soil ecology. Trends Ecol Evol 21:548–554

Högberg P, Högberg MN, Göttlicher SG, Beston NR, Keel SG, Metcalfe DB, Campbell C, Schindlbacher A, Hurry V, Lundmark T, Linder S, Näsholm T (2008) High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. New Phytol 177:220–228

Hood MA, van Dijk KV, Nelson EB (1998) Factors affecting attachment of *Enterobacter cloacae* to germinating cotton seed. Microb Ecol 36:101–110

Hütsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition- an important source for carbon turnover in soils. J Plant Nutr Soil Sci 165:397–407

Ibekwe AM, Grieve CM (2004) Changes in developing plant microbial community structure as affected by contaminated water. FEMS Microbiol Ecol 48:239–248

Ibekwe AM, Poss JA, Grattan SR, Grieve CM, Suarez D (2010) Bacterial diversity in cucumber (*Cucumis sativus*) rhizosphere in response to salinity, soil pH, and carbon. Soil Biol Biochem 42:567–575

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl Environ Microbiol 70:2667–2677

Idris A, Labuschagne N, Korsten L (2009) Efficacy of rhizobacteria for growth promotion in sorghum under greenhouse conditions and selected modes of action studies. J Agri Sci 147:17–30

Ikeda S, Okubo T, Takeda N, Banba M, Sasaki K, Imaizumi-Anraku H, Fujihara S, Ohwaki Y, Ohshima K, Fukuta Y, Eda S, Mitsui H, Hattori M, Sato T, Shinano T, Minamisawa K (2011) The genotype of the calcium/calmodulin-dependent protein kinase dene (CCaMK) determines bacterial community diversity in rice roots under paddy and upland field conditions. Appl Environ Microbiol 77:4399–4405

Inbar E, Green SJ, Hadar Y, Minz D (2005) Competing factors of compost concentration and proximity to root affect the distribution of Streptomycetes. Microb Ecol 50:73–81

Inceoğlu Ö, Abu Al-Soud W, Falcã Salles J, Semanov AV, van Elsas JD (2011) Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 6:e23321

Insunza V, Alström S, Eriksson KB (2002) Root bacteria from nematicidal plants and their biocontrol potential against trichodorid nematodes in potato. Plant Soil 241:271–278

Jack ALH, Rangarajan A, Culman SW, Sooksa-Nguan T, Thies JE (2011) Choice of organic amendments in tomato transplants has lasting effects on bacterial rhizosphere communities and crop performance in the field. Appl Soil Ecol 48:94–101

Jenkins MB, Franzluebbers AJ, Humayoun SB (2006) Assessing short-term responses of prokaryotic communities in bulk and rhizosphere soils to tall fescue endophyte infection. Plant Soil 289:309–320

Ji X, Lu G, Gai Y, Gao H, Lu B, Kong L, Mu Z (2010) Colonization of *Morus alba* L. by the plant-growth-promoting and antagonistic bacterium *Burkholderia cepacia* strain Lu10-1. BMC Microbiol 10:243

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil 321:5–33

Jossi M, Fromin N, Tarnawski S, Kohler F, Gillet F, Aragno M, Hamelin J (2006) How elevated pCO_2 modifies total and metabolically active bacterial communities in the rhizosphere of two perennial grasses grown under field conditions. FEMS Microbiol Ecol 55:339–350

Jung S, Park S, Kim D, Kim SB (2008) Denaturing gradient gel electrophoresis analysis of bacterial community profiles in the rhizosphere of *cry1AC*-carrying *Brassica rapa* subsp. *pekinensis*. J Microbiol 46:12–15

Kaiser O, Pühler A, Selbitschka W (2001) Phylogenetic analysis of microbial diversity in the rhizoplane of oilseed rape (*Brassica napus* cv. Westar) employing cultivation-dependent and cultivation-independent approaches. Microb Ecol 42:136–149

Kao-Kniffin J, Balser TC (2007) Elevated CO_2 differentially alters belowground plant and soil microbial community structure in reed canary grass-invaded experimental wetlands. Soil Biol Biochem 39:517–525

Kao-Kniffin J, Freyre DS, Balser TC (2010) Methane dynamics across wetland plant species. Aquat Bot 93:107–113

Kao-Kniffin J, Freyer DS, Balser TC (2011) Increased methane emissions from an invasive wetland plant under elevated carbon dioxide levels. Appl Soil Ecol 48:309–312

Karpouzas DG, Karatasas A, Spiridaki E, Rousidou C, Bekris F, Omirou M, Ehaliotis C, Papadopoulpus KK (2011) Impact of a beneficial and a pathogenic *Fusarium* strain on the fingerprinting-based structure of microbial communities in tomato (*Lycopersicon esculentum* Milll.) rhizosphere. Eur. J. Soil Biol 47:400–408

Khamna S, Yokota A, Lumyong S (2009) Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J Microb Biot 25:649–655

Kielak A, Pijl AS, van Veen JA, Kowalchuk GA (2008) Differences in vegetation composition and plant species identity lead to only minor changes in soil-borne microbial communities in a former arable field. FEMS Microbiol Ecol 63:372–382

Kiely PD, Haynes JM, Higgins CH, Franks A, Mark GL, Morrissey JP, O'Gara F (2006) Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microb Ecol 51:257–266

Kleineidam K, Košmrlj K, Kublik S, Palmer I, Pfab H, Ruser R, Fiedler S, Schloter M (2011) Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. Chemosphere 84:182–186

Klironomos JN, Rilling MC, Allen MF (1996) Below-ground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. Funct Ecol 10:527–534

Kloepper JW, Rodríguez-Kábana R, McInory JA, Young RW (1992) Rhizosphere bacteria antagonistic to soybean cyct (*Heteroera glycines*) and root-knot (*Meloidogyne incognita*) nematodes: identification by fatty acid analysis and frequency of biological control activity. Plant Soil 139:75–84

Kluepfel DA, McInnis TM, Zehr EI (1993) Involvement of root-colonizing bacteria in peach orchard soils suppressive of the nematode *Criconemella xenoplax*. Phytopathology 83:1240–1245

Knee EM, Gong FC, Gao M, Teplitski M, Jones AR, Foxworthy A, Mort AJ, Bauer WD (2001) Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. Mol Plant Microbe Interact 14:775–784

Köberl M, Müller H, Ramadan EM, Berg G (2011) Desert farming benefits from microbial potential in arid soils and promotes diversity and plant health. PLoS One 6:e24452

Kohler J, Knapp BA, Waldhuber S, Caravaca F, Roldán A, Insam H (2010) Effects of elevated CO2, water stress, and inoculation with *Glomus intraradices* or *Pseudomonas nemdocina* of lettuce dry matter and rhizosphere microbial and functional diversity under growth chamber conditions. J Soils Sediment 10:1585–1597

Kolton M, Meller Harel Y, Pasternak Z, Graber ER, Elad Y, Cytryn E (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl Environ Microbiol 77:4924–4930

Kowalchuk GA, Yergean E, Leveau JHJ, Sessitch A, Bailey M (2010) Plant associated microbial communities. In: Liu WT, Jansson JK (eds) Environmental molecular biology. Caister Academic Press, Norfolk, pp 131–148

Kozdrój J, Trevors JT, van Elsas JD (2004) Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. Soil Biol Biochem 36:1775–1784

Krechel A, Faupel A, Hallmann J, Ulrich A, Berg G (2002) Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Can J Microbiol 48:772–786

Kremer RJ, Means NE (2009) Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur J Agron 31:153–161

Krüger M, Frenzel P, Kemnitz D, Conrad R (2005) Activity, structure and dynamics of the methanogenic archaeal community in a flooded Italian rice field. FEMS Microbiol Ecol 51:323–331

Kuske CR, Barns SM, Busch JD (1997) Diverse uncultivated bacterial groups form soils of the arid southwestern United States that are present in many geographic regions. Appl Environ Microbiol 63:3614–3621

Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. Review. J Plant Nutr Soil Sci 163:421–431

Kuzyakov Y, Domanski G (2002) Model for rhizodeposition and CO_2 efflux from planted soil and its validation by ^{14}C pulse labeling of ryegrass. Pant Soil 239:87–102

Kuzyakov Y, Raskatov A, Kaupenjohann M (2003) Turnover and distribution of root exudates of *Zea mays*. Plant Soil 254:317–327

La Scola B, Birtles RJ, Mallet MN, Raoult D (1998) *Massilia timonae* gen. nov., sp. nov., isolated from blood of an immunocompromised patient with cerebellar lesions. J Clin Microbiol 36:2847–2852

Laheurte F, Berthelin J (1988) Effect of a phosphate solubilizing bacteria on maize growth and root exudation over four levels of labile phosphorus. Plant Soil 105:11–17

Larigauderie A, Reynolds JF, Strain BR (1994) Root response to CO_2 enrichment and nitrogen supply in loblolly pine. Plant Soil 165:21–32

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75:5111–5120

Lee SH, Ka JO, Cho JC (2008) Members of the phylum *Acidobacteria* are dominant and metabolically active in rhizosphere soil. FEMS Microbiol Ecol 285:263–269

Lehmann-Richter S, Großkopf R, Liesack W, Frenzel P, Conrad R (1999) Methanogenic archaea and CO_2-dependent methanogenesis on washed rice roots. Environ Microbiol 1:159–166

Leninger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

Lerner A, Hersckovitz Y, Baudoin E, Nazaret S, Moenne-Loccoz Y, Okon Y, Jurkevitch E (2006) Effect of *Azospirillum brasilense* inoculation on rhizobacterial communities analyzed by denaturing gradient gel electrophoresis and automated ribosomal intergenic spacer analysis. Soil Biol Biochem 38:1212–1218

Lewis JD, Ward JK, Tissue DT (2010) Phosphorus supply drives nonlinear responses of cottonwood (*Populus deltoids*) to increases in CO_2 concentration from glacial to future concentrations. New Phytol 187:438–448

Liljeroth E, van Veen JA, Miller HJ (1990) Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. Soil Biol Biochem 22:1015–1021

Lin Q, Zhao HM, Chen YX (2007) Effects of 2,4-dichlorophenol, pentachlorophenol and vegetation on microbial characteristics in a heavy metal polluted soil. J Environ Sci Heal B 42:551–557

Lindquist D, Murrill D, Burran WP, Winans G, Janda MJ, Probert W (2003) Characteristics of *Massilia timonae* and *Massilia timonae*-like isolates from human patients, with an emended description of the species. J Clin Microbiol 41:192–196

Lioussanne L, Perreault F, Jolicoeur M, St-Arnaud M (2010) The bacterial community of tomato rhizosphere is modified by inoculation with arbuscular mycrorrhizal fungi but unaffected by soil enrichment with mycorrhizal root exudates or inoculation with *Phytophthora nicotianae*. Soil Biol Biochem 42:473–483

Liu X, Zhao H, Chen S (2006) Colonization of maize and rice plants by strain *Bacillus megaterium* C4. Curr Microbiol 52:186–190

Liu W, Lu HH, Wu W, Wei QK, Chen YX, Thies JE (2008) Transgenic Bt rice does not affect enzyme activities and microbial composition in the rhizosphere during crop development. Soil Biol Biochem 40:475–486

Lopes MS, Foyer CH (2012) The impact of high CO_2 on plant abiotic stress tolerance. In: Slafer GA, Araus JL (eds) Crop stress management and global climate change. CABI, Wallingford, pp 85–100

Lottmann J, Heuer H, Smalla K, Berg G (1999) Influence of transgenic T4-lysozyme-producing potato plants on potentially beneficial plant-associated bacteria. FEMS Microbiol Ecol 29:365–377

Lottmann J, Heuer H, de Vries J, Mahn A, Düring K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33:41–49

Lu Y, Conrad R (2005) In situ isotope probing of methanogenic Archaea in the rice rhizosphere. Science 309:1088–1090

Lu Y, Lueders T, Friedrich MW, Conrad R (2005) Detecting active methanogenic populations on rice roots using stable isotope probing. Environ Microbiol 7:326–336

Lu Y, Wolf-Rainer A, Conrad R (2007) Spatial variation of active microbiota in the rice rhizosphere revealed by *in situ* stable isotope probing of phospholipid fatty acids. Environ Microbiol 9:474–481

Lübeck PS, Hansen M, Sørensen J (2000) Simultaneous detection of the establishment of seed-inoculated *Pseudomonas fluorescens* strain DR54 and native soil bacteria on sugar beet root surfaces using fluorescence antibody and in situ hybridization technique. FEMS Microbiol Ecol 33:11–19

Lucas-García JA, Domenech J, Santamaría C, Camacho M, Daza A, Gutierrez Mañero FJ (2004) Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. Environ Exp Bot 52:239–251

Lugtenberg B, Kamilova F (2009) Plant-Growth-Promoting Rhizobacteria. Annu Rev Microbiol 63:541–556

Lugtenberg BJJ, Dekkers LC, Bloemberg GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu Rev Phytopathol 39:461–490

Mahaffee WE, Kloepper JW (1997) Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). Microb Ecol 34:210–223

Malik KA, Bilal R, Mehnaz S, Rasul G, Mirza MS, Ali S (1997) Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. Plant Soil 194:37–44

Maloney PE, van Bruggen AHC, Hu S (1997) Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. Microb Ecol 34:109–117

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly divers and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60:157–166

Mariley L, Aragno M (1999) Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. Appl Soil Ecol 13:127–136

Marilley L, Vogt G, Blanc M, Aragno M (1998) Bacterial diversity in the bulk soil and rhizosphere fractions of *Lolium perenne* and *Trifolium repens* as revealed by PCR restriction analysis of 16S rRNA. Plant Soil 198:219–224

Marilley L, Hartwig UA, Aragno M (1999) Influence of an elevated atmospheric CO_2 content on soil and rhizosphere bacterial communities beneath *Lolium perenne* and *Trifolium repens* under field conditions. Microb Ecol 38:39–49

Marschner P, Baumann K (2003) Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant Soil 251:279–289

Marschner P, Timonen S (2005) Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Appl Soil Ecol 28:23–36

Marschner P, Crowley DE, Lieberei R (2001a) Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11:297–302

Marschner P, Yang CH, Lieberei R, Crowley DE (2001b) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445

Marschnner P, Neumann G, Kania A, Wiskopf L, Lieberei R (2002) Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). Plant Soil 246:167–174

Marschner P, Crowley D, Yang CH (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261:199–208

Martin JK, Foster RC (1985) A model system for studying the biochemistry and biology of the root-soil interface. Soil Biol Biochem 17:261–269

Mary B, Fresneau C, Morel JL, Mariotti A (1993) C and N cycling during decomposition of root mucilage, roots and glucose in soil. Soil Biol Biochem 25:1005–1014

Matilla MA, Espinosa-Urgel M, Rodrígez-Herva JJ, Ramos JL, Ramos-González MI (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. Genome Biol 8:R179

Mazzola M, Gu YH (2002) Wheat genotype-specific induction of soil microbial communities suppressive to disease incited by *Rhizoctonia solani* anastomosis group (AG)-5 and AG-8. Phytopathology 92:1300–1307

Meharg AA, Killham K (1995) Loss of exudates from the roots of perennial ryegrass inoculated with a range of micro-organisms. Plant Soil 170:345–349

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl Environ Microbiol 73:7259–7267

Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 331:1097–1100

Miethling R, Wieland G, Backhous H, Tebbe CC (2000) Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Mircob Ecol 41:43–56

Miller SCM, LiPuma JJ, Parke JL (2002) Culture-based and non-growth-dependent detection of the *Burkholderia cepacia* complex in soil environments. Appl Environ Microbiol 68:3750–3758

Mishra A, Chauhan PS, Chaudhry V, Tripathi M, Nautiyal CS (2011) Rhizosphere competent *Pantoea agglomerans* enhances maize (*Zea mays*) and chickpea (*Cicer arietinum* L.) growth, without altering the rhizosphere functional diversity. A Van Leeuw J Microb 100:405–413

Mogge B, Loferer C, Agerer R, Hutzler P, Hartmann A (2000) Bacteiral community structure and colonization patterns of *Fagus sylvatica* L. ectomycorrhizospheres as determined by fluorescence in situ hybridization and confocal laser scanning microscopy. Mycorrhiza 9:271–278

Montealegre CM, Van Kessel C, Blumenthal JM, Hur HG, Hartwig UA, Sadowsky MJ (2000) Elevated atmospheric CO_2 alters microbial population structure in a pasture ecosystem. Glob Change Biol 6:475–482

Montealegre CM, van Kessel C, Russelle MP, Sadowsky MJ (2002) Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. Plant Soil 243:197–207

Morgan JA, Mosier AR, Milchunas DG, LeCain DR, Nelson JA, Parton WJ (2004) CO_2 enhances productivity, alters species composition, and reduces digestibility of shortgrass steppe vegetation. Ecol Appl 14:208–219

Nagy ML, Pérez A, Garcia-Pichel F (2005) The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiol Ecol 54:233–245

Navarro-Noya YE, Jan-Roblero J, González-Chávez MDC, Hernández-Gama R, Hernández-Rodrígez C (2010) Bacterial communities associated with the rhizosphere of pioneer plants (*Bahia xylopoda* and *Viguiera linearis*) growing on heavy metals-contaminated soils. A Van Leeuw J Microb 97:335–349

Nelson DM, Cann IKO, Mackie RI (2010) Response of Archaeal communities in the rhizosphere of maize and soybean to elevated atmospheric CO_2 concentrations. PLoS One 5:e15897

Newman EI, Bowen HJ (1974) Patterns of distribution of bacteria on root surfaces. Soil Biol Biochem 6:205–209

Neumann G, Römheld V (2002) Root-induced changes in the availability of nutrients in the rhizosphere. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half, 3rd edn. Marcel Dekker, New York, pp 617–650

Nguyen C (2003) Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375–396

Nguyen LM, Buttner MP, Cruz P, Smith SD, Robleto EA (2011) Effects of elevated atmospheric CO_2 on the rhizosphere soil microbial communities in a Mojave Desert ecosystem. J Arid Enviro 75:917–925

Nichols D (2007) Cultivation gives context to the microbial ecologist. FEMS Microbiol Ecol 60:351–357

Nicol GW, Glover LA, Prosser JI (2003) The impact of grassland management on archaeal community structure in upland pasture rhizosphere soil. Environ Microbiol 5:152–162

Normander B, Prosser JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66:4372–4377

Ofek M, Ruppel S, Waisel Y (2007) Differences between bacterial associations with two root types of *Vicia faba* L. Plant Biosyst 141:352–362

Ofek M, Hadar Y, Minz D (2009) Comparison of effects of compost amendment and single-strain inoculation on root bacterial communities of young cucumber seedlings. Appl Environ Microbiol 75:6441–6450

Ofek M, Hadar Y, Minz D (2011) Colonization of cucumber seeds by bacteria during germination. Environ Microbiol 13:2794–2807

Offre O, Pivato B, Siblot S, Gamalero E, Corberand T, Lemanceau P, Mougel C (2007) Identification of bacterial groups preferentially associated with mycorrhizal roots of *Medicago truncatula*. Appl Environ Microbiol 73:913–921

Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimarães CT, Schaffert RE, Sá NMH (2009) Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. Soil Biol Biochem 41:1782–1787

Olsson PA, Wallander H (1998) Interactions between ectomycorrhizal fungi and the bacterial community in soils amended with various primary minerals. FEMS Microbiol Ecol 27:195–205

Ostle N, Whiteley AS, Bailey MJ, Sleep D, Ineson P, Manefield M (2003) Active microbial RNA turnover in a grassland soil estimated using a $^{13}CO_2$ spike. Soil Biol Biochem 35:877–885

Øvreås L, Torsvik V (1998) Microbial diversity and community structure in two different agricultural soil communities. Microb Ecol 36:303–315

Oyaizu-Masuchi Y, Komagata K (1988) Isolation of free-living nitrogen-fixing bacteria from the rhizosphere of rice. J Gen Appl Microbiol 34:127–164

Pakarinen J, Hyvärinen A, Salkinoja-Salonen M, Laitinen S, Nevalainen A, Mäkelä MJ, Haahtela T, von Hertzen L (2008) Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. Environ Microbiol 10:3317–3325

Paterson E, Rattray EAS, Killham K (1996) Effect of elevated atmospheric CO_2 concentration on C-partitioning and rhizosphere C-flow for three plant species. Soil Biol Biochem 28:195–201

Paterson E, Hall JM, Rattray EAS, Griffiths BS, Ritz K, Killham K (1997) Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. Glob Change Biol 3:363–377

Paterson E, Thornton B, Midwood AJ, Osborne SM, Millard P (2008) Atmopheric CO_2 enrichment and nutrient additions to planted soil increases mineralization of soil organic matter, but do not alter microbial utilization of plant- and soil C-sources. Soil Biol Biochem 40:2434–2440

Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, Webb RI, Hamage HK, Carroll BJ, Schenk PM, Schmidt S (2008) Plants can use protein as a nitrogen source without assistance from other organisms. P Natl Acad Sci USA 105:4524–4529

Paungfoo-Lonhienne C, Lonhienne TGA, Mudge SR, Schenk PM, Christie M, Carroll BJ, Schmidt S (2010a) DNA is taken up by root hairs and pollen, and stimulates root and pollen tube growth. Plant Physiol 153:799–805

Paungfoo-Lonhienne C, Rentsch D, Robatzek S, Webb RI, Sagulenko E, Näsholm T, Schmidt S, Lonhienne TGA (2010b) Turning the table: plants consume microbes as a source of nutrients. PLoS One 5:e11915

Pedraza RO, Bellone CH, Carrizo de Bellone S, Boa Sorte PMR, Teixeira KRDS (2009) *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. Eur J Soil Biol 45:36–43

Pereira EIP, Chung H, Scow K, Sadowsky MJ, van Kessel C, Six J (2011) Soil nitrogen transformations under elevated atmospheric CO_2 and O_3 during the soybean growing season. Environ Pollut 159:401–407

Philippot L, Kuffner M, Chèneby D, Depret G, Laguerre G, Martin-Laurent F (2006) Genetic structure and activity of the nitrate-reducers community in the rhizosphere of different cultivars of maize. Plant Soil 287:177–186

Phillips RP (2007) Towards a rhizo-centric view of plant-microbial feedbacks under elevated atmospheric CO_2. New Phytol 173:664–667

Phillips RP, Bernhardt ES, Schlesinger WH (2009) Elevated CO_2 increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response. Tree Physiol 29:1513–1523

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14:187–194

Picard C, Di Cello F, Ventura M, Fani R, Guckert A (2000) Frequency and biodiversity of 2,4-diacetylphloglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. Appl Environ Microbiol 66:948–955

Pisa G, Magnani GS, Weber H, Souza EM, Faoro H, Monteiro RA, Daros E, Baura V, Bespalhok JP, Pedrosa FO, Cruz LM (2011) Diversity of 16S rRNA genes from bacteria of sugarcane rhizosphere soil. Bra J Med Biol Res 44:1215–1221

Poole EJ, Bending GD, Whipps JM, Read DJ (2001) Bacteria associated with *Pinus sylvestris-Lactarius rufus* ectomycorrhizas and their effects on mycorrhiza formation in vitro. New Phytol 151:743–751

Poonguzhali S, Madhaiyan M, Yim WJ, Kim KA, Sa TM (2008) Colonization patterns of plant root and leaf surfaces visualized by use of green-fluorescent-marked strain of *Methylobacterium suomiense* and its persistence in rhizosphere. Appl Microbiol Biot 78:1033–1043

Poplawski AB, Mårtensson L, Wartiainen I, Rasmussen U (2007) Archaeal diversity and community structure in a Swedish barley field: specificity of the EK510R/(EURY498) 16S rDNA primer. J Microbiol Meth 69:161–173

Pregitzer KS, Zak DR, Maziasz J, DeForest J, Curtis PS, Lussenhop J (2000) Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. Ecol Appl 10:18–33

Probanza A, Mateos JL, Lucas García JA, de Felipe MR, Gutierrez-Mañero FJ (2001) Effect of inoculation with PGPR *Bacillus* and *Pisolithus tinctorius* on *Pinus pinea* L. growth, bacterial rhizosphere colonization, and mycorrhizal infection. Microb Ecol 41:140–148

Probanza A, Lucas-García JA, Ruiz Palomino M, Ramos B, Gutiérrez Mañero FJ (2002) *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. lichniformis* CECT 5106 and *B. pumilus* CECT 5105). Appl. Soil Ecol 20:75–84

Puente ME, Bashan Y, Li CY, Lebsky VK (2004) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol 6:629–642

Raaijmakers JM, Weller DM, Thomashow LS (1997) Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl Environ Microbiol 63:881–887

Racke J, Sikora RA (1992) Isolation, formulation and antagonistic activity of rhizobacteria toward the potato cyct nematode *Globodera pallida*. Soil Biol Biochem 24:521–526

Ramachandran VK, East AK, Karunakaran R, Downie JA, Poole PS (2011) Adaptation of *Rhizobium leguminosarum* to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. Genome Biol 12:R106

Ramakrishnan B, Lueders T, Dunfield PF, Conrad R, Friedrich MW (2001) Archaeal community structures in rice soils from different geographical regions before and after initiation of methane production. FEMS Microbiol Ecol 37:175–186

Ramos B, Lucas García JA, Probanza A, Domenech J, Gutierrez Mañero JF (2003) Influence of an indigenous European alder (*Alnus glutinosa* (L.) Gaertn) rhizobacterium (*Bacillus pumilis*) on the growth of alder and its rhizosphere microbial community structure in two soils. New For 25:149–159

Rangel-Castro JI, Killham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM, Ineson P, Meharg A, Prosser JI (2005a) Stable isotope probing analysis of the influence of liming on root exudates utilization by soil microorganisms. Environ Microbiol 7:828–838

Rangel-Castro JI, Prosser JI, Ostle N, Scrimgeour CM, Killham K, Meharg AA (2005b) Flux and turnover of fixed carbon in soil microbial biomass of limed and unlimed plots of an upland grassland ecosystem. Environ Microbiol 7:544–552

Rasche F, Hödl V, Poll C, Kandeler E, Gerzabek MH, van Elsas JD, Sessitsch A (2006) Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiol Ecol 56:219–235

Rattray EAS, Paterson E, Killham K (1995) Characterisation of the dynamics of C-partitioning within *Lolium perenne* and to the rhizosphere microbial biomass using ^{14}C pulse chase. Biol Fertil Soils 19:280–286

Richardt W, Mascariña G, Padre B, Doll J (1997) Microbial communities of continuously cropped irrigated rice fields. Appl Environ Microbiol 63:233–238

Riedewald F (2006) Bacterial adhesion to surfaces: the influence of surface roughness. PDA J Pharm Sci Technol 60:164–171

Rigamonte TA, Pylro VS, Daurte GF (2010) The role of mycorrhization helper bacteria in the establishment and action of ectomycorrhizae association. Bra J Microbiol 41:832–840

Rillig MC, Scow KM, Kilonomos JN, Allen MF (1997) Microbial carbon-substrate utilization in the rhizosphere of *Gutierrezia sorothrae* grown in elevated atmospheric carbon dioxide. Soil Biol Biochem 29:1387–1394

Rinan R, Nerg AM, Ahtoniemi P, Suokanerva H, Holopainen T, Kyrö E, Bååth E (2008) Plant-mediated effects of elevated ultraviolet-B radiation on peat microbial communities of a subarctic mire. Glob Change Biol 14:925–937

Ringelberg DB, Stair JO, Almeida J, Norby RJ, O'Neill EG, White DC (1997) Consequences of rising atmospheric carbon dioxide levels for the belowground microbiota associated with white Oak. J Environ Qual 26:495–503

Robleto EA, Borneman J, Triplett EW (1998) Effects of bacterial antibiotic production on rhizosphere microbial communities from a culture-independent perspective. Appl Environ Microbiol 64:5020–5022

Rodríguez-Díaz M, Rodelas-Gonzalés B, Pozo-Clemente C, Martínez-Toledo MV, González-López J (2008) A review on the taxonomy and possible screening traits of plant growth-promoting rhizobacteria. In: Pichtel AJ, Hayat S (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. Wiley-VCH, Weinheim, pp 55–80

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FAO, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

Roesti D, Gaur R, Johri BN, Imfeld G, Sharma S, Kawaljeet K, Aragno M (2006) Plant growth stage, fertilizer management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteia affect the rhizobacterial community structure in rain-fed what fields. Soil Biol Biochem 38:1111–1120

Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environ Pollut 83:155–189

Rondon MR, Goodman RM, Handelsman J (1999) The earth's bounty: assessing and accessing soil microbial diversity. Trends Biotechnol 17:403–409

Rønn R, Gavito M, Larsen J, Jakobsen I, Frederiksen H, Christensen S (2002) Response of free-living soil protozoa and microorganisms to elevated atmospheric CO_2 and presence of mycorrhiza. Soil Biol Biochem 34:923–932

Ros M, Pascual JA, Garcia C, Mernandez MT, Insam H (2006) Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biol Biochem 38:3443–3452

Rosenberg E, Zilberg-Rosenberg I (2011) Symbiosis and development: the hologenome concept. Birth Defects Res 93:56–66

Rosenberg E, Koren O, Reshef L, Efrony R, Zilberg-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362

Rovira AD (1965) Interactions between plant roots and soil microorganisms. Annu Rev Microbiol 19:241–266

Rovira AD, Campbell R (1974) Scanning electron microscopy of microorganisms on the roots of wheat. Microb Ecol 1:15–23

Rovira AD, Campbell R (1975) A scanning electron microscope study of interaction between micro-organisms and *Gaeumannomyces graminis* (Syn. *Ophiobolus graminis*) on wheat roots. Microb Ecol 2:177–185

Rovira AD, Newman EI, Bowen HJ, Campbell R (1974) Quantitative assessment of the rhizoplane microflora by direct microscopy. Soil Biol Biochem 6:211–216

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

Ruffel S, Freixes S, Balzergue S, Tillard P, Jeudy C, Martin-Magniette ML, van der Merwe MJ, Kakar K, Gouzy J, Fernie AR, Udvardi M, Salon C, Gojon A, Lepetit M (2008) Systemic signaling of the plant nitrogen status triggers specific transcriptome responses depending on the nitrogen source in *Medicago truncatula*. Plant Physiol 146:2020–2035

Rui YK, Yi GX, Zhao J, Wang BM, Li ZH, Zhai ZX, He ZP, Li QX (2005) Changes of Bt toxin in the rhizosphere of transgenic Bt cotton and its influence on soil functional bacteria. World J Microbiol 21:1279–1284

Ryan PR, Dessaux Y, Thomashow LS, Weller DM (2009) Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321:363–383

Salles JF, van Veen JA, van Elsas JD (2004) Multivariate analyses of *Burkholderia* species in soil: effect of crop and land use history. Appl Environ Microbiol 70:4012–4020

Sarathchandra SU, Bruch G, Cox NR (1997) Growth patterns of bacterial communities in the rhizoplane and rhizosphere of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) in long-term pasture. Appl. Soil Ecol 6:293–299

Sato A, Watanabe T, Unno Y, Purnomo E, Osaki M, Shinano T (2009) Analysis of diversity of diazotrophic bacteria associated with the rhizosphere of a tropical arbor, *Melastoma malabathricum* L. Microbes Environ 24:81–870

Schallmach E, Minz D, Jurkevitch E (2000) Culture-independent detection of changes in root-associated bacterial populations of common bean (*Phaseolus vulgaris* L.) following nitrogen depletion. Microb Ecol 40:309–316

Scheid D, Stubner S, Conrad R (2003) Effects of nitrate- and sulfate-amendment on the methanogenic populations in rice root incubations. FEMS Microbiol Ecol 43:309–315

Schenk PM, Carvalhais LC, Kazan K (2012) Unraveling plant–microbe interactions: can multi-species transcriptomics help? Trends Biotechnol 30:177–184

Scheublin TR, Sanders IR, Keel C, van der Meer JR (2010) Characterisation of microbial communities colonizing the hyphal surface of arbuscular mycorrhizal fungi. ISME J 4:752–763

Schloss PD, Handelsman J (2006) Toward a census of bacteria in soil. PLoS Comp Biol 2:e92

Schloter M, Winkler JB, Aneja M, Koch N, Fleischmann F, Pritsch K, Heller W, Stich S, Grams TEE, Göttlein A, Matyssek R, Munch JC (2005) Short term effects of ozone on the plant-rhizosphere-bulk soil system of young beech trees. Plant Biol 7:728–736

Schmalengerger A, Tebbe CC (2002) Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transtenic cultivar Bosphore. FEMS Microbiol Ecol 40:29–37

Schmalenberger A, Tebbe CC (2003) Genetic profiling of noncultivated bacteria from the rhizosphere of sugar beet (*Beta vulgaris*) reveal field and annual variability but no effect of a transgenic herbicide resistance. Can J Microbiol 49:1–8

Schortemeyer M, Hartwig UA, Hendrey GR, Sadowsky MJ (1996) Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). Soil Biol Biochem 28:1717–1724

Schwieger F, Tebbe CC (2000) Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in the rhizosphere of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) – Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. Appl Environ Microbiol 66:3553–3565

Scott JS, Knudsen GR (1999) Soil amendment effects of rape (*Brassica napus*) residues on pea rhizosphere bacteria. Soil Biol Biochem 31:1435–1441

Senthilkumar M, Anandham R, Madhaiyan M, Venkateswaran V, Sa T (2011) Endophytic bacteria: perspectives and applications in Agricultural crop production. In: Maheshwari DK (ed) Bacteria in agrobiology: crop ecosystems. Springer, Berlin/Heidelberg, pp 61–96

Sessitsch A, Kan FY, Pfeifer U (2003) Diversity and community structure of culturable *Bacillus* spp. populations in the rhizosphere of transgenic potatoes expressing the lytic peptide cecropin B. Appl. Soil Ecol 22:149–158

Sessitsch A, Gyamfi S, Tscherko D, Gerzabek MH, Kabdeler E (2004) Activity of microorganisms in the rhizosphere of herbicide treated and untreated transgenic glufosinate-tolerant and wildtype oilseed rape grown in containment. Plant Soil 266:105–113

Shanmugam V, Verma R, Rajkumar S, Naruka DS (2011) Bacterial diversity and soil enzyme activity in diseased and disease free apple rhizosphere soils. Ann Microbiol 61:765–772

Shiomi Y, Mishitama M, Onizuka T, Marumoto T (1999) Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive towards bacterial wilt. Appl Environ Microbiol 65:3996–4001

Siciliano SD, Germida JJ (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol Ecol 29:263–272

Simon HM, Dodsworth JA, Goodman RM (2000) Crenarchaeota colonize terrestrial plant roots. Environ Microbiol 2:495–505

Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36:147–155

Sliwinski MK, Goodman RM (2004) Comparison of Crenarchaeal consortia inhabiting the rhizosphere of diverse terrestrial plants with those in bulk soil in native environments. Appl Environ Microbiol 70:1821–1826

Söderberg KH, Olsson PA, Bååth E (2002) Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonization. FEMS Microbiol Ecol 40:223–231

Solís-Domínguez AF, Valentín-Vargas A, Chorover J, Maier RM (2011) Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings. Sci Total Environ 409:1009–1016

Somenahally AC, Hollister EB, Loeppert RH, Yan W, Gentry TJ (2011) Microbial communities in rice rhizosphere altered by intermittent and continuous flooding in fields with long-term arsenic application. Soil Biol Biochem 43:1220–1228

Stanfford WHL, Baker GC, Brown SA, Burton SG, Cowan DA (2005) Bacterial diversity in the rhizosphere of Proteaceae species. Environ Microbiol 7:1755–1768

Sun L, Qui F, Zhang X, Dai X, Dong X, Song W (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. Microb Ecol 55:415–424

Teixeira LCRS, Peixoto RS, Cury JC, Sul WJ, Pellizari VH, Tiedge J, Rosado AS (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty bay, maritime Antartica. ISME J 4:989–1001

Thies JE, Woomer PL, Singleton PW (1995) Enrichment of *Bradyrhizobium* spp. populations in soil due to cropping of the homologous host legume. Soil Biol. Biochem 27:633–636

Timonen S, Jørgensen S, Haahtela K, Sen R (1998) Bacterial community structure at defined locations of *Pinus sylvestris-Suillus bovinus* and *Pinus sylvestris-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can J Microbiol 44:499–513

Tiquia SM, Lloyd J, Herms DA, Hoitink HAJ, Michel FC Jr (2002) Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes. Appl Soil Ecol 21:31–48

Tobita H, Uemura A, Kitao M, Kitaoka S, Maruyama Y, Utsugi H (2011) Effects of elevated atmospheric carbon dioxide, soil nutrients and water conditions on photosymthetic and growth responses of *Alnus hirsute*. FunctPlant Biol 38:702–710

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelia exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304

Toljander JF, Santos-González JC, Tehler A, Finlay RD (2008) Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trail. FEMS Microbiol Ecol 65:323–338

Torsvik V, Sørheim R, Goksøyr J (1996) Total bacterial diversity in soil and sediment communities- a review. J Ind Microbiol 17:170–178

Treonis AM, Ostle NJ, Stott AW, Primrose R, Grayston SJ, Ineson P (2004) Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biol Biochem 36:533–537

Trivedi P, Duan Y, Wang N (2010) Huanglongbing, a systemic disease, restructures the bacterial community associated with citrus roots. Appl Environ Microbiol 76:3427–3436

Uren NC (2001) Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere. Marcel Dekker, New York, pp 19–40

Uroz S, Buée M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288

Van Elsas JD, Bersma FGH (2011) A review of molecular methods to study the microbiota of soil and the mycosphere. Eur J Soil Biol 47:77–87

Van Elsas JD, Garbeva P, Salles J (2002) Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation 13:29–40

Van Overbeek L, van Elsas JD (2008) Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.). FEMS Microbiol Ecol 64:283–296

Vandenkoornhuyse P, Mahé S, Ineson P, Staddon P, Ostle N, Cliquet JB, Francez AJ, Fitter AH, Young JPW (2007) Active root-inhabiting microbes identified by rapid incorporation of plant-drived carbon into RNA. Proc Natl Acad Sci USA 104:16970–16975

Vestergard M, Henry F, Rangel-Castro JI, Michelsen A, Prosser JI, Christensen S (2008) Rhizosphere bacterial community composition responds to arbuscular mycorrhiza, but not to reductions in microbial activity by foliar cutting. FEMS Microbiol Ecol 64:78–89

Vázquez MM, Cesar S, Azcon R, Barea JM (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15:261–272

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vetterlein D, Reinhold J (2004) Gradients in soil solution composition between bulk soil and rhizosphere- In situ measurement with changing soil water content. Plant Soil 258:307–317

Waisel Y, Eshel A (2002) Functional diversity of various constituents of a single root system. In: Waisel Y, Eshel A, Kafkafi U (eds) plant roots: the hidden half, 3rd edn. Marcel Dekker, New York, pp 157–174

Wamberg C, Christensen S, Jakobsen I, Müller AK, Sørensen SJ (2003) The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biol Biochem 35:1349–1357

Wand SJE, Midgley GF, Jones MH, Curtis PS (1999) Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO_2 concentration:

a meta-analytic test of current theories and perceptions. Glob Change Biol 5:723–741

Wasaki J, Rothe A, Kania A, Neumann G, Römheld V, Shinano T, Osaki M, Kandeler E (2005) Root exudation, phosphorus acquisition and microbial diversity in the rhizosphere of white lupine as affected by phosphorus supply and atmospheric carbon dioxide concentration. J Environ Qual 34:2157–2166

Watt M, McCully ME, Canny MJ (1994) Formation and stabilization of rhizosheaths of *Zea mays* L. Plant Physiol 106:179–186

Watt M, McCully ME, Kirkegaard JA (2003) Soil strength and rate of root elongation alter the accumulation of Pseudomonas spp. and other bacteria in the rhizosphere of wheat. Funct Plant Biol 30:483–491

Watt M, Hugenholtz P, White R, Vinall K (2006) Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence *in situ* hybridization (FISH). Environ Microbiol 8:871–884

Weinert N, Piceno Y, Ding GC, Meincke R, Heuer H, Berg G, Schloter M, Andersen G, Smalla K (2008) PhyloChip hybridization uncovered an enourmous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75:497–506

Weinert N, Meincke R, Gottwald C, Heuer H, Gomes NCM, Schloter M, Berg G, Smalla K (2009) Rhizosphere communities of genetically modified zeaxanthin-accumulating potato plants and their parent cultivar differ less than those of different potato cultivars. Appl Environ Microbiol 75:3859–3865

Weinert N, Meincke R, Gottwald C, Radi V, Dong X, Schloter M, Berg G, Smalla K (2010) Effects of genetically modified potatoes with increased zeaxanthin content on the abundance and diversity of rhizobacteria with in vitro antagonistic activity do not exceed natural variability among cultivars. Plant Soil 326:437–452

Weisskopf L, Fromin N, Tomasi N, Aragno M, Martinoia E (2005) Secretion activity of white lupin's cluster roots influences bacterial abundance, function and community structure. Plant Soil 268:181–194

Weisskopf L, Le Bayon RC, Kohler F, Page V, Jossi M, Gobat JM, Martinoia E, Aragno M (2008) Spatio-temporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: a one-year microcosm study on lupin and wheat. Soil Biol Biochem 40:1772–1780

Weisskopf L, Heller S, Eberl L (2011) *Burkholderia* species are major inhabitants of white lupin cluster roots. Appl Environ Microbiol 77:7715–7720

Werker E, Kislev M (1978) Mucilage on the root surface and root hairs of Sorghum: heterogeneity in structure, manner of production and site of accumulation. Ann Bot 42:809–816

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

Widmar F, Rasche F, Hartmann M, Fliessbach A (2006) Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of DOK long-term field experiment. Appl Soil Ecol 33:294–307

Will C, Thürmer A, Wollherr A, Nacke H, Herold N, Schrumpf M, Gutknecht J, Wubet T, Buscot F, Daniel R (2010) Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. Appl Environ Microbiol 76:6751–6759

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci USA 74:5088–5090

Wu WX, Liu W, Lu HH, Chen YX, Devare M, Thies J (2009a) Use of ^{13}C labeling to assess carbon partitioning in transgenic and nontransgenic (parental) rice and their rhizosphere soil microbial communities. FEMS Microbiol Ecol 67:93–102

Wu L, Ma K, Li Q, Ke X, Lu Y (2009b) Composition of Archaeal community in a paddy field as affected by rice cultivar and N fertilizer. Microb Ecol 58:819–823

Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66:345–351

Yang CH, Crowley DE, Menge JA (2001) 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiol Ecol 35:129–136

Yang JH, Liu HX, Zhu GM, Pan YL, Xu LP, Guo JH (2008) Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. J Appl Microbiol 104:91–104

Young IM, Crawford JW (2004) Interactions and self-organization in the soil-microbe complex. Science 304:1634–1637

Young IM, Crawford JW, Nunan N, Otten W, Spiers A (2008) Microbial distribution in soils: physics and scaling. Adv Agron 100:81–121

Zachow C, Tilcher R, Berg G (2008) Sugar beet-associated bacterial and fungal communities show a high indigenous antagonistic potential against plant pathogens. Microb Ecol 55:119–129

Zak DR, Pregitzer KS, King JS, Holmes WE (2000) Elevated atmospheric CO_2, fine roots and response of soil microorganisms: a review and hypothesis. New Phytol 147:201–222

Zhang NN, Sun YM, Li L, Wang ET, Chen WX, Yuan HL (2010) Effects of intercropping and *Rhizobium* inoculation on yield and rhizosphere bacterial community of faba bean (*Vicia faba* L.). Biol Fertil Soils 46:625–639

Zhang Y, Du BH, Jin ZG, Li ZH, Song HN, Ding YQ (2011a) Analysis of bacterial communities in rhizosphere soil of healthy and diseased cotton (*Gossypium* sp.) at different plant growth stages. Plant Soil 339:447–455

Zhang YZ, Wang ET, Li M, Li QQ, Zhang YM, Zhao SJ, Jia XL, Zhang LH, Chen WF, Chen WX (2011b) Effects of rhizobial inoculation, cropping systems and growth stages on endophytic bacterial community of soybean roots. Plant Soil 347:147–161

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol Rev 32:723–735

5 Tropical Soil Microbial Communities

Andrew Macrae · Rosalie R. R. Coelho · Raquel Peixoto · Alexandre S. Rosado
Instituto de Microbiologia Paulo de Góes, Universidade Federal do, Rio de Janeiro, Brazil

Introduction

In this chapter, we focus on tropical soil ecosystems and review some of their bacterial communities. We start by describing the tropics and tropical soils and then describe bacterial community diversity and function based on case studies of those soils. We end the chapter looking at the positive role soil bacteria can play in tropical agriculture and food production.

Tropical soils are those found between the Tropic of Cancer (latitude at 23½° North) and the Tropic of Capricorn (23½° South) imaginary lines around the Earth that delineate the zone where the sun's rays will be perpendicular for at least 1 day in the year. Between these latitudes lies an area that accounts for about 40% of the Earth's surface. If we look at a globe and focus on the area within the tropics and then remove the deserts and then the Atlantic, Pacific, and Indian oceans, what we are left with are the remnants of the World's tropical rainforests and savannahs. Approximately 40% of the World's population depends on those soils for a home and sustenance. Of 270 countries and island groups on the planet, 169 countries have all or a part of their territory within the tropics (IUCN 1988). Simplifying this we see that the tropics include Mexico, all Central American countries, all Caribbean countries, most South American countries, the vast majority of African countries, much of India and Southern Asia, a bit of China, all of Oceania, and a large part of Australia. To say that tropical soils are important would be an understatement, but what is a tropical soil and what determines its characteristics?

Soil is the thin layer of material on the Earth's surface made up of minerals and organic matter that serves as the natural growth media for land plants. Its formation and usefulness depend on five main factors: parent rock, climate, topography, biological interactions, and time. Human intervention also plays an increasingly important role especially in agricultural soils where fertility is managed. At the global scale, it is climate and time that explain the soil types that we see in the tropics today. Within the tropics, climate is often described as tropical humid or dry. Tropical humid can be further divided into: tropical wet with no dry season, tropical monsoonal with a short dry season and heavy rains in other months, and tropical savannah with a dry winter season. Tropical soils are generally old (>100,000 years) and often ancient (>10 million years). This length of time is important because it can take a 1,000 years for natural rainfall to dissolve/weather a cubic meter of limestone or a million years for similar volume of granite. The rocks beneath humid tropical soils have been exposed to sustained heat and high levels of precipitation for a long time and most of the soluble minerals and salts near the surface have been dissolved and elements transported via rivers to the sea. The minerals that we tend to see in humid tropical soils are those that resist weathering and that have been left behind. For example, bauxite (aluminum ore) might be considered a tropical soil. Aluminum is present in many primary rock forming minerals and many secondary clay minerals, these in turn weather further until all that remains are the least soluble elements of the original rock which include iron, aluminum, and silica oxides. Generally humid tropical soils are deep (>1 m) but generally not rich in bases. They are typically acidic (<pH 5), and their fertility is often dependent on the cycling of a thin layer (1–5 cm) of organic matter associated with the covering vegetation. In the dry tropical climates, namely, deserts, precipitation is rare. When rainfall does occur, it is normally short lived and solutes are often transported to the soil surface by the strong evaporation that follows such events. This can result in base rich, salty, and alkaline soils. We have seen that the different tropical climates can and do produce different types of soils from acidic soils in wet areas and neutral and alkaline soils in dry areas independent of the original parent rock. A combination of a limestone rock matrix and dry climate would produce an extremely alkaline soil (pH > 10) just as a weathered granite could produce a bauxitic soil that would be very acidic (pH < 4). Given the extreme variability of soil types found within the tropics, a meaningful correlation between latitude and tropical bacterial communities is unlikely. That noted, soil bacterial communities in the tropics have had millions of years to evolve in their habitats and to occupy almost every conceivable niche. They have had more time for coevolution than communities in soils in northern latitudes influenced by quaternary ice ages. Bacteria are found living on almost every surface and within almost every plant and animal. Given that all these life forms eventually die and become a part of the organic matter that is turned over in the soil, one might say that soils are the richest sources of bacterial biodiversity.

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_115,

After heat and light from the sun, both abundant in the tropics, the distribution and abundance of water determines primary production and drives ecosystems and their biomes. Topography plays its part, and within the tropics on a global scale, we recognize three main tropical basins: the Amazon, the Congo, and the Borneo-Mekong. Plants and animals in tropical rainforests have deservedly received a lot of research attention, their bacterial communities less so and perhaps that is because it is not the soil bacteria that drive destructive changes in tropical forests. Climate change (leading to fires) and/or human intervention for agricultural and industrial production is increasingly responsible for changes in tropical ecosystems (Nepstad et al. 1999). To understand tropical soil bacterial communities, it is helpful to focus on smaller geographical scales, for example, biomes, and then specific habitats within biomes where these organisms have had millions of years to coevolve with fungi, plants, and animals. It is helpful to look at community diversity and function based on specific case studies from the different tropical ecosystems. Given the relative large size of the tropics, only a very small number of case studies can be presented in this chapter. Those we have chosen we hope will generate interest in the reader to discover more. One would predict that bacterial communities that have evolved in specific habitats are unique and that convergent evolution would mean that similar environmental conditions around the World will have evolved different bacterial communities that exercise very similar functions.

We now look briefly at bacteria from desert soils with a focus on the hyper arid Atacama Desert, and then two semiarid ecosystems: the Brazilian Cerrado and Caatinga ecosystems. Afterward we take a special look at the Mangrove ecosystem before describing soil bacteria from humid tropical rainforests and a look at the global biogeography of bacteria.

Desert Soil Communities

Deserts pose a challenge to most life forms, and very few plants and animals have adapted to low water, high heat, and solar radiation. Organisms that have adapted are described as xerophiles, and their biology is very special. Perhaps the lack of abundant plant and animal life forms in deserts makes the forms that do live there more curious and at the same time easier to observe and study. In environments with very limited organic matter, it is the phototrophs that initiate carbon cycling and drive ecosystem development. The cyanobacteria and other bacteria generally wait and when water does become available, they move to the surface and fix and excrete carbon that is exploited by a heterotrophic bacterial food web which then provides the conditions for multicellular life forms. Garcia-Pichel and Pringault (2001) have studied cyanobacteria in desert soils and have noted that they track water. When there is abundant water at the surface, they are found there and as water is evaporated, they retreat deeper into the soils. Endolithic (within rock) habitats are frequently hypothesized as a means of surviving desiccation, extreme heat, extreme cold, and radiation (Stivaletta and Barbieri 2008). The presence of potential endolithic habitats on the planet Mars, similar to those found in deserts on Earth, motivates astrobiologists in their search for life from extraterrestrial environments. Azúa-Bustos et al. (2011) reported finding hypolithic cyanobacteria, archaea, and heterotrophic bacterial communities on the bottom surface of all translucent quartz rocks over 20 cm in length and on 80% of all other translucent pebbles in coastal regions of the Atacama Desert. This is quite amazing given that these areas do not see rainfall. The authors explain this bacterial diversity and richness, suggesting that sea fogs provide the moisture necessary for life and that the quartz pebbles protect the bacteria against high temperatures and excessive solar radiation. The Atacama Desert is famously inhospitable being one of the driest places on Earth. At its core, the desert receives less than 1 mm of precipitation per year and more often than not there is no precipitation. At its core, even the hypolithic bacterial communities described by Azúa-Bustos et al. (2011) are rare. Most life forms struggle to survive aridity; yet, there are many studies that describe rich bacterial biodiversity that can be isolated from desert soils (Okoro et al. 2009). These authors isolated a diverse group of actinobacterial strains belonging to the *Amycolatopsis*, *Lechevalieria*, and *Streptomyces* genera. Lester et al. (2007) isolated 20 bacterial strains from the Atacama Desert and identified them based on 16S rRNA gene sequences. The isolated strains belonged to eight phylogenetic groups including *Rhodopseudomonas* sp., *Sphingomonas* sp., *Mesorhizobium* sp., *Asticcacaulis* sp., *Bradyrhizobium* sp., *Bacillus subtilis*, *Bacillus pumilus*, and *Burkholderia* sp. It would be tempting to think that deserts are colonized by Gram positive bacteria, those bacteria that have the capability to survive dry and hot conditions in spore form, but it is clear that the Gram negative bacteria remain viable and cultivable from the hottest and driest soils on the planet. The ability to survive let alone thrive in such conditions belies highly adapted physiologies and with them potential for novel biotechnologies. In the Tataouine Desert of South Tunisia, Chanal et al. (2006) compared a culture-based community of bacteria with a molecular biology approach. They found their isolated strains were related to *Actinobacteria*, *Firmicutes*, *Proteobacteria* while none were related to *Archaea*. In their 16S rDNA clone library, they found greater diversity and sequences related to the non-thermophilic *Crenarchaeota* archaea and bacterial sequences dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria*. They also found sequences related to the *Thermus/Deinococcus* and identified two novel radiotolerant α-*Proteobacteria* strains.

In discussing soil bacterial biogeography and biodiversity, we are often asked the question: "Are all bacterial species everywhere"? The answer to which is no! No, for many reasons, one being that not all bacterial niches are exposed to winds that in theory could transport all species everywhere, and another, bacteria evolve faster in smaller numbers than it would be possible for them to be globally distributed! Fulthorpe et al. (2008) provide good molecular evidence that distantly sampled soils share relatively few common species. That noted, however, tropical soil bacteria and especially those from arid and

semiarid ecosystems are transported by tropical winds around the globe. Griffin et al. (2001) studying the effects of West African desert dust on the health of human populations in the Caribbean reported that air laden with African dust carried to Caribbean brought with it two to three times the number of infection-causing bacteria and fungi. Given the global wind circulation patterns, many soil bacteria have been transported globally. If wind transported bacteria come to rest in an environment in which they can adapt and survive, they will and some will indeed thrive! A lot of soil bacterial types are found in lots of different soils around the World but not all! Next we consider soil bacteria from arid and semiarid tropical ecosystems found in Brazil.

Semiarid Soil Communities

Semiarid biomes cover a large fraction of the Earth's land surface (Sprent and Gehlot 2010) and are commonly known as savannahs. Two examples of these are well known, the Brazilian Cerrado and the African savannah. Less well known is the Caatinga, one of the largest semiarid areas in the world which is found in Brazil.

The Brazilian Cerrado occupies a vast area of the South American Central Plateau which covers 2 million km^2 and is listed as 1 of the 25 global hotspots for biodiversity (Myers et al. 2000). Cerrado vegetation varies from treeless grasslands with small shrubs to semi-deciduous woodland that retains a distinctive herbaceous ground cover (Furley 1999). Precipitation in the Cerrado is highly seasonal, but is generally higher than 1,000 mm per year. Cerrado soils are generally dystrophic, with low cation exchange capacity and are acidic (pH values of 4.0–5.5) (Adámoli et al. 1987). These soils are typically rich in aluminum and with oxidized iron giving them their characteristic red and orange colors. They have low nutrient contents and are frequently subjected to natural burning. Despite all of this, these soils are extensively used in agriculture, especially for growing soybeans. In Africa, the situation is similar, where the savannah also occupies a large continental area between the deserts and tropical rainforests in central Africa (Sprent and Gehlot 2010). African savannahs typically comprise a mosaic of grass, shrub, and tree components (Furley 2007).

The name "Caatinga" refers to a very peculiar ecosystem covering about 735,000 km^2 of the northeastern region of Brazil. It is dry, almost a desert, with endemic xerophile plants that include small trees and bushes and patchy grasses. The region is characterized by high levels of insolation, high temperatures, scarce rain, and long periods of drought (Gorlach-Lira and Coutinho 2007). Precipitation in the Caatinga is <800 mm year, and this is normally concentrated in a very short unpredictable wet season that lasts 2–3 months. The Caatinga is one of the most degraded semiarid biomes on the planet with less than 1% of its area protected in permanent reserves (Santos et al. 2010b). The extremely dry conditions mean that for much of the time, the trees do not have leaves and those that do not lose their leaves are often sun bleached (Santos et al. 2010b).

Although the semiarid tropics can be considered a harsh environment for plants with limited species diversity, the same should not be said for their bacterial communities. Bacterial physiological diversity is a constant reminder of their plasticity and adaptability to almost any environment. From semiarid soils, it is possible to isolate and select soil bacteria that tolerate high temperatures (>40°C), survive high solar radiation, live well at low pH. This ability to thrive under harsh conditions indicates the genetic and associated enzymatic metabolic machinery required to thrive under such conditions (Parkinson and Coleman 1991). The capacity of a soil bacterial community to adapt varies as a function of its community members, in essence its genetic pool. Incredible diversity leads to incredible versatility which has been described as: "the biological buffer of the soil" (Santos et al. 2010b).

Caatinga soils are almost desert soils and their bacterial communities have not been studied in detail yet. From the Caatinga, Gorlach-Lira and Coutinho (2007) isolated 10^6 to 10^8 CFU/g of bacteria from dry soil. The bacterial communities that were cultivable were almost exclusively Gram positive spore-forming isolates and actinomycetes (Gorlach-Lira and Coutinho 2007). Given that culture media are selective, this finding tells us more about the groups of bacteria that grow under specific laboratory conditions than what is happening in soil. Peixoto et al. (2002) used the universal *rpoB* gene (RNA polymerase b-subunit gene), as an alternative to the 16S rRNA gene *rrs*, to evaluate total bacterial soil community profiles of Cerrado soils. In a later study and using the same approach, the same group compared bacterial community profiles under tillage and no tillage with forest soils in the Cerrado (Peixoto et al. 2006). Their results demonstrated that the PCR-DGGE method was sensitive enough to detect reproducible changes in soil bacterial communities and that the no-tillage soil community profile resembled the forest soil profile. The authors indicated that given the vast bacterial diversity in any given soil, that studies should focus on functionally important groups. For example, in agricultural soils, an interesting functional group to study would be the plant-growth-promoting rhizobacteria (PGPR).

In another survey using molecular methods to study bacterial diversity in Cerrado soil, Quirino et al. (2009) compared the bacterial composition of a native *senso stricto* Cerrado area with a Cerrado area converted into pasture and agricultural land. Their results showed differences between the two areas and greater bacterial diversity in the native area. Using the 16S rDNA-based approach, they observed that the species richness of the bacterial community associated with the native community was approximately 10 times greater than that of Cerrado converted to pasture! The largest group of bacteria represented in the Cerrado sensu stricto soil were sequences from α-Proteobacteria (26.4%), followed by Acidobacteria (22.2%) and Actinobacteria (19.4%). The other groups of bacteria identified were β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, unaffiliated Proteobacteria, Fibrobacteres, Planctomycetes, Chloroflexi, Verrucomicrobia, and unknown bacteria. Each of the latter groups represented between 1.4% and 5.6% of the total number of clones. Analysis of sequences

derived from Cerrado sensu stricto soil converted to pasture showed that the most prevalent group was the Actinobacteria, representing 34.3% of sequences. The Acidobacteria were also very abundant (20.0%), as well as representatives of α-Proteobacteria (11.4% of sequences). Representatives of the phylum Planctomycetes were identified (8.6% of sequences) as well as Chloroflexi (7.1% of sequences). Other groups such as β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, Fibrobacteres, Bacteroidetes, and Gemmatimonadetes were retrieved at lower frequencies (varying from 1.4% to 4.3%). Unknown soil bacteria corresponded to 4.3% of sequences. Verrucobacteria and unaffiliated proteobacterial sequences were not identified in pasture soil, and Bacteroidetes and Gemmatimonadetes were not identified in the Cerrado sensu stricto.

Studying tropical soil bacterial community structures in soils that have been under long-term cultivation, Peixoto et al. (2010) studied "all" soil bacteria and separately the Pseudomonas group to determine the effects of land management. They studied long-term cultivation with either conventional tillage or no tillage and compared their results with a non-cultivated soil. The authors demonstrated that between soils, the microbial community profiles were significantly different and that differences between conventional tillage, no tillage, and the uncultivated areas could be correlated to chemical and biochemical variations in the soils. These results provide a small but important piece of information about bacterial communities in the Cerrado and demonstrate that the effects of change in land use can be measured and compared at the bacterial community level. Using this approach would facilitate monitoring a globally important biodiversity hotspot. Much of the Cerrado ecosystem has been converted to agriculture such that the remnants should be protected. Natural vegetation has been cleared for soybean production much of which benefits from biological nitrogen fixation (BNF) of root nodule–forming bacteria. BNF in Brazil is managed and used on a scale not seen anywhere elsewhere on the Earth. Areas of the size of small European countries are planted with soya where as much as 20% of its nitrogen requirement is gained through deliberate inoculation with nitrogen-fixing strains.

Legume-nodulating bacteria, rhizobia, live as saprophytes in tropical soil and in facultative symbiosis with plants. They induce the formation of root nodules, where they fix atmospheric nitrogen and provide it to the plant in exchange for carbon compounds (Masson-Boivin et al. 2009). Biological nitrogen fixation is the main process for getting nitrogen into soils in natural ecosystems, with the greatest contribution occurring from fixation by rhizobial strains associated with leguminous plants. Identifying bacterial strains that can induce nodulation across a wider range of tropical soils is of great importance for global food production and reducing the energy and climate change costs of fertilizer production. In the past, it was assumed that rhizobia were restricted to the Alphaproteobacteria. More recently legumes have been observed to be nodulated by Betaproteobacteria, for example, *Ralstonia taiwanensis* and *Burkholderia* sp. (Suárez-Moreno et al. 2011). Dos Reis Jr et al. (2010) undertook an extensive survey of nodulation in the legume genus *Mimosa* in the Cerrado and the Caatinga. In their study, the authors found that nodulation is a generic characteristic of the genus *Mimosa*, and that *Burkholderia* strains are the predominant symbionts in two of the major centers of diversity of this genus. Nodulated *Mimosa* species can fix N_2 within their native environments, and therefore, they may make a valuable contribution to the N cycle of the fragile ecosystems of the Cerrado and the Caatinga. These findings indicate that other fragile semiarid and desert ecosystems such as those found in Africa, Asia, and Australia could be reforested with the help of bacterial communities isolated in situ or from Brazil.

Rhizobial isolates from Caatinga legumes such as *Cratylia mollis* Mart. Ex Benth, *Calliandra depauperata* Benth., and *Mimosa tenuiflora* (Will.) have been studied by Teixeira et al. (2010). Nodules were collected from field plantations and plants cultivated in a greenhouse experiment using Caatinga soil. Bacterial strains isolated from the soil were analyzed morphologically and by ARDRA. A high diversity of both slow- and fast-growing rhizobia was observed. Freitas et al. (2010) also studied biological nitrogen fixation in tree legumes of the Brazilian semiarid Caatinga. The tree species with the greatest N_2 fixation capacity were identified as *Mimosa tenuiflora*, *Mimosa arenosa*, and *Piptadenia stipulacea*. The mean BNF contributions of nitrogen to these plant species were very high, varying from 27 to 68%. In situations of native vegetation regeneration, when plant succession is dominated by nitrogen-fixing species, fixation can reach up to 130 kg of nitrogen ha/year (Freitas et al. 2010). Another frequent observation is that some soil root bacteria opt for an endophytic mode of life and with time might be considered permanent endosymbionts.

Endophytic bacteria occupy internal tissues of plants without causing damage to their hosts. They can play an important role in agriculture by conferring advantages to a plant's development by producing phytohormones and siderophores, increasing resistance to pathogens and parasites, and promoting biological nitrogen fixation as well as antibiotic production. Understanding the diversity of plant-bacterial associations and their role in plant development is necessary if these associations are to be manipulated to increase crop production, conserve biodiversity, and to sustain tropical agro-ecosystems in developing countries (Magnani et al. 2010). The study of endophytes is extremely exciting and we are beginning to understand better the contribution of soil bacteria to the secondary metabolism of plants. It seems that soil bacteria can confer medicinal properties among many other properties to plant formulations. Many plants species from the Caatinga are widely known and used in folk medicine and for commercial manufacturing of phytotherapeutic products (Albuquerque et al. 2007). In this sense, the number of studies on medicinal plants from the semiarid region of northeast Brazil is substantial, with most studies focusing on listing plants together with their therapeutic indication, manner of use, and the plant parts used (Silva et al. 2011a). However, it is now well known that in some instances, the isolated bioactive compound is not from the plant but,

instead, from bacteria inhabiting the plants. Examples of this phenomenon include the medicinal plant snakevine (*Kennedia nigriscans*) (Castillo et al. 2002), not to mention the kakadumycins that are produced by *Streptomyces* sp. NRRL 30566, an endophyte of *Grevillea pteridifolia* (Castillo et al. 2003). Latex from *Hancornia speciosa*, a very common plant found in the Caatinga and Cerrado, has antifungal activity (Silva et al. 2011b). The identification of the 16S rRNA sequences from latex indicated that bacteria belonging to the genera *Enterobacter, Escherichia, Klebsiella*, and *Bacillus* were all present within the plant latex and possibly responsible for its antifungal activity. It seems that if we look for them, we will often find tropical soil bacteria within tropical plants.

One of the main purposes of studying bacterial diversity is linked to the possibility of finding biotechnological applications from these organisms. As has been highlighted, bacteria are extremely versatile and are able to perform various metabolic pathways unique to their Kingdom. The identification of bacteria that can improve quality of life as well as protecting soil, plant, and water resources is a goal for the international scientific community. Tropical soil bacteria have a special contribution to make to environmental protection, agriculture, medicine, and a host of other industries. Santos et al. (2010b) have discussed the question of biotechnological bacteria associated with Caatinga plants. They highlighted the kind of research that is underway in Brazil and Australia where dry-adapted plant-growth-promoting bacteria can help with plant cultivation in semiarid agricultural lands. Such bacteria protect new roots from desiccation and help initial plant development (Santos et al. 2010b).

Actinobacteria are found in all soils globally. Studies concerning their diversity in temperate soils, especially in North America, Europe, and North Asia, started in middle of the last century, when the group of Selman Waksman discovered streptomycin, produced by *Streptomyces griseus*. Bioprospecting to identify new metabolites of commercial value has not stopped since. The genus *Streptomyces* has more described species than any other soil bacterium, and many of these species were described more than 40 years ago.

One of the first reports on actinomycetes isolated from Brazil's tropical soils was published in the 1970s by Coelho and Drozdowicz (1978). Cerrado soil was investigated, and around 10^6CFU actinomycetes/gram dry weight soil were detected and around a hundred strains isolated. These isolates were tested for the production of bioactive compounds antagonistic to the hemoflagellate *Trypanosoma cruzi*, the etiological agent of Chagas disease, and an important medical problem in the tropics (Coelho et al. 1995). In that study, nine strains were selected that secrete compounds that completely inhibited parasite replication in axenic medium. Latter Gomes et al. (1999) and Semêdo et al. (2001) used the dispersion and centrifugation technique (Hopkins et al. 1991) to isolate actinomycete strains not only from Cerrado, but also from Atlantic Rainforest soil. They hoped that this procedure would facilitate the isolation of microbial cells more firmly adhered to the soil particles, not normally isolated using the traditional dilution plate technique. Some of the isolates were screened for antimicrobial and enzymatic activities, and many of them were considered promising (Semêdo et al. 2001). From the Cerrado soil, a *Streptomyces cyaneus* strain (Petinate et al. 1999a, b) was able to produce a serine-proteinase. Several strains from this same soil presented endochitinase activity comparable to those from a commercial chitinase strain (Gomes et al. 2000); two of the selected strains, with high chitinase and proteinase activity, were described as a new species: *Streptomyces lunalinharesii* (Souza et al. 2008). *Streptomyces lunalinharesii* is active against several phytopathogenic fungi and promising in the biocontrol of these fungi (Gomes et al. 2001). *Streptomyces* strain IMPPG 594, which has not been described yet, is a promising proteolytic strain (De Azeredo et al. 2003, 2004), also able to degrade keratin (De Azeredo et al. 2006). Esposito et al. (1998) also isolated actinomycetes from Cerrado soil and three strains from soil treated with the herbicide 2,4-D that were also able to degrade Diuron in vitro. All strains showed protease and urease activity. They describe an actinomycete strain that produced manganese peroxidase, that could be potentially related to the degradation of Diuron. *Paenebacillus* strains isolated from Cerrado soil demonstrate antimicrobial activity against some fungi (Tupinamba et al. 2008). New species with N_2 fixation properties including *Paenibacillus brasiliensis* (Von der Weid et al. 2002) have been described. Given that the Cerrado and the Caatinga are very unique and old ecosystems with unique and diverse vegetation, it is to be expected the same follows for their associated rhizobacterial communities. Tropical soils under natural vegetation offer a rich source of new bacterial species able to produce enzymes of commercial importance. It is worthwhile repeating that soil bacterial diversity is not strongly linked to latitude but more to the vegetation covering these soils and so as we move into the more humid tropics with abundant light and water, we find increasing plant, insect, and animal diversity and with it coevolved bacterial diversity. But before looking at rainforests, another important tropical ecosystem that should be mentioned is the mangrove forest.

Mangrove Soil/Sediment Communities

Mangroves are the natural interface between estuarine, marine, and terrestrial environments and found throughout the tropics. They are a transitional coastal ecosystem occupied by terrestrial plants (Kathiresan and Qasim 2005), plants in soils that become unconsolidated tidal sediments along coasts. Sahoo and Dhal (2009) estimated that there are more than 14.5 million hectares of mangrove forest, in the Indo-Pacific region (6.9 million), Africa (3.5 million), and the Americas (4.1 million). Brazil, Indonesia, and Australia are the countries with most intact mangroves (Aksornkoae et al. 1984; Holguin and Bashan 2001). Different to tropical rainforests, which can have as many as 400 tree species in a hectare, mangrove forests in Brazil are dominated by just six tree species from three genera (Ghizelini et al. 2012, in press). Estimates of microbial diversity

inhabiting mangrove ecosystems are high, both in the soil/sediment and in mangrove plant rhizospheres. These microorganisms are fundamental for the maintenance of productivity, conservation, and recovery of this ecosystem, being key components of the biogeochemical cycles (Holguin and Bashan 2001; Das et al. 2006) and important source of biotechnological targets (Santos et al. 2011a, b).

Oxygen availability is a driver for bacterial community structure in mangroves especially as the transition from a soil with horizons changes to tidal sediment where anoxic conditions prevail. At the water's edge, mangrove sediments are generally anoxic except for the very superficial layers where root networks and crab dens permit the flow of air (Kristensen et al. 2008). Santos et al. (2011b) suggest that the aerobic microbial community consumes debris deposited on the surface and anaerobic microorganisms are fed by debris that are buried by members of the mesofauna and macrofauna (Alongi 2002; Kristensen and Alongi 2006; Kristensen et al. 2008).

Mangrove microbial communities are heterogeneously distributed within mangroves and between different mangroves (Peixoto et al. 2010; Santos et al. 2010a, 2011a, b). These differences can be explained by sharp environmental gradients over short spatial scales that include pollutants, reductive-oxidative balance (redox state), pH, and nutrient distribution (Peixoto et al. 2010). The aerobic/anaerobic interface is a critical boundary that characterizes soil community structures. Lessa et al. (unpublished data) have shown that anaerobic communities at 5–10-cm and 10–20-cm depth are similar and very different to communities at the surface and to a depth of 5 cm.

Santos and colleagues (2010a, 2011a), using clone libraries and pyrosequencing, described very diverse microeukaryotic and bacterial communities from pristine mangrove sediments at the *Restinga da Marambaia*, Rio de Janeiro. Among the dominant bacterial groups in the natural sediment, the authors highlighted Proteobacteria as the dominant phyla, followed by Firmicutes and Acidobacteria (Santos et al. 2011a). Other authors have also described the dominance of Proteobacteria in Brazilian and other tropical mangroves sediments. Dias and colleagues (2010) used clone libraries and DGGE to indicate the dominance of Proteobacteria, followed by Acidobacteria, in sediments of a not disturbed mangrove in Ilha do Cardoso (São Paulo, Brazil). Ghosh et al. (2010) described the dominance of Proteobacteria clones (58%) in a gene library analysis of a mangrove sediment of Sudarban (India), the world's largest mangrove forest, shared by Bangladesh and India. In China, the dominance of Proteobacteria clones, about 67%, was revealed by Liang and colleagues (2007). As we have seen from other tropical soil environments, there would appear to be no shortage of bacterial diversity. The challenge that faces us is managing and protecting ecosystems so that this diversity remains. The bacteria that occupy soils and sediments are probably the best equipped to remediate pollution and other environmental impacts. Given that mangroves are the natural interface between the land and the sea in the tropics, they also are net receivers of a lot of pollution from rivers and the sea.

The majority of oil that converges on coastal ecosystems in the tropics impacts mangroves. Microorganisms are the key drivers of the degradation of many carbon sources, including petroleum hydrocarbons (Santos et al. 2011b). The impact of oil contamination on mangrove sediment microbial diversity has been described using molecular techniques. At the phylum level, the dominance of the proteobacteria in mangrove sediments is not affected by oil pollution; however, there are significant changes at the genus level where new members of the total community dominate in the presence of oils (Liang et al. 2007; Dias et al. 2010; Ghosh et al. 2010; Santos et al. 2011a). For example, Santos and colleagues (2011a) have shown that Proteobacteria phylum dominates sediments before and after the simulated oil spill, but at a finer resolution, the order Chromatiales and the genus *Haliea* decrease when exposed to 2% and 5% oil. Conversely, three other genera, *Marinobacterium*, *Marinobacter*, and *Cycloclasticus*, all increased their prevalence after oil contamination. In this study, the molecular methods point to oil-sensitive bacteria that could be used for monitoring pollution levels, and identify groups of bacteria that seem to enjoy and thrive in the presence of oil, and that may have a role in bioremediation.

There are many studies that have been conducted to develop or improve bioremediation strategies to be applied in oil-impacted mangroves, mostly in vitro, with very few field applications described in the literature (Burns et al. 2000; Duke et al. 2000; Ramsay et al. 2000; Ke et al. 2003; Guo et al. 2005; Yu et al. 2005a, b; Luan et al. 2006; Brito et al. 2009). Odokuma and Dickson (2003) tested different bioremediation strategies in a mangrove along the New Calabar River in Rivers State in the Niger Delta of Nigeria and indicated that biostimulation associated with tilling was the best oil bioremediation approach for tropical mangroves.

Recently, Peixoto and colleagues (2011a, b) evaluated different strategies of bioremediation in an oil-contaminated mangrove in Bahia State (Brazil) in situ. They demonstrated impressive plant recovery in areas under biostimulation together with a bacterial bioaugmentation strategy. The bioaugmentation also contributed to the plant recovery and growth. Known plant-growth-promoting rhizobacteria (PGPR) were found to be efficient oil degraders. Recent studies on biostimulation and bioaugmentation in tropical ecosystems confirm their global applicability for oil biodegradation. Ron Atlas and others had first described their application in temperate and cold climates when cleaning up after the Exxon Valdez Oil spill (Bragg et al. 1994). Carmo and colleagues (2011) have described a range of oil-degrading bacterial isolates from mangrove sediments and mangrove plants' rhizospheres and also detected a predominance of Proteobacteria (81%) in contaminated sediments. Other phyla observed included Actinobacteria (7%) and Firmicutes (12%). All isolated bacteria were tested to evaluate their PGPR characteristics and the authors proposed a bacterial consortium to be used in oil-impacted mangrove bioremediation. The proposed consortium contained strains from the *Pseudomonas*, *Acinetobacter*, *Azospirillum*, *Paenibacillus*, and *Ralstonia* genera and this mixture in culture media degraded

39–97% of total petroleum hydrocarbons (TPH) in 7 days. It is worthy to highlight the importance of bioaugmentation and biostimulation as promising alternatives to recover oil-impacted mangroves in tropical and subtropical areas. Bioaugmentation and biostimulation are sustainable and efficient technologies to be used after oil spills reach sediments and coastal soils to minimize the impact of high concentrations of oil in mangrove areas. Moving in land from the mangroves and in humid tropical areas, we encounter rainforests.

Rainforest Soil Communities

Tropical rainforest research rightly attracts significant research funding as these hugely diverse ecosystems are the product of 25 million years of coevolution and succession. High light energy and lots of precipitation have provided ideal conditions for primary production, multiple successions, niche creation, and complex and rich plant, animal, and microbial communities. If we agree that microbial communities live on and in all other species, and if we accept that tropical rainforest ecosystems are home to the largest number of plant and insect species, and if each of these has its own unique microbial flora, then one can assume that bacterial communities in forest soils are likely to be incredibly diverse. We have already seen impressive diversity in desert, arid, and semiarid soils, and this does not change for rainforest soils. Molecular biology techniques involving high-throughput sequencing methods have made it possible to identify vast bacterial and fungal biodiversity from soils around the World. International research collaborations mean that soil samples from the tropics, where the latest technologies are not readily available, have been included in some biodiversity studies and global ecosystems have been compared. Fierer and Jackson (2006) investigated 98 distinct soil samples across North and South America using the T-RLFP method and concluded that bacterial community composition was not related to latitude, nor temperature, but was mostly related to ecosystem type and that there was a correlation with soil pH. In 2007, Roesch et al. published the first pyrosequencing study of soil bacterial diversity in a transect crossing North and South America. Against a scenario where bacterial species richness in soil had been estimated at 2,000 to eight million species in 1 g of soil, they sequenced 139,000 partial V9 domain 16S rDNA sequences from four locations. Using a 3% difference to define species level operational taxonomic units (OTU) (1% would have been a better choice), their approach detected significant bacterial diversity in all soils and a land use effect on soil bacterial diversity. Their forest soil was richer in the number of phyla than their agricultural soils that were comparatively genus rich but phylum poor. Another observation was that greater diversity was seen in Archaea from the arable soils. A year later, a deeper analysis of the same data was published by Fulthorpe et al. (2008) who made an important observation. They observed that very distantly sampled soils share very few of the same species. At 100% and 97% sequence similarity, synonymous to genus and species level similarity, 1.5% and 4.1% of the OTUs were found in all soils and 88% and 74% were unique to a particular soil. This analysis, though not surprising, demonstrates and confirms the incredible and probably habitat specific soil diversity. A question their findings raise is what do we do with all the diversity? These authors then identified, among the 130,000 sequence types, relatives that have been isolated on culture medium and compared the top ten genera for each soil. Interestingly between 30% and 37% of sequence reads fell within genera that have been cultured and can be studied. The authors suggest that soil microbiologists should turn their attention to isolating bacteria from genera that have been very infrequently studied and seem to be important in soil. In the case of the Brazilian soil, the important and poorly studied genera were: *Chitinophaga, Acidobacterium, Nevskia, Hydrocarboniphaga, and Dyadobacter.* Other high-throughput sequencing studies have opened our eyes even further to the incredible bacterial biodiversity that is in all soils. Fulthorpe et al. (2008) conclude that they would need to sequence greater than a million reads from each soil to get an accurate measure of OTU at the 97% similarity level, but they also question the value of doing so especially given that 97% similarity would group together very different bacteria. They rightly warn about PCR-based approaches to diversity studies. They state that sequences which are more abundant in soil DNA extract will be preferentially amplified, and that rare sequences may not be detected. The authors discuss the real possibility of an invisible tail of many rare species that may be present in all soils awaiting the right environmental conditions for them to appear in abundance.

Urich et al. (2008) described a meta-transcriptome approach to study "total" soil diversity and function as a method to circumvent PCR-based biases. In a pioneering study on soil biodiversity they extracted soil RNA from a grassland soil in Germany and made cDNA from the rRNA and mRNA in their sample. They then used tags and pyrosequencing to sequence the cDNA avoiding the use of PCR. Their data is particularly interesting because it shows comparable results between LSU and SSU rRNAs and as well as providing data on the eukaryotes that were present in the soil. Of the 100,000 mRNA sequences, only half could be ascribed to known functions, which begs the question: What else is happening in soils that we do not know about? The phylum level biodiversity they found was similar to that reported by Roesch et al. (2007) but a deeper analysis of their 180,000 ribosomal sequences and a comparison with Fulthorpe et al. (2008) data has not been made and would help to answer some of the questions surrounding PCR biases. PCR-free approaches to community studies represent the next step in better understanding soil bacterial communities and one would expect to read reports from tropical soils in the near future.

In tropical Brazil, Faoro et al. (2010) described 11 soil bacterial communities from a 26-km transect beneath Atlantic Rainforest. As with the previously cited pyrosequencing studies, at the phylum level, about 10 phyla are strongly represented and then there is a tail of many other phyla with fewer sequences. What we have gained from the high-throughput sequencing methods is a better idea of the immense bacterial diversity in

all soils and an idea of a very long tail of rare species. When extracting RNA from soils becomes as easy and as reliable as extracting DNA from soils, then one can predict that the metatranscriptome approach combined with high-throughput sequencing will not only shine a light on which communities are present in a soil but also what they are doing. PCR-free methods are likely to make PCR approaches to bacterial biogeography redundant. Recognizing soil bacterial biodiversity and conserving it are important; they guarantee soil function for future generations. Soil function and fertility protect water resources and provide food resources, and these are the very real and practical outcomes of understanding soil bacteria.

Roles of Tropical Soil Bacteria

Fulthorpe et al. (2008) suggested that microbiologists might wish to isolate the dominant bacterial groups found in biodiversity studies to understand their roles and physiology better. It is a fact that as molecular methods to study microbiology have rapidly evolved in the last 20 years, the ability of researchers in most tropical countries to have access to those technologies has been limited. Nevertheless, isolation and cultivation methods, and screening for novel bacteria of agricultural importance, have probably progressed further in tropical countries than in temperate climates. Baldani and Baldani (2005) have written an excellent review of biological nitrogen fixation (BNF) with grass plants from Brazilian soils. In the review, 40 years of research led by Johanna Dobereiner is carefully recorded and it is a story of nitrogen, and the tropical soil bacteria studied in Seropedica, Rio de Janeiro. Johanna Dobereiner is no longer with us, but her legacy continues through her students who now are research leaders. Baldani and Baldani (2005) describe the key findings and methods used to isolate and employ free-living and associated nitrogen fixers with sugarcane and other crop plants. Their review provides a blueprint of how one might identify and employ tropical soil bacteria to improve crop production in arid and semiarid savannah conditions. First references to nitrogen fixing by endophytes (associated bacteria) are cited and date back to the 1980s. The review finishes with modern plant and bacterial genome projects where the genetics of plant bacterial signaling and plant promotion are being successfully understood. Dobereiner's "children" now produce tropical soil bacterial inoculants using *Herbaspirillum seropedicae* strains to promote the growth of maize, rice, sorghum, and sugarcane. Slowly Brazil is sharing this technology with tropical countries in Africa, which is led by Embrapa. Bacterial strain and plant variety specific interactions for *Gluconacetobacter diazotrophicus* with sugarcane, and *Azorhizobium caulinodans* and wheat, are beginning to be understood (Tadra-Sfeir et al. 2011). The unfulfilled promise initially shown by *Azospirillum brasiliensis*, that once made Johanna Dobereiner famous, has resulted in success with other related bacteria isolated from tropical soils. It seems likely that the strain specific and intimate relationships that bacteria share with tropical grasses have evolved in tropical savannahs over millions of years. These relationships will become increasingly important as we rely on these ecosystems to feed much of the World's human population. Rice, sorghum, sugarcane, wheat, and beans all benefit through BNF and tropical soil bacteria.

When thinking about bacterial communities across the range of tropical soil types a theme that repeats is time. We recognise long and slow geological time that has influenced tropical soil formation and to a certain extent has determined its biota. We also recognize microbial time which is quick and responsive, some soil bacteria can produce three generations in a day. We have large and long geological time in contrast with the small and fast of bacteria. This results in incredible evolutionary power and soil bacterial communities in the tropics can and do respond quickly to changes in soil management. The biggest impact on a soil is typically when a farmer cultivates it, fertilizes it, or manages its pH. Peixoto et al.'s studies have shown that changing from tillage to direct drilling agriculture, which protects soil organic matter, results in community changes that are readily detectable and seem to recuperate pre-cultivation communities. Effects of tillage and types of tillage can be seen as changes in genetic profiles with DGGE and by pyrosequencing (Roesch et al. 2007). Roesch and every other group who has used high-throughput sequencing have indicated the vast alpha diversity (total species richness/OTU richness) of tropical and other soils. It would appear that there is more than enough bacterial diversity in tropical soils and implicitly functional redundancy. If so, human changes to soils may not be an issue in terms of bacterial biodiversity. However, the question is whether human interventions are diminishing the capacity of tropical soil bacteria to respond to new soil conditions or simply favoring another community of bacteria within the same soil. How much functional redundancy is there in tropical soils? How many times can they be disturbed and still function? These questions remain to be answered.

Given that soils are a nonrenewable resource (on a human time scale), ideally, they must be managed to sustain agricultural productivity and some must be preserved with native vegetation as biodiversity reserves. The perception that global land use is not sustainable is not just a national concern but a worldwide concern. Within this context, there is a growing demand for tools to assess soil quality in order to alert authorities about the direction that natural systems of production are taking and their likely consequences. In temperate regions, the impacts of different types of agricultural management on soil properties have been investigated, but in tropical regions, relatively few studies have been conducted on the impacts of different management types on soil microbial communities. Microbiological data currently available for use in Brazil indicate that the microbiological parameters might be useful to assess soil quality. Several studies developed in laboratories at Federal University of Rio de Janeiro and in partnership with other institutions show that bacterial diversity can be used as a quick indicator of soil quality. Molecular biology tools can be used to indicate changes in microbial community structure caused by different agricultural practices in tropical soils (Peixoto et al. 2002; Peixoto et al. 2006; Peixoto et al. 2011a, b). These same methods can be used to reveal

functionally important groups that can be monitored, and changes correlated with type of soil management. Soil quality concepts can be applied to environmental monitoring programs to evaluate if a given practice will deteriorate, stabilize, or improve soil ecosystem functions. Soil quality is obviously a concept in constant development, and it is foreseeable that this will remain so for some time to come. Given that soil quality is strongly influenced by microbe-mediated processes, and soil function can be related to diversity, it is likely that microbial community signatures have the potential to serve as early signals of soil degradation or soil improvement. Analysis of tillage treatments offers possibilities for exploring the relationships among soil organic matter, organic matter turnover, and carbon fluxes, since no-tillage practices generally accumulate more organic matter than conventional tillage (Salinas-García et al. 2002). Thus, analysis of microbial communities in these soil treatments can provide data that may elucidate the links between the soil biotic and abiotic factors. Peixoto et al. (2006) hypothesize that soil structural improvement resulting from the conversion to zero tillage agriculture helps create the environmental conditions needed for the reestablishment of part of the native microbial genotypes repressed by the soil degradation caused by conventional agricultural systems.

Assessments of biodiversity remain important because it is believed that in soils with greater biodiversity, the possibility of finding new classes of microorganisms that act in important processes, such as degradation of pesticides and maintenance of microbial processes under conditions of environmental stress, is higher. Preserving natural environments and different biomes provides a source of genetic diversity to protect against anthropogenic damage caused by industrial and agricultural practices. The tropics are home to the richest and most diverse ecosystems in the World, and it is safe to assume that is also the case for the tropical soil bacteria. This diversity is now recognized, is extremely useful, and should be protected for future generations.

References

Adámoli J, Macêdo J, Azevedo LG, Netto JM (1987) Caracterização da região dos Cerrados. In: Goedert WJ (ed) Solos dos Cerrados – Tecnologias e estratégias de manejo. Nobel e Embrapa, São Paulo e Brasília, pp 33–74

Aksornkoae S, Arroyo C, Blasco F, Burbridge PR, Tuck CH, Cintron G et al (1984) Handbook for mangrove area management. United Nations Environment Program and East-West Center, Environment and Policy Institute, Honolulu, Hawaii, p 256

Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Lins Neto EMF, Melo JG, Santos JP (2007) Medicinal plants of the *caatinga* (semi-arid) vegetation of NE Brazil: a quantitative approach. J Ethnopharmacol 114:325–354

Alongi DM (2002) Present state and future of the world's mangrove forests. Aust Inst Marine Sci 29:331–349

Azúa-Bustos A, González-Silva C, Mancilla RA, Salas L, Gómez-Silva B (2011) Hypolithic cyanobacteria supported mainly by fog in the coastal range of the Atacama Desert. Microb Ecol 61(3):568–581

Baldani JI, Baldani VLD (2005) History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience. Anais da Academia Brasileira de Ciências 77(3):549–579. doi:10.1590/S0001-37652005000300014

Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368:413–418. doi:10.1038/368413a0

Brito EM, Duran R, Guyoneaud R, Goni-Urriza M, Garcia de Oteyza T, Crapez MA et al (2009) A case study of in situ oil contamination in a mangrove swamp (Rio De Janeiro, Brazil). Mar Pollut Bull 58:418–423

Burns KA, Codi S, Duke NC (2000) Gladstone, Australia Field Studies: Weathering and Degradation of Hydrocarbons in Oiled Mangrove and Salt Marsh Sediments With and Without the Application of an Experimental Bioremediation Protocol. Mar Pollut Bull 41:392–402

Carmo FL, Santos HF, Ferreira EM, van Elsas JD, Rosado AS, Peixoto RS (2011) Bacterial Structure and Characterization of Plant Growth Promoting and Oil Degrading Bacteria from the Rhizospheres of Mangrove Plants. J Microbiol 49(535–543):201

Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robison R, Condron MA, Teplow DB, Stevens D, Yaver D (2002) Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on Kennedia nigriscans. Microbiology 148:675–685

Castillo UF, Harper JK, Strobel GA, Sears J, Alesi K, Ford E, Lin J, Hunter M, Maranta M, Ge H, Yaver D, Jensen JB, Porter H, Robison R, Millar D, Hess WM, Condron M, Teplow D (2003) Kakadumycins, novel antibiotics from Streptomyces sp. NRRL 30566, an endophyte of Grevillea pteridifolia. FEMS Microbiol Lett 29:183–190

Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin T (2006) The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. Environ Microbiol 8:514–525. doi:10.1111/j.1462-2920.2005.00921.x

Coelho RRR, Drozdowicz A (1978) The occurrence of actinomycetes in a cerrado soil in Brazil. Rev Ecol Biol Sol 15:459–473

Coelho RRR, Lopes A, Semêdo LTAS, Cruz FS (1995) Culture filtrates of actinomycetes isolated from tropical soils inhibit *Trypanosoma cruzi* replication in vitro. Rev Microbiol 26:307–313

Das S, Lyla PS, Ajmal Khan S (2006) Marine microbial diversity and ecology: importance and future perspectives. Curr Sci 90:1325–1335

De Azeredo LAI, Castilho LR, Leite SGF, Freire DMG, Coelho RRR (2003) Protease production by *Streptomyces* sp. isolated from Brazilian Cerrado soil. Optimization of culture medium employing statistical experimental design. Appl Biochem Biotechnol 105–108:749–755

De Azeredo LAI, Freire DMGF, Soares RMA, Leite SGF, Coelho RRR (2004) Production and partial characterization of thermophilic proteases from *Streptomyces* sp. isolated from Brazilian Cerrado soil. Enz Microb Technol 34:354–358

De Azeredo LAI, Lima MB, Coelho RRR, Freire DMG (2006) A low cost fermentation medium for thermophilic protease production by Streptomyces sp 594 using feather meal and corn steep liquor. Curr Microbiol 53:335–339

Dias AC, Andreote FD, Rigonato J, Fiore MF, Melo IS, Araújo WL (2010) The bacterial diversity in a Brazilian non-disturbed mangrove sediment. Antonie Van Leeuwenhoek 98:541–551

Dos Reis FB Jr, Simon SF, Gross E, Boddey RM, Elliott GN, Neto NE, Loureiro M de F, de Queiroz LP, Scotti MR, Chen W-M et al (2010) Nodulation and nitrogen fixation by *Mimosa* spp in the Cerrado and Caatinga biomes of Brazil. New Phytol 186:934–946

Duke NC, Burns KA, Swannell RPJ, Dalhaus O, Rupp RJ (2000) Dispersant use and a bioremediation strategy as alternate means of reducing impacts of large oil spills on mangroves: the Gladstone Field Trials. Mar Pollut Bull 41:403–412

Esposito E, Paulillo SM, Manfio GP (1998) Biodegradation of the herbicide diuron in soil by indigenous actinomycetes. Chemosphere 37:541–548

Faoro H, Alves AC, Souza EM, Rigo LU, Cruz LM, Al-Janabi SM (2010) Influence of soil characteristics on the diversity of bacteria in the southern Brazilian. Atlantic Forest. Appl Environ Microbiol 76:4744–4749

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103:626–631. dx.doi.org/10.1073/pnas.0507535103

Freitas ADS, Sampaio EVSB, Santos CERS, Fernandes AR (2010) Biological nitrogen fixation in tree legumes of the Brazilian semi-arid caatinga. J Arid Environ 74:344–349

Fulthorpe RR, Roesch LFW, Riva A, Triplett EW (2008) Distantly sampled soils carry few species in common. ISME J V2:901–910

Furley PA (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. Global Ecol Biogeogr 8:223–241

Furley PA (2007) Tropical savannas and associated forests: vegetation and plant ecology. Prog Phys Geogr 31:203–211

Garcia-Pichel F, Pringault O (2001) Microbiology: Cyanobacteria track water in desert soils. Nature 413:380–381. doi:10.1038/35096640

Ghizelini AM, Mendonca-Hagle LCS, Macrae A (2012) Microbial diversity in Brazilian mangrove sediments – a mini review. Brazilian J Microbiol (in press)

Ghosh A, Dey N, Bera A, Tiwari A, Sathyaniranjan KB, Chakrabarti K, Chattopadhyay D (2010) Culture independent molecular molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. Saline Syst 6:1

Gomes RC, Semêdo LTAS, Linhares AA, Guimarães ACC, Alviano CS, Linhares LF, Coelho RRR (1999) Efficiency of the dispersion and differential centrifugation technique in the isolation of chitinolytic actinomycetes from soil. W J Microbiol Biotechnol 15:47–50

Gomes RC, Semêdo LTAS, Soares RMA, Alviano CS, Linhares LF, Coelho RRR (2000) Chitinolytic activity of actinomycetes from a cerrado soil and their potential. Lett Appl Microbiol 30:146–150

Gomes RC, Semêdo LTAS, Soares AS, Alviano CS, Coelho RRR (2001) Purification of a thermostable endochitinase from *Streptomyces* sp RC 1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. J Appl Microbiol 90:653–661

Gorlach-Lira K, Coutinho HDM (2007) Population dynamics and extracellular enzymes actrivity of mesophilic and thermophilic bacteria isolated from semi-arid soil of Northeastern Brazil. Brazilian J Microbiol 38:1–7

Griffin DW, Garrison VH, Herman JR, Shinn EA (2001) African desert dust in the Caribbean atmosphere: microbiology and public health. Aerobiologia 17(3):203–213. doi:10.1023/A:1011868218901

Guo CL, Zhou HW, Wong YS, Tam NFY (2005) Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. Mar Pollut Bull 51:1054–1061

Holguin GVP, Bashan Y (2001) The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biol Fertil Soils 33:265–278

Hopkins DW, MacNaughton SJ, O'Donnell AG (1991) A dispersion and differential centrifugation technique for representatively sampling microorganisms from soil. Soil Biol Biochem 23:217–225

IUCN (1988) Tropical countries from the *IUCN* list. www.nhm.ac.uk/hosted_sites/. . ./tropctry.htm. The following list is taken from Plants in Danger: What do we know? Published . . . TBG02/tropctry Issue 1–15.04.1988 – BJO (site visited on 15/01/12)

Kathiresan K, Qasim SZ (2005) Biodiversity of mangrove ecosystems. Hindustan Publishing Corporation, New Delhi, p 251

Ke L, Wang WQ, Wong TWY, Wong YS, Tam NFY (2003) Removal of pyrene from contaminated sediments by mangrove microcosms. Chemosphere 51:25–34

Kristensen E, Alongi DM (2006) Control by fiddler crabs (*Uca vocans*) and plant roots (*Avicennia marina*) on carbon, iron and sulfur biogeochemistry in mangrove sediment. Limnol Oceanogr 51:1557–1571

Kristensen EBS, Dittmar T, Marchand C (2008) Organic carbon dynamics in mangrove ecosystems: A review. Aquatic Botany 89:201–219

Lester ED, Satomi M, Ponce A (2007) Microflora of extreme arid Atacama Desert soils. Soil Biol Biochem 39(2):704–708

Liang JB, Chen YQ, Lan CY, Tam NFY, Zan QJ, Huang LN (2007) Recovery of novel bacterial diversity from mangrove sediment. Mar Biol 150:739–747

Luan TG, Yu KSH, Zhong Y, Zhou HW, Lan CY, Tam NFY (2006) Study of metabolites from the degradation of polycyclic aromatic hydrocarbons (PAHs) by bacterial consortium enriched from mangrove sediments. Chemosphere 65:2289–2296

Magnani GS, Didonet CM, Cruz LM, Picheth CF, Pedrosa FO, Souza EM (2010) Diversity of endophytic bacteria in Brazilian sugarcane. Genet Mol Res 9(1):250–258

Masson-Boivin C, Giraud E, Perret X, Batut J (2009) Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends Microbiol 17:458–466

Myers N, Mittermeier RA, Mittermeier CG, de Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–856

Nepstad DC, Verssimo A, Alencar A, Nobre S, Lima E, Lefebvre P, Schlesinger P, Potterk C, Moutinho P, Mendoza E, Cochrane M, Brooks V (1999) Large-scale impoverishment of Amazonian forests by logging and fire. Nature 398:505–508

Odokuma LO, Dickson AA (2003) Bioremediation of a Crude Oil Polluted Tropical Mangrove Environment. J Appl Sci Environ Manag 7:23–29

Okoro CK, Brown R, Jones AL, Andrews BA, Asenjo JA (2009) Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie Van Leeuwenhoek 95(2):121–133

Parkinson D, Coleman DC (1991) Microbial communities, activity and biomass. Agric Ecosyst Environ 24(1):3–33

Peixoto RS, Coutinho HLC, Rumjanek NG, Macrae A, Rosado AS (2002) Use of rpoB and 16 S rRNA genes to analyse bacterial diversity of a tropical soil using PCR and DGGE. Lett Appl Microbiol 35(4):316–320

Peixoto RS, Coutinho HLC, Madari B, Machado PLOA, Rumjanek NG, van Elsas JD, Seldin L, Rosado AS (2006) Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. Soil Tillage Res 90:16–28

Peixoto RS, Chaer GM, Franco N, Reis Junior FB, Mendes IC, Rosado AS (2010) A decade of land use contributes to changes in the chemistry, biochemistry and bacterial community structures of soils in the Cerrado. Antonie Van Leeuwenhoek 98:403–413

Peixoto R, Chaer GM, Carmo FL, Araújo FV, Paes JE, Volpon A, Santiago GA, Rosado AS (2011a) Bacterial communities reflect the spatial variation in pollutant levels in Brazilian mangrove sediment. Antonie Van Leeuwenhoek 99(2):341–354

Peixoto RS, Carmo FL, Santos HF, Andrade LL, Paes JE, Cury J, Rosado AS (2011b) Biomonitoramento: Bioindicadores microbianos da presença de óleo em manguezais. Microbiologia in Foco 14:8–13

Petinate SDG, Branquinha MH, Coelho RRR, Vermelho AB, De Simone G (1999a) Purification and partial characterization of an extracellular serine-proteinase of *Streptomyces cyaneus*, isolated from Brazilian Cerrado soil. J Appl Microbiol 87:557–563

Petinate SDG, Martins RM, Coelho RRR, Meirelles MN, Branquinha MH, Vermelho AB (1999b) Influence of growth medium in proteinase and pigment production by *Streptomyces cyaneus*. Mem Inst Oswaldo Cruz 94:173–177

Quirino BF, Pappas G Jr, Tagliaferro A, Collevatti RG, Leonardecz E, Silva MRSS, Bustamante MMC, Kruger RH (2009) Molecular phylogenetic diversity of bacteria associated with soil of the savanna-like Cerrado vegetation. Microbiol Res 164:59–70

Ramsay MA, Swannell RPJ, Shipton WA, Duke NC, Hill RT (2000) Effect of bioremediation community in oiled mangrove sediments. Mar Pollut Bull 41:413–419

Roesch LFW, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Daroub SH, Carmargo FAO, Farmeire WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J V1:283–290

Sahoo K, Dhal NK (2009) Potential microbial diversity in mangrove ecosystem: A review. Indian J Mar Sci 38:249–256

Salinas-García JR, Velázquez-García JJ, Gallardo-Valdez M, Díaz-Mederos P, Caballero-Hernández F, Tapia-Vargas LM, Rosales-Robles E (2002) Tillage effects on microbial biomass and nutrient distribution in soils under rain-fed corn production in central-western Mexico. Soil Tillage Res 66:143–152

Santos HF, Carmo FL, Cury J, Rosado AS, Peixoto RS (2010a) 18 S rDNAsequences from microeukaryotes reveal oil indicators in mangrove sediment. PLoS One 5(8):e12437

Santos SN, Kavamura VN, Silva JL, Melo IS, Andreote FD (2010b) Plant growth promoter rhizobacteria in plants inhabiting harsh tropical environments and its role in agricultural improvements. In: DK Maheshwari (Org) Plant growth and health promoting bacteria, 1 edn, vol 1. Springer, Berlin, pp 251–272

Santos HF, Carmo FL, Cury J, Lopes AL, Tiedje J, Van Elsas JF, Rosado AS, Peixoto RS (2011a) Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: Bacterial proxies for oil pollution. PLoS One 6(3):e16943

Santos HF, Carmo FL, Paes JE, Rosado AS, Peixoto RS (2011b) Bioremediation of mangroves impacted by petroleum. Water Air Soil Poll 216:329–350

Semêdo LTAS, Linhares AA, Gomes RC, Manfio GP, Alviano CS, Linhares LF, Coelho RRR (2001) Isolation and characterization of actinomycetes from Brazilian tropical soils. Microbiol Res 155:291–299

Silva MIG, Melo CTV, Vasconcelos LF, Carvalho AMR, Sousa FCF (2011a) Bioactivity and potential therapeutic benefits of some medicinal plants from the Caatinga (semi-arid) vegetation of Northeast Brazil: a review of the literature. Brazilian J Pharmacogn Rev Bras Farmacogn 22:193–207

Silva TF, Coelho MRR, Vollu RE, Goulart FRV, Alviano DS, Alviano CS, Seldin L (2011b) Bacterial community associated with the trunk látex of *Hancornia speciosa*. Antonie Van Leeuwenhoek 99(3):523–532

Souza RF, Coelho RRR, Macrae A, Soares RMA, Nery DCM, Semêdo LTAS, Alviano CS, Gomes RC (2008) *Streptomyces lunalinharesii* sp. nov., a chitinolytic streptomycete isolated from cerrado soil in Brazil. Int J Syst Evol Microbiol 58(12):2774–2778

Sprent JI, Gehlot HS (2010) Nodulated legumes in arid and semi-arid environments: are they important? Plant Ecol Divers 3:211–219

Stivaletta N, Barbieri R (2008) Endoliths in Terrestrial Arid Environments: Implications for Astrobiology, Cellular Origin, Life in Extreme Habitats and Astrobiology, 1, Volume 12, From Fossils to Astrobiology, Part 2. Pages 3:319–333

Suárez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonça-Previato L, James EK, Venturi V (2011) Common features of environmental and potentially beneficial plant-associated Burkholderia. Microb Ecol 62:241–248

Tadra-Sfeir MZ, Souza EM, Faoro H, Müller-Santos M, Baura VA, Tuleski TR, Rigo LU, Yates MG, Wassem R, Pedrosa FO, Monteiro RA (2011) Naringenin regulates expression of genes involved in cell wall synthesis in *Herbaspirillum seropedicae*. Appl Environ Microbiol 77(6):2180–2183

Teixeira FCP, Borges WL, Rumjanek NG, Xavier GR (2010) Characterization of indigenous rhizobia from Caatinga. Brazilian Journal of Microbiology 41:201–208

Tupinamba GS, Silva AJR, Souto-Padrón TCBS, Alviano CS, Seldin L, Alviano DS (2008) Antimicrobial activity of *Paenibacillus polymyxa* SCE2 against some mycotoxin-producing fungi. J Appl Microbiol 105:1044–1053

Urich T, Lanzén A, Qi K, Huson DH, Schleper C, Schuster SC (2008) Simultaneous Assessment of Soil Microbial Community Structure and Function through Analysis of the Meta-Transcriptome. PLoS One 3(6):e2527. doi:10.1371/journal.pone.0002527

Von der Weid I, Duarte GF, van Elsas JD, Seldin L (2002) Paenibacillus brasilensis sp. nov., a novel nitrogen-fixing species isolated from the maize rhizosphere in Brazil. Int J Syst Evol Microbiol 52:2147–2153

Yu KS, Wong AH, Yau KW, Wong YS, Tam NF (2005a) Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments. Mar Pollut Bull 51:1071–1077

Yu SH, Ke L, Wong YS, Tam NF (2005b) Degradation of polycyclic aromatic hydrocarbons by a bacterial consortium enriched from mangrove sediments. Environ Int 31:149–154

6 Freshwater Microbial Communities

Jakob Pernthaler
Limnological Station, Institute of Plant Biology, University of Zurich, Kilchberg, Switzerland

> ▶ Jordan, writing on bacteria in Ward and Whipple's "Freshwater Biology" states: "There is no special and characteristic class of "water bacteria," but germs from the air, from the soil, from decomposing animal and plant substances and from the healthy and diseased tissues of animals and plants may at times find their way into water"... (Henrici 1933).

Introduction

Freshwaters have been termed the "blood of society" in reference to their crucial importance for an adequate supply of water of reasonable quality for agriculture, industry, and domestic use (Wetzel 2000). It is estimated that >1,000 gT of freshwater resources have been annually consumed for these human activities during the last decades on a global scale, and even higher volumes of wastewater have been concomitantly released into the environment (Hoekstra and Mekonnen 2012). Moreover, despite the small fraction (<1%) of the Earth's surface occupied by inland waters, they nevertheless play a crucial role in global biogeochemical processes, in particular the cycling of carbon (Cotner and Biddanda 2002; Tranvik et al. 2009). Thus, inland waters not only affect climate at a regional, but also at a global scale.

Prokaryotes are a significant biomass component in lacustrine ecosystems; they are centrally involved in the degradation of pollutants and they dominate biogeochemical processes. Bacterial respiration in inland waters represents a single large sink of organic carbon comparable in size to that of marine habitats. The net metabolic balance of freshwater ecosystems (i.e., if they release or sequester carbon dioxide) is related to the nature and size of the dominant pools of organic carbon that is available for respiration by pelagic and benthic bacteria and *Archaea* (Ask et al. 2009; Tranvik et al. 2009; Berggren et al. 2012). Lakes and rivers are typically more productive than the open oceanic realms, and in addition receive higher quantities of dissolved and particulate organic carbon and dissolved inorganic carbon (DOC, POC, and DIC, respectively) from terrestrial sources (Pace et al. 2004; Tranvik et al. 2009). While riverine export of organic matter represents a substantial source of terrestrial DOC and POC to the oceans, much of this material is old and likely more refractory (Raymond and Bauer 2001). Thus, the major part of the labile organic matter of terrestrial origin is rapidly consumed and respired already within freshwater systems (Berggren et al. 2010; Pollard and Ducklow 2011). Moreover, although the rate of carbon storage in inland water sediments exceeds that of the ocean floors (Tranvik et al. 2009), most lakes are small and shallow (Downing et al. 2006). As a consequence, much of the sediment is in contact with the upper mixed water layers, favoring the remineralization of carbon over burial. Thus, the global rate at which carbon dioxide is emitted from—frequently supersaturated—inland waters into the atmosphere is likely of a similar order of magnitude as that of fossil fuel combustion or ocean carbon dioxide consumption (Battin et al. 2009; Tranvik et al. 2009). In addition, freshwater systems are also crucially involved in the production and cycling of the potent greenhouse gas methane (Tranvik et al. 2009). Most methane generated within hypolimnetic sediments that is not released via gas bubbles is transformed into carbon dioxide in the oxic realms of lakes or in some cases even anaerobically (Schubert et al. 2011). However, due to the frequency of shallow, productive habitats featuring anoxic zones, such as wetlands or flood plains, more methane is altogether released into the atmosphere from freshwaters than from the oceans

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_40,

(Tranvik et al. 2009). Recently, methane production has even been found in fully oxygenated epilimnetic waters of an oligotrophic lake, possibly caused by metabolic interactions between methanogenic archaea and autotrophs (Grossart et al. 2011).

Not surprisingly, the interest about prokaryotes in freshwater systems has originated from bacteriological studies on pathogens in drinking water systems during the 1880s (Hamlin 1990) and from investigations about wastewater processing (Dunbar 1908), whereas topics of basic science were addressed only later by microbiologists. The early investigations about the occurrence, seasonality, and depth distribution of lacustrine bacteria in lakes used for drinking water generation (Minder 1920; Fred et al. 1924) were conducted with the then available tools, that is, cultivation and the counting of colony-forming bacteria on solid media. However, already in the 1930s, one of the pioneers of freshwater microbial ecology, A.T. Henrici, recognized that it would not be possible to "sythesize a picture of the various organisms living and working together" from bacteriological studies on pure cultures only (Henrici 1933). Henrici himself developed more direct approaches to microscopically investigate the morphological diversity of "periphyton" bacteria attaching to submersed glass slides (*Fig. 6.1*), while his contemporary A. Rasumov, and later H. Jannasch and J. G. Jones, developed and improved filtration-based methods to obtain a correct quantitative appreciation of the total abundances of free-living bacteria in freshwaters (Rasumov 1932; Jannasch 1958; Jones 1974). In parallel, the interest in the rates at which heterotrophic bacteria transform Lindeman's "ooze" (Lindeman 1942), that is, the dissolved organic matter (DOM) pool, provided methodology to investigate the incorporation of particular DOM components (e.g., sugars and amino acids) by single bacterial cells and whole microbial assemblages (Wright and Hobbie 1965; Brock and Brock 1968) that were later adapted to investigate microbial growth and carbon production at the community level (Simon 1987). More recently, novel strategies for bacterial isolation (Bruns et al. 2003; Hahn et al. 2004; Page et al. 2004) and field studies by a variety of cultivation-independent molecular approaches (Muylaert et al. 2002; Zwart et al. 2002; Yannarell and Triplett 2004; Salcher et al. 2008; Eiler et al. 2012)—culminating in the genome sequencing of single microbial cells (Martinez-Garcia et al. 2012)—have led to a wealth of new information about the diversity, ecology, and physiology of the most common taxa of *Bacteria* and *Archaea* in freshwater habitats.

At this stage, one might ask if "freshwater microbial communities" indeed exist as separate entities, that is, (1) if there are prokaryotic taxa that exclusively occur in freshwaters and are not merely introduced by runoff from the vast pool of terrestrial microbial diversity and (2) if the various microbial taxa found in inland water habitats regularly associate into recognizable units that may legitimately be referred to as communities. The former question can be readily answered in the affirmative, and several examples for clearly autochthonous freshwater bacterial taxa will be presented in detail later. Moreover, a recent meta-analysis of microbial co-occurrence patterns across habitats, based on all available environmental 16S rRNA gene sequences, also seems to provide evidence for the latter statement, that is, the existence of numerous distinct association patterns of species-like operational taxonomic units (OTUs) within particular habitat types, including freshwaters (Chaffron et al. 2010).

Diversity of Freshwater Habitats

Most microbial ecologists studying inland waters likely live in the Northern hemisphere and tend to focus on surface water habitats that are conspicuous and/or of local relevance. In analogy with the "Taxonomy of taxonomists" issue (Gaston and May 1992), this may have led to a somewhat biased view on freshwater microbial assemblages. While it has been estimated that less than 0.01% of the bacteria in lacustrine aquatic environments actually grow in suspension (Costerton et al. 1995), the best-studied freshwater microbial communities are probably the easily accessible epilimnetic bacterioplankton assemblages of temperate lakes (Newton et al. 2011), whereas considerably less is known about microbes in subsurface, benthic or littoral habitats, in tropical regions, or in rivers and streams. Moreover, although technically "freshwater" at least during some periods, the microbial communities in terrestrial-aquatic ecotones (e.g., wetlands, floodplains) are commonly studied from a perspective of soil microbiology (Gutknecht et al. 2006). Although a large part of this chapter will be focused on the planktonic microbial assemblages in the surface layers of lakes, the author would nevertheless like to draw the reader's attention to the great diversity of freshwater habitats by presenting a few selected examples.

Subsurface Aquatic Habitats

The subsurface aquatic habitats are considered to harbor a hundred to a thousand times more microbial biomass than surface waters, although arguably with lower growth potential due to a general shortage of energy sources (Griebler and Lueders 2009). At one end of the spectrum, there are deep anoxic subsurface habitats that are entirely devoid of organic carbon sources, but that may nevertheless provide chemical energy for microbial growth in the form of geologically produced hydrogen. Such hydrothermal systems harbor microbial assemblages in which bacteria are substantially outnumbered by methanogenic *Archaea* (Chapelle et al. 2002), and that have been presented as potential models for biogenic methane production on Mars (Krashnopolsky et al. 2004). By contrast, methanogens typically only form a small proportion of the total microbes in other groundwater habitats that are richer in organic carbon (Chapelle et al. 2002). At the other end, the bacterial assemblages in the hyporheic zone (i.e., the transition zone between surface running waters and the adjacent groundwater systems) in parts resemble those of surface riverine sediments and even exhibit seasonal fluctuations (Hullar et al. 2006). While seasonality has so far not been observed in deeper aquifers, spatial heterogeneity may nevertheless contribute to a high

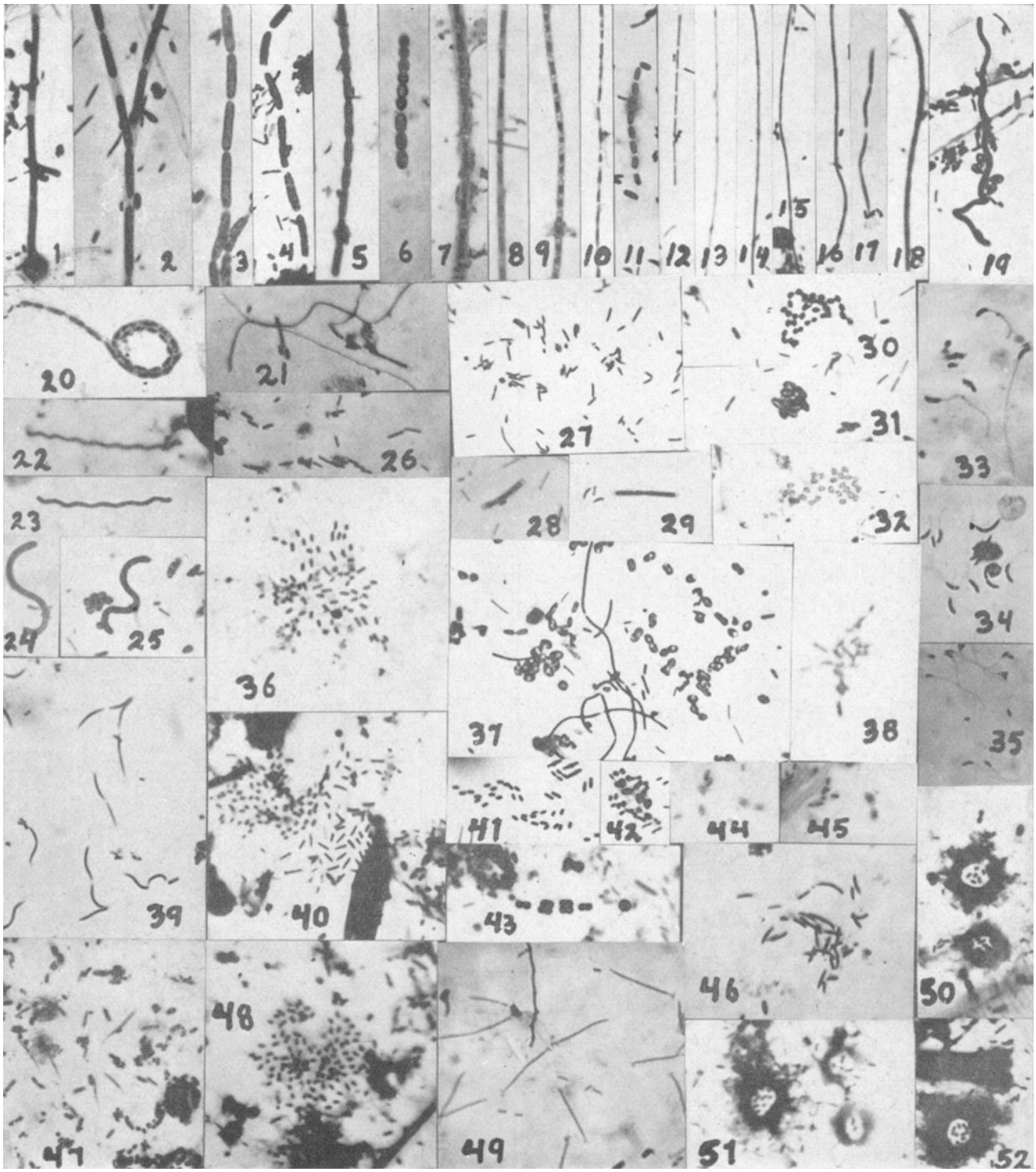


Fig. 6.1
Morphological diversity of freshwater "periphyton" bacteria that grow on submersed slides, as observed by A.T. Henrici (Reproduced from Henrici 1933)

overall diversity in such habitats (Griebler and Lueders 2009). For example, the microbial communities in three subsurface pools located within the same karst cave system only shared 1 out of 150 operational taxonomic units (OTUs, 16S rRNA similarity of 97%) (Shabarova and Pernthaler 2010). A variety of chemolithoautotrophic *Proteobacteria* have been found in groundwater habitats, and their metabolic versatility is suggested by the diversity of their ribulose-1,5-bisphosphate

carboxylase/oxygenase genes (Alfreider et al. 2012). Other physiological groups of bacteria that may be present in aquifers are denitrifiers and sulfate reducers (Griebler and Lueders 2009). Subsurface waters are also known to contain extremely small bacteria that pass through filters of 0.2 µm pore size and are affiliated with exotic phylogenetic lineages (e.g., the candidate divisions OD1, OP3, and OP11) (Miyoshi et al. 2005). However, to date, there is no clear consensus which bacteria should be considered typical for pristine groundwater systems. On the one hand it has been pointed out that cultivation-independent analyses of groundwater diversity may essentially draw a similar picture as isolation-based approaches, that is, a dominance of different *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* (Griebler and Lueders 2009). No "pure" subclades of exclusively subsurface origin were found in the candidate phylum OP3 (Shabarova and Pernthaler 2010). On the other hand, endokarst habitats have been proposed to nevertheless harbor truly autochthonous microbial communities because they contain conspicuously large proportions of bacterial genotypes that are only distantly related to known rRNA sequence types (Farnleitner et al. 2005; Pronk et al. 2009).

River and Stream Habitats

Although microbes in the pelagic zone of rivers are introduced from a multitude of sources, such as terrestrial habitats (farm land, urban settlements, forest soils etc.), biofilms, and sediments, their community composition nevertheless appears to be roughly comparable to that of freshwater lakes (Kirchman et al. 2004; Crump and Hobbie 2005; Winter et al. 2007; Ghai et al. 2011b). Rivers exhibit gradual longitudinal transitions in major biogeochemical features such as the quotient of production to respiration (Vannote et al. 1980), and anthropogenic influences such as point sources of organic input or geomorphological changes (damming) may locally affect productivity. As a consequence, the concentrations and composition of DOC and nutrients as well as the levels of primary production in the pelagic zone of different reaches of large rivers greatly vary (Kirchman et al. 2004; Winter et al. 2007). These continuous and discontinuous transition patterns are also reflected in the successions of the heterotrophic microbial assemblages and may lead to substantial variation of both, microbial activity patterns and community composition. Microbial hydrolysis patterns of DOC in various reaches of the Hudson River were related to differences in community structure of the studied assemblages, which in turn appeared to be longitudinally affected by DOC availability (Kirchman et al. 2004). This agrees with a metagenomic analysis of microbial carbon metabolism in the pristine upper realms of the Amazon River that suggested specialization of the assemblage for the incorporation and processing of allochthonous DOC from plant material (Ghai et al. 2011b). Regional variations in the levels of inorganic nutrients in river water may indirectly affect microbial assemblages via primary production (Winter et al. 2007), but might also have more direct effects: High anthropogenic input of ammonium into the River Seine favored the growth of nitrifying bacteria that were locally introduced by the effluents of a wastewater treatment plant (Cébron et al. 2004). The apparent bacterial richness in the River Danube was found to be positively correlated with phosphate (Winter et al. 2007); it was suggested that phosphorus limitation might lead to local blooms of bacteria that are specialists for this nutrient.

Biofilms are a dominant microbial growth form in numerous aquatic systems. They have been referred to as "microbial landscapes" to conceptualize their mesoscale heterogeneity and their biotic and abiotic interactions with the surrounding larger landscape (Battin et al. 2007). Biofilms are of particular importance in streams, where they perform key ecosystem functions and provide a matrix-enclosed, attached habitat for epilithic algae and pro- and eukaryotic microbial heterotrophs. As described above, for riverine bacterioplankton, longitudinal changes are also observed in stream biofilm assemblages that parallel the gradual geomorphological and/or biogeochemical transitions within the habitat. The taxonomic and functional properties of microbial community composition and function of alpine stream biofilms transformed with distance from the source, likely due to changes in DOC availability (Battin et al. 2001): While allochthonous macromolecules transiently stored in the polysaccharide matrix represented the major substrate for microbial heterotrophs in the upper realms, the exudates of the phytobenthos produced within the biofilm became increasingly important downstream. The structure and function of stream biofilms is moreover affected by flow velocity and direction: Multidirectional heterogeneous flow patterns led to more architectural differentiation of biofilms, to higher patchiness and genotypic diversity of experimental biofilm microbial assemblages, which in turn resulted in a higher amount of DOC components that could be utilized (Singer et al. 2010). Stream biofilm communities are exposed to high rates of immigration, but do not appear to be defined by such mass effects: While the attached communities from different streams were less diverse than their respective suspended stream water assemblages, they showed more similarity to each other, suggesting a selection of species that were particularly well adapted for this habitat type (Besemer et al. 2012). Among these specialized stream biofilm taxa, members of the *Flavobacteriaceae* appear to be of particular importance (O'Sullivan et al. 2002), as well as betaproteobacterial genera (Battin et al. 2001) such as *Acidovorax* (Besemer et al. 2012).

Special Habitats Within Lake Ecosystems

The neuston layer, that is, the air-water interface, represents a zone in lakes that is characterized by a specific microbiota. The surface microlayers of alpine lakes were characterized by higher concentrations of DOC of smaller molecular size, and microbial cell numbers, particularly of *Betaproteobacteria*,

tended to be higher in the bacterioneuston than in the subjacent water layer (Hörtnagl et al. 2010). *Archaea*, presumably *Thaumarchaeota*, were also found to be enriched in the neuston layer of high mountain lakes (Auguet and Casamayor 2008). The neuston dweller *Nevskia ramosa*, a deeply branching *Gammaproteobacterium* (Glöckner et al. 1998), has been known for more than 100 years prior to its isolation for its conspicuous morphology and growth pattern: It forms flat rosettes with a bush-like appearance which are colonies of dichotomously branched rod-shaped cells on slime stalks that are slightly bent at the tips (Stürmeyer et al. 1998). Adaptation in this bacterium to the neuston lifestyle included highly effective photorepair mechanisms, which may compensate for its higher exposure to solar UV radiation (Sommaruga 2001).

Although the presence or absence of macrophytes may be decisive for the overall state of a shallow lake ecosystem (Scheffer et al. 1993), little is known about close microbial associations with aquatic plants compared to the interactions between microbes and the phytoplankton. The plant-attached bacterial communities appear to differ from the bacterial communities in the surrounding environments, and differences were also found between the microbial assemblages colonizing leaves and roots of *Valisneria americana* (Crump and Koch 2008). The same study also reported that some epiphytic microbial taxa were always present on aquatic macrophytes from different habitat types (freshwater, brackish, marine), for example, genotypes related to methylotrophic *Betaproteobacteria*, hinting at a core set of possibly mutualistic microbial epibionts on aquatic plants.

In meromictic lakes, a recurrent wind-driven complete vertical turnover of the pelagic zone is impeded by the geomorphology of the surrounding catchment and/or hydrochemical gradients (e.g., salinity), resulting in a permanently stratified water column. A special feature of many meromictic lakes is a stable transition zone between a mixed oxic upper water layer and a subjacent anoxic sulfidic layer, which may be paralleled by a steep gradient of ambient light intensity. This redox transition zone typically harbors a slowly growing but dense and diversified microbial community (Overmann et al. 1999) with a relatively constant and compact vertical stratification of bacterial populations. It provides a favorable environment for the growth of, for example, anoxygenic phototrophic sulfur bacteria that compete for sulfide with chemolithoautotrophic sulfur oxidizers (Casamayor et al. 2008). The chemocline of the meromictic Lake Cadagno in the Swiss Alps featured prominent populations of autotrophic *Chromatiaceae* (Gammaproteobacteria) from the genera *Chromatium*, *Amoebobacter*, and *Lamprocystris* (Tonolla et al. 1999), as well as green non-sulfur bacteria from the *Chloroflexus* group, which are known for photoautotrophic or photoheterotrophic growth (Bosshard et al. 2000). Phototrophic consortia containing two types of bacteria in a regularly structured association are also typical and abundant inhabitants of the oxygen-sulfide interface of freshwaters. In these consortia, a colorless central bacterium, presumably a chemolithotroph, is surrounded by red or green epibionts to which it provides mobility (Overmann et al. 1998).

Many lakes develop an anoxic hypolimnetic layer at least transiently, characterized by higher total prokaryotic densities and/or biomasses and larger sized cells than in the epilimnion (Cole et al. 1993). Archaeal methanogenesis usually dominates anaerobic carbon mineralization in this zone (Canfield et al. 2005). Methane produced in the anoxic hypolimnion may in fact represent a major contribution to the total microbial carbon budget: the majority of total microbial biomass in the epilimnion of an oxygen-stratified humic lake was temporarily found in methane-oxidizing (type I) gammaproteobacteria (Taipale et al. 2011).

Benthic habitats play an important part in lake metabolism and may even dominate the whole-lake production of new biomass in clear water lakes (Ask et al. 2009). Aerobic and anaerobic microbial degradation and transformation of POC and DOC in sediments are central to the carbon cycling in freshwater systems, and the deposition of organic material and bacterial activities in the sediment are often tightly coupled (Sander and Kalff 1993). Depending on the redox state and hydrochemistry, organic matter degradation is performed by vertically stratified, phylogenetically diverse (Wobus et al. 2003) microbial assemblages that comprise a great variety of physiological groups (Schink 1989), including—but not limited to—denitrifying bacteria, methano- and methylotrophs, fermenting and syntrophic bacteria, manganese-, iron-, and sulfate-reducers, and methanogens.

Major Groups of Freshwater Microbes

Over the last 15 years, the analysis of freshwater microbial assemblages by molecular methods has fundamentally changed our view of which bacterial lineages are important in these habitats. First, collections of 16S rRNA genes from lake samples (Hiorns et al. 1997; Methé et al. 1998) and microscopic quantifications using rRNA-targeted fluorescent oligonucleotide probes (Glöckner et al. 1999) revealed fundamental differences in the composition of freshwater and marine bacterioplankton communities, as well as difference between bacteria in freshwaters and soils (Warnecke et al. 2004). Subsequently, a series of progressively detailed meta-analyses of published rRNA genes (Glöckner et al. 2000; Zwart et al. 2002; Newton et al. 2011) have provided a coherent phylogenetic framework of the major microbial taxa in freshwater habitats, culminating in the recent concept of typical freshwater bacterial "tribes" (i.e., species-like clusters of sequence types with a similarity of $\geq$97% and with internal coherence granted by complete linkage clustering (Newton et al. 2011)). While any "guide to [...] freshwater lake bacteria" (Newton et al. 2011) might require periodic revision, a repetition of the thorough work of Newton and coworkers would at this stage reveal little novelty. Therefore, this chapter does not present a comprehensive phylogenetic analysis of all phylogenetic groups of bacteria that occur in

freshwater habitats, but instead puts a focus on the contrasting properties of selected bacterial lineages that are known to be numerically abundant or otherwise of importance for ecological processes in freshwater pelagic environments.

Phototrophic Bacteria

Cyanobacteria are oxygenic phototrophs that are ubiquitous in many freshwater habitats. They are among the best-studied prokaryotes in lakes, likely because they are relatively amenable to cultivation and often exhibit conspicuous morphologies. Moreover, there is considerable public interest in freshwater cyanobacteria since they tend to form pronounced surface blooms in eutrophied shallow waters that are considered a nuisance, negatively affect drinking water production, and even may represent a health risk due to the release of toxic secondary metabolites from decaying cyanobacterial cells (Huisman et al. 2005). Planktonic cyanobacteria have been broadly categorized into four types (Dokulil and Teubner 2000): species (1) that are able to fix N_2, (2) that inhabit the layer of strongest thermal stratification (the metalimnion) and are able to regulate their buoyancy, (3) turbulence specialists present in the epilimnetic layers, including the picocyanobacteria (Callieri 2007), and (4) colony- or aggregate-forming species. In accordance with their outstanding importance for water quality, there are numerous reviews that discuss in detail various aspects of cyanobacterial ecology (Dokulil and Teubner 2000; Huisman et al. 2005; Reynolds 2006; Callieri 2007).

Besides the sulfur and non-sulfur bacteria that inhabit the chemocline of meromictic lakes (see above), aerobic anoxygenic phototrophs (AAPs) containing bacteriochlorophyll *a* also represent an important bacterioplankton component in epilimnetic water layers of temperate freshwaters (Masin et al. 2008). Microscopic analysis revealed a morphologically diverse set of AAPs in various lakes that were particularly abundant in oligotrophic habitats, where they represented between 10% and 80% of the total bacterial biomass. By contrast, AAPs were only a minor fraction of total cells in more eutrophic systems (Masin et al. 2008). Cultured representatives of AAPs from alpine lakes were affiliated with the freshwater alpha IV subgroup (Glöckner et al. 2000) of *Alphaproteobacteria* (Gich and Overmann 2006). *puf* operon sequences—indicative of the anoxygenic phototrophic lifestyle—were found in isolates from oligotrophic Crater Lake that belonged to the beta I subgroup of *Betaproteobacteria* (Page et al. 2004) and that were closely related to genotypes obtained from a high mountain lake (Pernthaler et al. 1998). Recently, single-cell genomic analysis has provided evidence that *Betaproteobacteria* from the beta II subgroup (related to *Polynucleobacter* sp.) may also contain *puf* genes and may in fact be amongst the numerically most important AAPs in freshwaters (Martinez-Garcia et al. 2012).

Finally, bacterial rhodopsins represent another means of utilizing light energy in a photoheterotrophic context that appears to be common in freshwaters, and some of the most abundant freshwater bacteria possess rhodopsin genes (Atamna-Ismaeel et al. 2008; Sharma et al. 2009; Martinez-Garcia et al. 2012).

Ultramicrobacteria

The majority of free-living bacteria in the epilimnetic layers of freshwater lakes are conspicuously small (Cole et al. 1993; Posch et al. 2009). While this has initially been interpreted as a response to starvation, in analogy with the phenotypic transformations of many cultivable bacteria (Morita 1997), it is increasingly realized that a small cell size may in fact be an intrinsic feature of some planktonic freshwater bacteria that is maintained almost independently of their growth state (Hahn 2003; Hahn et al. 2003). The numerically abundant microbes in the pelagic zone of many freshwaters are so-called ultramicrobacteria that rarely exceed cell sizes of 1 μm. Both, the high in situ abundances (Burkert et al. 2003; Hahn et al. 2005; Warnecke et al. 2005; Allgaier and Grossart 2006b; Salcher et al. 2011a) and the cosmopolitan distribution (Hahn 2003; Warnecke et al. 2004; Salcher et al. 2011a) of relatively few phylogenetic lineages of ultramicrobacteria strongly point to their evolutionary success and functional significance at the ecosystem level.

There are at least three distinct monophyletic lineages of quantitatively significant and widely distributed freshwater ultramicrobacteria: Betaproteobacteria of the genus *Polynucleobacter* (Hahn 2003), Gram-positive *Actinobacteria* from the acI lineage (Hiorns et al. 1997; Glöckner et al. 2000; Warnecke et al. 2004), and members of the alphaproteobacterial LD12 clade (Bahr et al. 1996; Zwart et al. 2002; Salcher et al. 2011a). Preliminary evidence suggests that freshwater ultramicrobacteria may possess relatively small genomes, for example, a *Polynucleobacter necessarius* ssp. *asymbioticus* strain had the smallest genome of all so far sequenced strains from the family it is affiliated with (*Burkholderiaceae)* (Hahn et al. 2012). Since bacterial growth in more oligotrophic freshwater habitats may be limited by nutrient concentrations (phosphate), such a minimal genome could be a key for the success of these slowly growing ultramicrobacteria. However, genome streamlining may also result in the loss of physiological versatility, potentially pushing ultramicrobacteria toward microdiversification and specialization (Hahn and Pöckl 2005; Newton et al. 2007; Jezbera et al. 2011; Hahn et al. 2012). Besides cell size, the three lineages of ultramicrobacteria share other important physiological features, such as a free-living lifestyle or a slow response to sudden changes in growth conditions. At the same time, they also possess distinct group-specific traits that might allow for their coexistence in an unstructured planktonic habitat.

acI *Actinobacteria*

Actinobacteria are generally considered to be Gram-positive bacteria with a high mol% G + C content of genomic DNA. Interestingly, this does not seem to apply for the numerically

most important freshwater actinobacterial acI lineage (Warnecke et al. 2004), which, according to a recent metagenomic analysis, are low GC (Ghai et al. 2011a). Members of the acI lineage have first been described in the water column of mountain lakes (Hiorns et al. 1997; Glöckner et al. 2000), but have since then been detected in a variety of freshwater systems ranging from hot springs to floodplains (Zwart et al. 2002; Warnecke et al. 2004). AcI *Actinobacteria* are particularly abundant in the epilimnion of lakes of different trophic state (oligo- to eutrophic, dystrophic), where they frequently outnumber all other prokaryotic groups (Warnecke et al. 2005; Allgaier and Grossart 2006b; Salcher et al. 2010). They may also be present in brackish waters, for example, the coastal Baltic Sea (Holmfeldt et al. 2009), but are never found in soils.

The acI lineage is rather diverse; it has been further split into 3 (Warnecke et al. 2004), 11, and eventually 13 (Newton et al. 2011) separate clades or tribes, some of which exhibited pH-dependent differences in their distribution patterns in Wisconsin lakes (Newton et al. 2007). The three originally defined subgroups of acI *Actinobacteria* can be microscopically distinguished by specific oligonucleotide probes (Warnecke et al. 2005), facilitating detailed studies about their population dynamics (Allgaier and Grossart 2006b) and ecophysiology. While bacteria from these lineages coexisted in the same environment (a meromictic humic lake), they also clearly differed in their vertical distribution patterns (forming maxima in the oxic or anoxic water layers, respectively) and in their uptake preferences for low-molecular-weight substances (Buck et al. 2009). In general, acI *Actinobacteria* are proficient consumers of various labile organic carbon compounds, such as glucose or amino acids (Buck et al. 2009; Salcher et al. 2010), and are capable of de novo synthesis of arginine from glutamate (Philosof et al. 2009). Moreover, they are in fact photoheterotrophs and possess an *Actinobacteria*-specific bacteriorhodopsin variant (Sharma et al. 2009).

One conspicuous feature of acI *Actinobacteria* is their apparent success in the presence of high protist-induced mortality. Bacteria from this lineage are typically underrepresented in the food vacuoles of heterotrophic nanoflagellates (Jezbera et al. 2006), which may be in part due to their minute cell size (Šimek and Chrzanowski 1992). Higher resistance to predation was mediated by the presence of an intact S-layer in closely related, albeit cultivable, freshwater *Actinobacteria* (Hahn et al. 2003; Tarao et al. 2009), suggesting that similar mechanisms might also apply to acI *Actinobacteria*. Members of the acI lineage were rapidly enriched in experimental bacterial assemblages upon addition of a predator that caused high mortality of their competitors (Pernthaler et al. 2001). It is intriguing to set this observation in the context of recent findings about the physiological properties of acI *Actinobacteria*: Some members of this lineage are proficient consumers of *N*-acetyl glucosamine (NAG) and di-NAG, as shown in experimental enrichments (Beier and Bertilsson 2011) and in lake water samples (Eckert et al. 2011). It is conceivable that acI *Actinobacteria* may profit double from predation, that is, from a reduction of competition and from the cell-wall-derived organic matter of bacteria that are consumed by heterotrophic nanoflagellates.

Polynucleobacter necessarius ssp. *Asymbioticus*

To date, the genus *Polynucleobacter* represents the only cultivable group of freshwater ultramicrobacteria. The isolation of first strains from this lineage coincided with first data about its abundances in lake water (Burkert et al. 2003; Hahn 2003). *Polynucleobacter* spp. are probably the best-studied group of environmentally relevant bacteria from freshwater plankton, and a wealth of information about their in situ population dynamics (Hahn et al. 2005; Wu and Hahn 2006a, b; Salcher et al. 2008), ecophysiology (Grossart et al. 2008; Alonso et al. 2009; Buck et al. 2009), biogeography (Jezberova et al. 2010; Jezbera et al. 2011), and even about specific genomic features (Hahn et al. 2012) is available. Probably the most widespread of several species of *Polynucleobacter, P. necessarius*, is divided into a subspecies that lives in symbiosis with a benthic ciliate (*Euplotes*) and a free-living subspecies, *P. necessarius* ssp. *asymbioticus* (Hahn et al. 2009). The latter (frequently termed PnecC) is a phylogenetically narrow group exhibiting >99% of 16S rRNA sequence similarity (Hahn et al. 2005). Nevertheless, PnecC bacteria occur across a wide range of acidic, circumneutral or alkaline freshwater habitat types (Jezberova et al. 2010), including dystrophic ponds, rivers and lakes (Hahn et al. 2005; Grossart et al. 2008; Buck et al. 2009; Ghai et al. 2011b), oligo- to eutrophic lakes (Crump et al. 2003; Wu and Hahn 2006a), and brackish lagoons (Alonso et al. 2009). The highest abundances of these bacteria are typically found in aerobic and humic-rich habitats (Jezberova et al. 2010), but PnecC may, for example, also form prominent populations in sub- to anoxic environments (Salcher et al. 2008, 2011b; Buck et al. 2009). It has been estimated that the total global contribution of *P. necessarius* ssp. *asymbioticus* to freshwater bacterioplankton might be as high as 20% (Jezberova et al. 2010).

Due to its ubiquity and high phylogenetic similarity, PnecC also represents an excellent model to study intra-taxon ecological diversification, that is, a specialization of particular lineages to specific environmental conditions. A survey of 121 habitats using 16S–23S ITS targeted probes to discriminate between 13 PnecC ecotypes revealed pronounced habitat preferences of some of these genotypes that were related to hydrochemical parameters such as pH, conductivity, DOC, and oxygen concentrations (Jezbera et al. 2011). A single genotypic lineage occurring in a shallow dystrophic pond exhibited a highly passive lifestyle, which nevertheless allowed for transient population maxima of almost 50% of the total bacterioplankton assemblage (Hahn et al. 2012). This suggests that genome streamlining and a specialization to particular environmental settings may not only be an adaptation to uniform and constant environmental conditions, but also represents a successful strategy in rather variable habitat types.

Notwithstanding the apparent specialization of *P. necessarius* ssp. *asymbioticus*, there are also indications of some metabolic flexibility upon change of growth conditions, which probably exceeds that of other freshwater ultramicrobacteria. Upon transfer from a transparent humic matter-dominated river into a brackish lagoon, PnecC bacteria exhibited a complex pattern of metabolic up- and downshifts, for example, a decrease in

DNA synthesis in parallel with a stimulation of NAG incorporation (Alonso et al. 2009). Members of this lineage can be readily enriched in dilution cultures or filtrates of humic-rich waters (Burkert et al. 2003; Grossart et al. 2008), and numerous of *P. necessarius* ssp. *asymbioticus* strains have been isolated following prior acclimatization to higher substrate and nutrient levels (Hahn 2003; Hahn et al. 2004).

LD12 *Alphaproteobacteria*

A "freshwater SAR11" lineage, subsequently renamed LD12 (Zwart et al. 2002), was discovered in 1996 in an Arctic lake (Bahr et al. 1996). LD12 is a monophyletic sibling group of a dominant lineage of free-living ultramicrobacteria in marine surface waters (Rappé et al. 2002); it likely originated from rare transition events of these marine SAR11 bacteria into freshwaters (Logares et al. 2010). LD12-related rRNA gene sequences have hitherto only been detected in freshwater habitats (Zwart et al. 2002; Eiler and Bertilsson 2004; Salcher et al. 2011a). To date, there is only limited information about their abundance, seasonality, and metabolic traits. rRNA-based surveys point to their presence in a wide range of systems, and to a key role of pH in controlling their distribution and quantitative significance in lake bacterioplankton communities (Stepanauskas et al. 2003). LD12 bacteria in two prealpine lakes exhibited annually recurring spatiotemporal distribution patterns, with distinct population maxima in the surface layers during the summer months when water temperatures exceeded 15°C (Salcher et al. 2011a). The same study could furthermore establish the small cell size of LD12 bacteria, and provide evidence for their adaptation to oligotrophic conditions, that is, a slow but efficient substrate uptake already at low concentrations. Alphaproteobacterial proteorhodopsin sequences from freshwaters have been assigned to LD12 bacteria based on phylogenomic comparisons (Atamna-Ismaeel et al. 2008); so far this has not been directly confirmed, for example, by single-cell genomic approaches (Martinez-Garcia et al. 2012).

Other Freshwater Ultramicrobacteria

In addition to these lineages, there are other, less studied groups of freshwater ultramicrobacteria. *Actinobacteria* from the cultivable "Luna" lineage (named for their typical selenoid cell shape) are highly resistant to flagellate predation (Hahn et al. 2003; Tarao et al. 2009). Moreover, as discussed above, for PnecC, the "Luna" lineage represents another example of pronounced ecotype diversification: "Luna" strains with identical rRNA gene sequences were highly variable with respect to their optimal growth temperature (Hahn and Pöckl 2005). The betaproteobacterial LD28 (beta IVa) lineage—so far without cultured representatives—is one of several clades of putatively methylotrophic bacteria (*Methylophilaceae*) in the pelagic zone of freshwater habitats that are closely related to the marine OM43 lineage (Rappe et al. 1997; Salcher et al. 2008; Shabarova and Pernthaler 2010). Members of LD28 were shown to be ultramicrobacteria by microscopic analysis; their proportions were highest in the oxygenated hypolimnetic layers of a mesotrophic prealpine lake (Salcher et al. 2011b). Finally, the hypolimnetic zone of deep lakes harbors another lineage of intriguing prokaryotes with minute cell size that have not yet received adequate attention, *Thaumarchaeota* that are phylogenetically closely related to ammonia-oxidizing archaeal lineages from soils and marine waters (Keough et al. 2003; Urbach et al. 2007; Callieri et al. 2009).

Opportunistically Growing Freshwater Bacteria

There are various dichotomous models of pro- and eukaryotic lifestyles. The microflora in soils has been categorized into zymogenous (allochthonous, copiotrophic) and autochthonous (oligotrophic) species, and the two extremes of the r/K continuum are often referred to as opportunistic versus equilibrium strategists (Grime 1979). The existence of these two strategies in freshwaters is suggested by the contrasting temporal persistence patterns of different pelagic microbes, as, for example, observed in a humic lake (Newton et al. 2006). While the freshwater ultramicrobacteria probably represent variants of an equilibrium-like lifestyle, the pelagic zone of lakes, ponds, and rivers also harbors microbes with larger cell sizes that are physiologically and phylogenetically rather diverse and exhibit various traits of the opportunistic growth type. Such bacterial taxa typically only form small and/or transient populations during periods characterized by rapid changes in growth conditions or other forms of disturbance. Representatives of this growth type are more readily cultivable than the ultramicrobacteria (Cousin et al. 2008), as they are often able to colonize solid surfaces and decompose particulate organic matter (e.g., lake snow).

The R-BT065 Clade of *Limnohabitans*

The betI-A clade of *Betaproteobacteria* (Newton et al. 2011) harbours several species of the recently described genus *Limnohabitans* (Hahn et al. 2010) that appears to be virtually omnipresent in freshwaters. A core group within this genus can be discriminated microscopically and has consequently received particular attention: the R-BT065 clade, a monophyletic lineage consisting of two subclades that together contain most of the available 16S rRNA gene sequences affiliated with *Limnohabitans* (Šimek et al. 2001; Newton et al. 2011). R-BT065 bacteria are found in habitats with strikingly contrasting properties, such as UV-exposed ultraoligotrophic high mountain lakes, dystrophic lakes and rivers, turbid brackish lagoons, or eutrophic reservoirs (Warnecke et al. 2005; Salcher et al. 2008, 2011b; Alonso et al. 2009; Šimek et al. 2010a), with a tendency to form smaller population sizes in more humic-rich systems (Šimek et al. 2010a).

Members of the R-BT065 lineage are opportunistic in that they can readily outgrow other bacterial lineages in predator-free filtrates incubated at in situ substrate conditions

(Šimek et al. 2006). They rapidly incorporate various low-molecular-weight model DOC substrates (Horňák et al. 2008; Alonso et al. 2009; Buck et al. 2009), and often the vast majority of cells from this lineage are metabolically active (Horňák et al. 2008; Salcher et al. 2008). R-BT065 bacteria appear to profit from phytoplankton exudates; they formed distinct maxima in parallel with a spring phytoplankton bloom in a temperate lake (Eckert et al. 2011), and they were typical "satellite bacteria" in cultures of eukaryotic algae such as cryptophytes and chlorophytes (Šimek et al. 2011). Moreover, the growth of both, natural *Limnohabitans* populations and selected pure cultures was favored when a cyanobacterial primary producer was maintained at suboptimal light conditions (Horňák et al. 2012). In general, R-BT065 bacteria appear to be substantially more vulnerable to predation by bacterivorous protists than, for example, acI *Actinobacteria*, as deduced from field experiments (Šimek et al. 2001) and from the analysis of protistan food vacuole content (Jezbera et al. 2006). However, while R-BT065 bacteria initially were found to be disproportionally affected by protistan predation after food web manipulation (Šimek et al. 2001), they seemed to be capable of compensating mortality by higher growth in other studies (Šimek et al. 2005, 2006). One explanation for such contradictory observations might lie in specific adaptation at the level of ecotypes that could not be discriminated in the above studies. This is, for example, suggested by the growth patterns of closely related sympatric *Limnohabitans* strains at various regimes of competition, predation, and viral mortality (Šimek et al. 2010b): While one of the studied strains was significantly more vulnerable to viruses obtained from its native habitat, it also exhibited enhanced resistance to protistan grazing.

Recently, it has been reported that *Limnohabitans* spp. from the R-BT065 lineage are also a major component of the gut flora of *Daphnia* (Freese and Schink 2011). This finding adds another intriguing ecological dimension to the role of *Limnohabitans* as an opportunistic member of freshwater bacterioplankton.

Flavobacteriaceae

The *Flavobacteriaceae* are known as members of the planktonic microbial assemblages of lakes, streams, and rivers (Riemann and Winding 2001; Eiler and Bertilsson 2007; Winter et al. 2007; Zeder et al. 2009; Besemer et al. 2012), as inhabitants of river and stream biofilms (O'Sullivan et al. 2002; Besemer et al. 2012), and they are also found on suspended organic aggregates in lakes and rivers (Böckelmann et al. 2000; Allgaier and Grossart 2006a; Lemarchand et al. 2006). Some genotypes may concomitantly feature large attached and suspended subpopulations, whereas others appear to favor a predominantly attached or free-living lifestyle (Riemann and Winding 2001). Many *Flavobacteriaceae* from freshwater habitats are cultivable (Cousin et al. 2008; Zeder et al. 2009), which might be regarded as another indication for a predominantly opportunistic growth strategy.

While there is ample qualitative information about the occurrence and diversity of *Flavobacteriaceae* in different habitats and at various environmental conditions, as deduced from isolation, from collections of rRNA genes, and from molecular fingerprinting (van Hannen et al. 1999; Riemann and Winding 2001; Zwart et al. 2002; Allgaier and Grossart 2006a; Lemarchand et al. 2006; Winter et al. 2007; Cousin et al. 2008; Besemer et al. 2012), comparatively little is known about their population dynamics. One reason may be the high phylogenetic diversity with this lineage: any particular lake will likely only harbor a subset of the numerous flavobacterial clades (Eiler and Bertilsson 2007). Moreover, if studied at a higher taxonomic resolution, individual flavobacterial population tend to form only small and transient populations (Eiler and Bertilsson 2007; Zeder et al. 2009). Transient peaks of *Flavobacteria* of up to 30% of the total bacterioplankton 16S rRNA gene numbers were observed in four eutrophic Scandinavian lakes (Eiler and Bertilsson 2007). While these maxima coincided with periods of high microbial heterotrophic activity, there was only a weak relationship to primary producers. However, *Flavobacteria* have also been quantitatively associated with phytoplankton blooms: Members of a cultivable flavobacterial lineage formed a small but highly active population during a spring phytoplankton bloom in a prealpine lake, and the growth of these bacteria was disproportionally stimulated in 0.2 μm prefiltered lake water at maximal ambient chlorophyll a concentrations (Zeder et al. 2009). This agrees with the observation that microcosms amended with carbon, nitrogen, and phosphorus induced blooms of bacteria affiliated with *F. aquatile* only during the spring season (Newton and McMahon 2011). Ecophysiological information about freshwater *Flavobacteriaceae* is generally scarce. Bacteria from this lineage do not seem to incorporate low-molecular-weight organic compounds such as amino acids (Salcher et al. 2010). Recent experiments suggest that some *Flavobacteria* are involved in the hydrolysis and solubilization of chitin (Beier and Bertilsson 2011), and rapid incorporation of NAG by these bacteria was observed at the onset of a spring phytoplankton bloom (Eckert et al. 2011). Moreover, there is indirect (phylogenomically induced) evidence that some *Flavobacteriaceae* might profit from light by means of proteorhodopsins (Atamna-Ismaeel et al. 2008).

Finally, from a biogeographic vewpoint, it is intriguing to speculate if the large populations of freshwater *Flavobacteria* that are transported by large rivers into estuaries and marine habitats (Winter et al. 2007) might in fact serve as continuous inocula for the evolution of these bacteria in coastal waters, as is suggested by several lineages of *Flavobacteriaceae* that harbor numerous closely related genotypes from freshwater, brackish, and marine habitats (Alonso et al. 2007).

Other Putatively Opportunistic Bacteria

The above discussed groups are but two comparatively well-studied examples of opportunistic microbial growth types that occur in freshwater habitats. Likely, there are numerous other lineages of such "tychoplanktic" bacteria that more or less regularly invade the pelagic environments, as suggested by the high

diversity of particle-associated microbes (Allgaier and Grossart 2006a; Lemarchand et al. 2006). Lake snow aggregates host large populations of bacteria affiliated with *Alphaproteobacteria, such as Sphingomonas* or *Brevundimonas*, or with various *betaproteobacterial* lineages known from wastewater treatment systems (Riemann and Winding 2001; Schweitzer et al. 2001; Allgaier and Grossart 2006a), and all these groups are also known as "typical lake bacteria" (Zwart et al. 2002; Newton et al. 2011). Short-lived blooms of *Bacteroidetes* other than *Flavobacteriaceae* have also been observed in lake water, for example, of *Cyclobacteriaceae* (Eckert et al. 2011). Shallow aquatic habitats are often intrinsically less stable with respect to hydrology and other physicochemical parameters and may thus specifically favor ecotypes that can rapidly adapt to changing growth conditions: extreme blooms of single bacterial species (transiently forming >90% of total microbial abundances) were observed in a shallow wind-exposed subtropical coastal lagoon (Piccini et al. 2006). It is conceivable that many bacteria from terrestrial habitats that are sporadically introduced into the aquatic environment (e.g., during flooding events) may also grow opportunistically: The addition of sterile water to dried river sediments resulted in the rapid dominance of the planktonic environment by various genotypes of *Bacilli* that were equally rapidly eliminated by the subsequent rise of protistan predators (Fazi et al. 2008).

Filamentous Bacteria

Bacterioplankton communities in lakes and ponds temporarily harbor substantial numbers of protist-inedible filamentous bacterial morphotypes from various phylogenetic lineages (Jürgens and Stolpe 1995; Jürgens et al. 1999; Schauer and Hahn 2005; Eckert et al. 2011) (◉ *Fig. 6.1*), whereas such thread-like bacteria are typically absent in coastal or offshore marine habitats. This difference cannot solely be explained by the contrasting availability of organic carbon or nutrients in the two biomes, since the habitat range of filamentous freshwater bacteria encompasses hypertrophic ponds (Sommaruga and Psenner 1995), meso- to eutrophic systems (Jürgens and Stolpe 1995; Pernthaler et al. 2004), but also ultraoligotrophic high mountain lakes (Pernthaler et al. 1998). Filamentous bacteria rapidly grow to high biomasses after the rise of bacterivorous nanoflagellates, for example, during the decline of the spring phytoplankton bloom in temperate lakes (Pernthaler et al. 2004; Eckert et al. 2011) or as a consequence of experimental food web manipulation (Jürgens et al. 1999; Šimek et al. 2001). However, while they appear largely immune against protistan foraging (an exception to this rule is presented in (Wu et al. 2004)), and in some instances also against viral attacks (Šimek et al. 2007), these bacteria are highly susceptible to the grazing of filter-feeding cladocerans (in particular *Daphnia* sp.) (Jürgens et al. 1994; Pernthaler et al. 2004). This vulnerability, together with the concomitant elimination of bacterivorous protists by metazooplankton, typically results in a rapid decline of filamentous morphotypes at the onset of the "clear water phase" in lakes (Jürgens and Stolpe 1995). Thus, from a carbon cycle perspective, the filamentous bacteria might be regarded as a freshwater-specific shortcut for the transfer of DOC to the metazooplankton without the energy-consuming channeling through at least one additional trophic level. The SOL clade of *Saprospiraceae* ("Soletti" being an Austrian brand of pretzels with close morphological resemblance to the SOL bacteria) is a particularly common and well-studied lineage of thread-like bacteria in freshwater plankton (Schauer and Hahn 2005). It consists of three distinct subclades that exhibit ecological differentiation with respect to habitat preference (Schauer et al. 2005). Two of the subclades (HAL, LD2) also had contrasting seasonal growth patterns in a mesotrophic lake (Schauer et al. 2006). A pronounced bloom of filaments from the LD2 subclade (up to 40% of total bacterioplankton biomass) was observed during a period of high protistan grazing, and it was terminated by the rise of cladoceran zooplankton (Pernthaler et al. 2004).

Factors Shaping Microbial Communities of Freshwater Environments

Various biotic interactions, such as predation, competition, symbiosis, or parasitism, are likely to strongly affect the community composition and activity of freshwater microbial assemblages. Judging from the conspicuously high abundances and biomasses of grazing-resistant bacteria (i.e., of Gram-positive *Actinobacteria*) or from the ubiquity of specific anti-predator adaptations, many of which are, moreover, exclusive to the freshwater biome, one might conclude that defense against predation-induced mortality is of particular importance for microbes in lacustrine habitats (Hahn et al. 2000; Salcher et al. 2005; Corno and Jurgens 2006; Jezberova and Komarkova 2007; Blom et al. 2010). At the same time, the pronounced fluctuations of numerous short-lived microbial populations in any particular habitat at comparatively constant total prokaryotic cell numbers (Pernthaler and Posch 2009) invite a variety of alternative explanations such as fierce interspecific competition enforced by an overall carrying capacity for microbial growth, or a "killing-the-winner" scenario caused by viral lysis (Weinbauer and Höfle 1998; Thingstad 2000). Moreover, heterotrophic prokaryotes in the pelagic zone of freshwaters are in an uneasy relationship with eukaryotic primary producers: On the one hand, bacteria are often superior competitors for growth-limiting nutrients, whereas on the other hand they depend on the phytoplankton as a source of organic carbon (Currie and Kalff 1984; Mindl et al. 2005).

Apart from such biotic factors, there are typical system properties of inland waters that, if taken together, set a rather unique stage for microbial existence, and that, therefore, may help to explain why freshwater microbial assemblages seem to be more than a mere extension of the terrestrial microbiota. These factors can be roughly divided into aspects of variability and disturbance, aspects that link aquatic and terrestrial biomes, and aspects that are related to the discontinuous nature of freshwater habitats.

Internal Habitat Variability

Freshwaters fundamentally differ from open ocean habitats in that they typically exhibit substantially higher internal spatiotemporal variability. Some of this variability is predictable and recurring; for example, temperature, and seasonality is certainly among the strongest drivers of microbial successions in all but tropical surface waters, groundwater, or extreme environments, for example, hot springs (Ferris and Ward 1997; Griebler and Lueders 2009). Seasonality is observed for autotrophic prokaryotic taxa, such as the cyanobacterial or anoxygenic autotrophic picoplankton (Callieri 2007; Masin et al. 2008), but also for various heterotrophic bacteria that thrive in the epilimnion of lakes and in rivers such as *Polynucleobacter*, LD12, acI *Actinobacteria*, or filamentous *Saprospiraceae* (Allgaier and Grossart 2006b; Schauer et al. 2006; Wu and Hahn 2006b; Salcher et al. 2011a). Seasonality can also be observed at the level of whole microbial communities, driven, for example, by temperature, water column mixing, or by change of the dominant substrate source (Crump et al. 2003; Shade et al. 2007). However, there are also reports that the community-level seasonal development of bacterioplankton assemblages in lakes might not be identical in consecutive years, especially during periods of stable thermal stratification (Boucher et al. 2006; Shade et al. 2007). This is not surprising, as numerous microbial taxa exhibit somewhat stochastic occurrence and/or persistence patterns that seem to be more related to intrinsic factors, such as the quantitative and qualitative annual development of primary producers or the microbial food web, than to temperature (Kent et al. 2004; Newton et al. 2006). Seasonality nevertheless unites freshwater systems within a regional climatic context, for example, it synchronizes the development of microbial communities in neighboring rivers (Crump and Hobbie 2005). This synchronicity may in fact only be an indirect response of bacterioplankton assemblages to extrinsic (climatic) factors, mediated via the action of these factors on the development of the phytoplankton communities (Kent et al. 2007).

Besides seasonality, freshwater microbial assemblages are exposed to numerous other, less predictable sources of variability, such as the physicochemical transition from the pelagic to the littoral zone, point sources of DOC and nutrient input (Cébron et al. 2004; Winter et al. 2007), or extreme storm events (Jones et al. 2008). In fact, seasonality may actually modulate the response of microbial assemblages to other types of disturbances (Newton and McMahon 2011). Larger lakes, and particularly reservoirs, also feature pronounced horizontal variation of both microbial productivity and community composition that can be related to gradients of phytoplankton productivity and microbial food web structure (Šimek et al. 2005; Horňák et al. 2006, 2008; Salcher et al. 2011b). Significant horizontal variability of microbial community composition may already be found at a horizontal scale of 100 m (Yannarell and Triplett 2004).

Interactions Between Aquatic Environments and the Catchment

Freshwater habitats are tightly embedded in catchment-related processes. A considerable fraction of the newly produced bacterial biomass in the pelagic and benthic zones of lakes is based on allochthonous organic carbon (Pace et al. 2004; Ask et al. 2009). Traditionally, such terrestrial input into freshwaters has been considered more chemically recalcitrant and thus less available for bacterial consumption (Benner 2003). However, recent evidence suggests that large amounts of readily consumable low-molecular-weight substrates may be released from the catchment into lakes, thereby substantially supporting bacterial growth (Berggren et al. 2012). Moreover, DOC of terrestrial origin is known to become increasingly bioavailable due to photochemical degradation (Bertilsson and Tranvik 1998). Freshwater microbes can thus exploit substrates that originate from different ecosystem processes, and their community composition is visibly affected by the dominant source of organic matter (algal-derived vs. terrestrial) (Crump et al. 2003), or the contribution of either source to total DOM (Jones et al. 2009). For example, pelagic *Actinobacteria* were selectively favored by the addition of allochthonous DOC or humic material, whereas the growth of *Betaproteobacteria* (e.g., *Limnohabitans* spp.) was promoted by algal-derived DOC (Haukka et al. 2005; Perez and Sommaruga 2006; Šimek et al. 2011).

In addition to this biogeochemical relationship, freshwaters are also continuously inoculated with microbial communities that originate from the catchment and thus in parts from terrestrial runoff. It has been suggested that bacterial community composition will be mainly controlled by surface inflow in boreal lakes with water retention times <200 days, because the import rates of new bacteria into such systems will grossly exceed the potential maximal growth rates of autochthonous strains (Lindström et al. 2006). Similar conclusions about the effects of hydraulic retention times on bacterioplankton diversity were reached for Himalayan high mountain lakes (Sommaruga and Casamayor 2009). A predominantly terrestrial origin was also proposed for particle-attached bacteria in a human-impacted coastal lagoon with low water residence time (LaMontagne and Holden 2003). The similarity between microbial assemblages in arctic lakes and streams was found to decrease with the degree of landscape separation, indicating that "terrestrial inputs influence lake bacterial communities not only by modifying the chemical conditions of lakes, but also by introducing organisms that thrive under those conditions" (Crump et al. 2007).

Freshwaters as Discontinuous Habitats

While aquatic habitats are undoubtedly linked with the surrounding watershed in terms of terrestrial runoff and microbial dispersal, there are also powerful intrinsic factors that select for the success of particular microbial species ("species sorting").

Contrasting physicochemical and/or biotic characteristics may create strikingly different growth scenarios for pelagic microbes even in neighboring aquatic habitats, as shown, for example, for shallow lakes distinguished by alternative stable states (turbid vs. clear) (Scheffer et al. 1993) and different nutrient loadings (Van der Gucht et al. 2005, 2007). Various other intrinsic parameters have also been found to affect local bacterial community structure in synoptically studied sets of freshwater habitats, for example, lake trophy (primary productivity) (Lindström 2000; Kolmonen et al. 2011), pH (Stepanauskas et al. 2003; Schauer et al. 2005), DOC composition (Jones et al. 2009), primary producers (Kent et al. 2007), UV transparency (Warnecke et al. 2005), or trophic cascades within food webs (Muylaert et al. 2002). Moreover, only a minimal influence on the autochthonous microbial assemblages of lakes was observed by bacterial species that were introduced via atmospheric transport (Jones and McMahon 2009), suggesting the importance of local factors for a successful establishment of dispersing microbes. As a consequence, lake bacteria may show clear biogeographic distribution patterns along regional environmental gradients if analyzed at appropriate phylogenetic resolution (as outlined earlier for ecotypes of *P. necessarius* ssp. *asymbioticus*). For a concise and balanced presentation of the current debate about the respective roles of local and regional factors on microbial community composition, a recent review by Lindström and Langenheder is recommended (Lindström and Langenheder 2012).

Outlook

The study of freshwater prokaryotes has developed into a creative and prolific scientific discipline that is by and large able to answer important questions about which microbes thrive in which habitats, and that increasingly proceeds to addressing the more complex issue of how these microbes actually function, evolve, and interact under natural settings. In some aspects, freshwater microbiologists have even been at the forefront of the methodological revolution that has transformed environmental microbiology over the last two decades. Nevertheless, it is conceivable that future research on freshwater microbial assemblages might profit to a larger extent by interactions with other microbiological and ecological disciplines than by novel technologies only. For example, cultured representatives of freshwater bacteria that are known to be of environmental relevance may well represent future model organisms for studies in biochemistry, experimental evolution, or theoretical ecology. Novel sensing technology for the continuous or spatially resolved monitoring of freshwater habitats might fruitfully interact with equally high-throughput approaches to assess changes in DOC composition, microbial diversity, population dynamics, or activities. Last but not least, microbiologists studying freshwater systems might be increasingly pressed to contribute to the understanding of how to manage these precious resources for future generations.

Acknowledgments

My thanks go to my wife and little daughter for tolerating that I finished this chapter during our holidays.

References

Alfreider A, Schirmer M, Vogt C (2012) Diversity and expression of different forms of RubisCO genes in polluted groundwater under different redox conditions. FEMS Microbiol Ecol 79:649–660

Allgaier M, Grossart HP (2006a) Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. Aquat Microb Ecol 45:115–128

Allgaier M, Grossart HP (2006b) Diversity and seasonal dynamics of Actinobacteria populations in four lakes in northeastern Germany. Appl Environ Microbiol 72:3489–3497

Alonso C, Warnecke F, Amann R, Pernthaler J (2007) High local and global diversity of Flavobacteria in marine plankton. Environ Microbiol 9:1253–1266

Alonso C, Zeder M, Piccini C, Conde D, Pernthaler J (2009) Ecophysiological differences of betaproteobacterial populations in two hydrochemically distinct compartments of a subtropical lagoon. Environ Microbiol 11:867–876

Ask J, Karlsson J, Persson L, Ask P, Bystrom P, Jansson M (2009) Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. Ecology 90:1923–1932

Atamna-Ismaeel N, Sabehi G, Sharon I, Witzel KP, Labrenz M, Jurgens K et al (2008) Widespread distribution of proteorhodopsins in freshwater and brackish ecosystems. ISME J 2:656–662

Auguet JC, Casamayor EO (2008) A hotspot for cold crenarchaeota in the neuston of high mountain lakes. Environ Microbiol 10:1080–1086

Bahr M, Hobbie JE, Sogin ML (1996) Bacterial diversity in an arctic lake: a freshwater SAR11 cluster. Aquat Microb Ecol 11:271–277

Battin TJ, Wille A, Sattler B, Psenner R (2001) Phylogenetic and functional heterogeneity of sediment biofilms along environmental gradients in a glacial stream. Appl Environ Microbiol 67:799–807

Battin TJ, Sloan WT, Kjelleberg S, Daims H, Head IM, Curtis TP, Eberl L (2007) Microbial landscapes: new paths to biofilm research. Nat Rev Microbiol 5:76–81

Battin TJ, Luyssaert S, Kaplan LA, Aufdenkampe AK, Richter A, Tranvik LJ (2009) The boundless carbon cycle. Nat Geosci 2:598–600

Beier S, Bertilsson S (2011) Uncoupling of chitinase activity and uptake of hydrolysis products in freshwater bacterioplankton. Limnol Oceanogr 56:1179–1188

Benner R (2003) Molecular indicators of bioavailability of dissolved organic matter. In: Findlay SEG, Sinsabaugh RL (eds) Aquatic ecosystems: interactivity of dissolved organic matter. Academic, San Diego, pp 121–137

Berggren M, Laudon H, Haei M, Strom L, Jansson M (2010) Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. ISME J 4:408–416

Berggren M, Lapierre J-F, del Giorgio PA (2012) Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. ISME J 6:984–993

Bertilsson S, Tranvik LJ (1998) Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. Limnol Oceanogr 43:885–895

Besemer K, Peter H, Logue JB, Langenheder S, Lindström ES, Tranvik LJ, Battin TJ (2012) Unraveling assembly of stream biofilm communities. ISME J 6:1–10

Blom JF, Horňák K, Šimek K, Pernthaler J (2010) Aggregate formation in a freshwater bacterial strain induced by growth state and conspecific chemical cues. Environ Microbiol 12:2486–2495

Böckelmann U, Manz W, Neu TR, Szewzyk U (2000) Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe river of Germany by cultivation and molecular methods. FEMS Microbiol Ecol 33:157–170

Bosshard PP, Santini Y, Gruyter D, Stettler R, Bachofen R (2000) Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. FEMS Microbiol Ecol 31(2):173–182

Boucher D, Jardillier L, Debroas D (2006) Succession of bacterial community composition over two consecutive years in two aquatic systems: a natural lake and a lake-reservoir. FEMS Microbiol Ecol 55:79–97

Brock ML, Brock TD (1968) The application of micro-autoradiographic techniques to ecological studies. Mitt Int Verein Limnol 15:1–29

Bruns A, Nübel U, Cypionka H, Overmann J (2003) Effect of signal compounds and incubation conditions on the culturability of freshwater bacterioplankton. Appl Environ Microbiol 69:1980–1989

Buck U, Grossart HP, Amann R, Pernthaler J (2009) Substrate incorporation patterns of bacterioplankton populations in stratified and mixed waters of a humic lake. Environ Microbiol 11:1854–1865

Burkert U, Warnecke F, Babenzien D, Zwirnmann E, Pernthaler J (2003) Members of a readily enriched beta-proteobacterial clade are common in surface waters of a humic lake. Appl Environ Microbiol 69:6550–6559

Callieri C (2007) Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. Freshwater Rev 1:1–28

Callieri C, Corno G, Caravati E, Rasconi S, Contesini M, Bertoni R (2009) Bacteria, Archaea, and Crenarchaeota in the epilimnion and hypolimnion of a deep holo-oligomictic lake. Appl Environ Microbiol 75:7298–7300

Canfield DE, Kristensen E, Thamdrup B (eds) (2005) Aquatic geomicrobiology. Elsevier/Academic Press, San Diego

Casamayor EO, Garcia-Cantizano J, Pedros-Alio C (2008) Carbon dioxide fixation in the dark by photosynthetic bacteria in sulfide-rich stratified lakes with oxic-anoxic interfaces. Limnol Oceanogr 53:1193–1203

Cébron A, Coci M, Garnier J, Laanbroek HJ (2004) Denaturing gradient gel electrophoretic analysis of ammonia-oxidizing bacterial community structure in the lower Seine River: impact of Paris wastewater effluents. Appl Environ Microbiol 70:6726–6737

Chaffron S, Rehrauer H, Pernthaler J, von Mering C (2010) A global network of coexisting microbes from environmental and whole-genome sequence data. Genome Res 20:947–959

Chapelle FH, O'Neill K, Bradley PM, Methe BA, Ciufo SA, Knobel LL, Lovley DR (2002) A hydrogen-based subsurface microbial community dominated by methanogens. Nature 415:312–315

Cole JJ, Pace ML, Caraco NF, Steinhart GS (1993) Bacterial biomass and cell-size distributions in lakes—more and larger cells in anoxic waters. Limnol Oceanogr 38:1627–1632

Corno G, Jurgens K (2006) Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. Appl Environ Microbiol 72:78–86

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilm. Annu Rev Microbiol 49:711–745

Cotner JB, Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5:105–121

Cousin S, Brambilla E, Yang J, Stackebrandt E (2008) Culturable aerobic bacteria from the upstream region of a karst water rivulet. Int Microbiol 11:91–100

Crump BC, Hobbie JE (2005) Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnol Oceanogr 50:1718–1729

Crump BC, Koch EW (2008) Attached bacterial populations shared by four species of aquatic angiosperms. Appl Environ Microbiol 74:5948–5957

Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69:2253–2268

Crump BC, Adams HE, Hobbie JE, Kling GW (2007) Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. Ecology 88:1365–1378

Currie DJ, Kalff J (1984) Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microb Ecol 10:205–216

Dokulil MT, Teubner K (2000) Cyanobacterial dominance in lakes. Hydrobiologia 438:1–12

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegl RG et al (2006) The global abundance and size distribution of lakes, ponds, and impoundments. Limnol Oceanogr 51:2388–2397

Dunbar C (1908) Principles of sewage treatment. C. Griffin, London

Eckert EM, Salcher MM, Posch T, Eugster B, Pernthaler J (2011) Rapid successions affect microbial *N*-acetyl-glucosamine uptake patterns during a lacustrine spring phytoplankton bloom. Environ Microbiol. doi:10.1111/j.1462-2920.2011.02639.x, online early

Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6:1228–1243

Eiler A, Bertilsson S (2007) Flavobacteria blooms in four eutrophic lakes: linking population dynamics of freshwater bacterioplankton to resource availability. Appl Environ Microbiol 73:3511–3518

Eiler A, Heinrich F, Bertilsson S (2012) Coherent dynamics and association networks among lake bacterioplankton taxa. ISME J 6:330–342

Farnleitner AH, Wilhartitz I, Ryzinska G, Kirschner AKT, Stadler H, Burtscher MM et al (2005) Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities. Environ Microbiol 7:1248–1259

Fazi S, Amalfitano S, Piccini C, Zoppini A, Puddu A, Pernthaler J (2008) Colonization of overlaying water by bacteria from dry river sediments. Environ Microbiol 10:2760–2772

Ferris MJ, Ward DM (1997) Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl Environ Microbiol 63:1375–1381

Fred EB, Wilson FC, Davenport A (1924) The distribution and significance of bacteria in Lake Mendota. Ecology 5:322–339

Freese HM, Schink B (2011) Composition and Stability of the Microbial Community inside the Digestive Tract of the Aquatic Crustacean Daphnia magna. Microb Ecol 62:882–894

Gaston KJ, May RM (1992) Taxonomy of Taxonomists. Nature 356:281–282

Ghai R, McMahon KD, Rodriguez-Valera F (2011a) Breaking a paradigm: cosmopolitan and abundant freshwater actinobacteria are low GC. Environ Microbiol Rep 3. doi:10.1111/j.1758-2229.2011.00274.x

Ghai R, Rodriguez-Valera F, McMahon KD, Toyama D, Rinke R, de Oliveira TCS et al (2011b) Metagenomics of the Water Column in the Pristine Upper Course of the Amazon River. PLoS One 6:e23785

Gich F, Overmann J (2006) *Sandarakinorhabdus limnophila* gen. nov., sp nov., a novel bacteriochlorophyll a-containing, obligately aerobic bacterium isolated from freshwater lakes. Int J Syst Evol Microbiol 56:847–854

Glöckner FO, Babenzien H-D, Amann R (1998) Phylogeny and identification in situ of Nevskia ramosa. Appl Environ Microbiol 64:1895–1901

Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl Environ Microbiol 65:3721–3726

Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. Appl Environ Microbiol 66:5053–5065

Griebler C, Lueders T (2009) Microbial biodiversity in groundwater ecosystems. Freshwat Biol 54:649–677

Grime J (1979) Evidence for the existance of three primary strategies in plants and its releveance to ecological and evolutionary theory. Am Nat 111:1169–1194

Grossart HP, Jezbera J, Horňák K, Hutalle KML, Buck U, Šimek K (2008) Top-down and bottom-up induced shifts in bacterial abundance, production and community composition in an experimentally divided humic lake. Environ Microbiol 10:635–652

Grossart HP, Frindte K, Dziallas C, Eckert W, Tang KW (2011) Microbial methane production in oxygenated water column of an oligotrophic lake. Proc Natl Acad Sci USA 108:19657–19661

Gutknecht JLM, Goodman RM, Balser TC (2006) Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant Soil 289:17–34

Hahn MG (2003) Isolation of strains belonging to the cosmopolitan *Polynucleobacter necessarius* cluster from freshwater habitats located in three climatic zones. Appl Environ Microbiol 69:5248–5254

Hahn M, Pöckl M (2005) Ecotypes of planktonic Actinobacteria with identical 16S rRNA genes adapted to thermal niches in temperate, subtropical, and tropical freshwater habitats. Appl Environ Microbiol 71:766–773

Hahn MW, Moore ERB, Hofle MG (2000) Role of microcolony formation in the protistan grazing defense of the aquatic bacterium Pseudomonas sp MWH1. Microb Ecol 39:175–185

Hahn MW, Lunsdorf H, Wu QL, Schauer M, Hofle MG, Boenigk J, Stadler P (2003) Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. Appl Environ Microbiol 69:1442–1451

Hahn MW, Stadler P, Wu QL, Pockl M (2004) The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. J Microbiol Methods 57:379–390

Hahn MW, Pockl M, Wu QLL (2005) Low intraspecific diversity in a Polynucleobacter subcluster population numerically dominating bacterioplankton of a freshwater pond. Appl Environ Microbiol 71:4539–4547

Hahn MW, Lang E, Brandt U, Wu QL, Scheuerl T (2009) Emended description of the genus *Polynucleobacter* and the species *P. necessarius* and proposal of two subspecies, *P. necessarius* subspecies *necessarius* subsp. nov. and *P. necessarius* subsp. *asymbioticus* subsp. nov. Int J Syst Evol Microbiol 59:2002–2009

Hahn MW, Kasalicky V, Jezbera J, Brandt U, Jezberova J, Šimek K (2010) *Limnohabitans curvus* gen. nov., sp nov., a planktonic bacterium isolated from a freshwater lake. Int J Syst Evol Microbiol 60:1358–1365

Hahn MW, Scheuerl T, Jezberová J, Koll U, Jezbera J, Šimek K et al (2012) The passive yet successful way of planktonic life: genomic and experimental analysis of the ecology of a free-living Polynucleobacter population. PLos one 7:e32772

Hamlin C (1990) A science of impurity: water analysis in nineteenth century Britain. University of California Press, Berkeley

Haukka K, Heikkinen E, Kairesalo T, Karjalainen H, Sivonen K (2005) Effect of humic material on the bacterioplankton community composition in boreal lakes and mesocosms. Environ Microbiol 7:620–630

Henrici AT (1933) Studies of freshwater bacteria I: a direct microscopic technique. J Bacteriol 25:277–287

Hiorns WD, Methe BA, Nierzwicki-Bauer SA, Zehr JP (1997) Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl Environ Microbiol 63:2957–2960

Hoekstra AY, Mekonnen MM (2012) The water footprint of humanity. Proc Natl Acad Sci USA 109:3232–3237

Holmfeldt K, Dziallas C, Titelman J, Pohlmann K, Grossart HP, Riemann L (2009) Diversity and abundance of freshwater Actinobacteria along environmental gradients in the brackish northern Baltic Sea. Environ Microbiol 11:2042–2054

Horňák K, Jezbera J, Nedoma J, Gasol JM, Šimek K (2006) Effects of resource availability and bacterivory on leucine incorporation in different groups of freshwater bacterioplankton, assessed using microautoradiography. Aquat Microb Ecol 45:277–289

Horňák K, Jezbera J, Šimek K (2008) Effects of a Microcystis aeruginosa bloom and bacterivory on bacterial abundance and activity in a eutrophic reservoir. Aquat Microb Ecol 52:107–117

Horňák K, Zeder M, Blom JF, Posch T, Pernthaler J (2012) Suboptimal light conditions negatively affect the heterotrophy of *Planktothrix rubescens* but are beneficial for accompanying *Limnohabitans* spp. Environ Microbiol 14:765

Hörtnagl P, Perez MT, Sommaruga R (2010) Living at the border: a community and single-cell assessment of lake bacterioneuston activity. Limnol Oceanogr 55:1134–1144

Huisman J, Matthijs CP, Visser PM (2005) Harmful cyanobacteria. Springer, Dordrecht

Hullar MAJ, Kaplan LA, Stahl DA (2006) Recurring seasonal dynamics of microbial communities in stream habitats. Appl Environ Microbiol 72:713–722

Jannasch HW (1958) Studies on planktonic bacteria by means of a direct membrane filter method. J Gen Microbiol 18:609–620

Jezbera J, Horňák K, Šimek K (2006) Prey selectivity of bacterivorous protists in different size fractions of reservoir water amended with nutrients. Environ Microbiol 8:1330–1339

Jezbera J, Jezberova J, Brandt U, Hahn MW (2011) Ubiquity of Polynucleobacter necessarius subspecies asymbioticus results from ecological diversification. Environ Microbiol 13:922–931

Jezberova J, Komarkova J (2007) Morphological transformation in a freshwater Cyanobium sp induced by grazers. Environ Microbiol 9:1858–1862

Jezberova J, Jezbera J, Brandt U, Lindström ES, Langenheder S, Hahn MW (2010) Ubiquity of Polynucleobacter necessarius ssp asymbioticus in lentic freshwater habitats of a heterogenous 2000 km2 area. Environ Microbiol 12:658–669

Jones JG (1974) Some Observations on Direct Counts of Freshwater Bacteria Obtained with a Fluorescence Microscope. Limnol Oceanogr 19:540–543

Jones SE, McMahon KD (2009) Species-sorting may explain an apparent minimal effect of immigration on freshwater bacterial community dynamics. Environ Microbiol 11:905–913

Jones SE, Chiu CY, Kratz TK, Wu JT, Shade A, McMahon KD (2008) Typhoons initiate predictable change in aquatic bacterial communities. Limnol Oceanogr 53:1319–1326

Jones SE, Newton RJ, McMahon KD (2009) Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environ Microbiol 11:2463–2472

Jürgens K, Stolpe G (1995) Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. Freshwat Biol 33:27–38

Jürgens K, Arndt H, Rothhaupt KO (1994) Zooplankton-mediated change of bacterial community structure. Microb Ecol 27:27–42

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

Kent AD, Jones SE, Yannarell AC, Graham JM, Lauster GH, Kratz TK, Triplett EW (2004) Annual patterns in bacterioplankton community variability in a humic lake. Microb Ecol 48:550–560

Kent AD, Yannarell AC, Rusak JA, Triplett EW, McMahon KD (2007) Synchrony in aquatic microbial community dynamics. ISME J 1:38–47

Keough BP, Schmidt TM, Hicks RE (2003) Archaeal nucleic acids in picoplankton from great lakes on three continents. Microb Ecol 46:238–248

Kirchman DL, Dittel AI, Findlay SEG, Fischer D (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. Aquat Microb Ecol 35:243–257

Kolmonen E, Haukka K, Rantala-Ylinen A, Rajaniemi-Wacklin P, Lepisto L, Sivonen K (2011) Bacterioplankton community composition in 67 Finnish lakes differs according to trophic status. Aquat Microb Ecol 62:241–U249

Krashnopolsky VA, Maillard JP, Owen TC (2004) Detection of methane in the martian atmosphere: evidence for life? Icarus 172:537–547

LaMontagne MG, Holden PA (2003) Comparison of free-living and particle-associated bacterial communities in a coastal lagoon. Microb Ecol 46:228–237

Lemarchand C, Jardillier L, Carrias JF, Richardot M, Debroas D, Sime-Ngando T, Amblard C (2006) Community composition and activity of prokaryotes associated to detrital particles in two contrasting lake ecosystems. FEMS Microbiol Ecol 57:442–451

Lindeman RL (1942) The trophic-dynamic aspect of ecology. Ecology 23:399–418

Lindström ES (2000) Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microb Ecol 40:104–113

Lindström ES, Langenheder S (2012) Local and regional factors influencing bacterial community assembly. Environ Microbiol Rep. doi:10.1111/j.1758-2229.2011.00257.x

Lindström ES, Forslund M, Algesten G, Bergstrom AK (2006) External control of bacterial community structure in lakes. Limnol Oceanogr 51:339–342

Logares R, Bråte J, Heinrich F, Shalchian-Tabrizi K, Bertilsson S (2010) Infrequent transitions between saline and fresh waters in one of the most abundant microbial lineages (SAR11). Mol Biol Evol 27:347–357

Martinez-Garcia M, Swan BK, Poulton NJ, Gomez ML, Masland D, Sieracki ME, Stepanauskas R (2012) High-throughput single-cell sequencing identifies photoheterotrophs and chemoautotrophs in freshwater bacterioplankton. ISME J 6:113–123

Masin M, Nedoma J, Pechar L, Koblizek M (2008) Distribution of aerobic anoxygenic phototrophs in temperate freshwater systems. Environ Microbiol 10:1988–1996

Methé BA, Hiorns WD, Zehr JP (1998) Contrasts between marine and freshwater bacterial community composition: analyses of communities in Lake George and six other Adirondack lakes. Limnol Oceanogr 43:368–374

Minder L (1920) Zur Hydrophysik des Zürich- und Walensees, nebst Beitrag zur Hydrochemie und Hydrobakteriologie des Zürichsees. Arch Hydrobiol 12:122–194

Mindl B, Sonntag B, Pernthaler J, Vrba J, Psenner R, Posch T (2005) Effects of phosphorus loading on interactions of algae and bacteria: reinvestigation of the 'phytoplankton-bacteria paradox' in a continuous cultivation system. Aquat Microb Ecol 38:203–213

Miyoshi T, Iwatsuki T, Naganuma T (2005) Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters. Appl Environ Microbiol 71:1084–1088

Morita RY (1997) Bacteria in oligotrophic environments: starvation-survival lifestyle. Chapman Hall, New York

Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M, Vyverman W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68:4740–4750

Newton RJ, McMahon KD (2011) Seasonal differences in bacterial community composition following nutrient additions in a eutrophic lake. Environ Microbiol 13:887–899

Newton RJ, Kent AD, Triplett EW, McMahon KD (2006) Microbial community dynamics in a humic lake: differential persistence of common freshwater phylotypes. Environ Microbiol 8:956–970

Newton RJ, Jones SE, Helmus MR, McMahon KD (2007) Phylogenetic ecology of the freshwater Actinobacteria acI lineage. Appl Environ Microbiol 73:7169–7176

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49

O'Sullivan LA, Weightman AJ, Fry JC (2002) New degenerate Cytophaga-Flexibacter-Bacteroides-specific 16S ribosomal DNA-targeted oligonucleotide probes reveal high bacterial diversity in River Taff epilithon. Appl Environ Microbiol 68:2093-b

Overmann J, Tuschak C, Frostl JM, Sass H (1998) The ecological niche of the consortium "Pelochromatium roseum". Arch Microbiol 169:120–128

Overmann J, Coolen MJL, Tuschak C (1999) Specific detection of different phylogenetic groups of chemocline bacteria based on PCR and denaturing gradient gel electrophoresis of 16S rRNA gene fragments. Arch Microbiol 172:83–94

Pace ML, Cole JJ, Carpenter SR, Kitchell JF, Hodgson JR, Van de Bogert MC et al (2004) Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:240–243

Page KA, Connon SA, Giovannoni SJ (2004) Representative freshwater bacterioplankton isolated from Crater Lake, Oregon. Appl Environ Microbiol 70:6542–6550

Perez MT, Sommaruga R (2006) Differential effect of algal- and soil-derived dissolved organic matter on alpine lake bacterial community composition and activity. Limnol Oceanogr 51:2527–2537

Pernthaler J, Posch T (2009) Microbial food webs. In: Encyclopedia of inland waters. Elsevier, Oxford, pp 244–251

Pernthaler J, Glockner FO, Unterholzner S, Alfreider A, Psenner R, Amann R (1998) Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. Appl Environ Microbiol 64:4299–4306

Pernthaler J, Posch T, Šimek K, Vrba J, Pernthaler A, Glockner FO 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, Zollner 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

Philosof A, Sabehi G, Beja O (2009) Comparative analyses of actinobacterial genomic fragments from Lake Kinneret. Environ Microbiol 11:3189–3200

Piccini C, Conde D, Alonso C, Sommaruga R, Pernthaler J (2006) Blooms of single bacterial species in a coastal lagoon of the southwestern Atlantic Ocean. Appl Environ Microbiol 72:6560–6568

Pollard PC, Ducklow H (2011) Ultrahigh bacterial production in a eutrophic subtropical Australian river: does viral lysis short-circuit the microbial loop? Limnol Oceanogr 56:1115–1129

Posch T, Franzoi J, Prader M, Salcher MM (2009) New image analysis tool to study biomass and morphotypes of three major bacterioplankton groups in an alpine lake. Aquat Microb Ecol 54:113–126

Pronk M, Goldscheider N, Zopfi J (2009) Microbial communities in karst groundwater and their potential use for biomonitoring. Hydrogeol J 17:37–48

Rappe MS, Kemp PF, Giovannoni SJ (1997) Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol Oceanogr 42:811–826

Rappé MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633

Rasumov AS (1932) Die direkte Methode der Zählung der Bakterien im Wasser und ihre Vergleichung mit der Kochschen Plattenkultur Methode. Microbiol, Moskow 1:131

Raymond PA, Bauer JE (2001) Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. Nature 409:497–500

Reynolds CS (2006) Ecology of phytoplankton. Cambridge University Press, Cambridge, UK

Riemann L, Winding A (2001) Community dynamics of free-living and particle-associated bacterial assemblages during a freshwater phytoplankton bloom. Microb Ecol 42:274–285

Salcher MM, Pernthaler J, Psenner R, Posch T (2005) Succession of bacterial grazing defense mechanisms against protistan predators in an experimental microbial community. Aquat Microb Ecol 38:215–229

Salcher MM, Pernthaler J, Zeder M, Psenner R, Posch T (2008) Spatio-temporal niche separation of planktonic Betaproteobacteria in an oligo-mesotrophic lake. Environ Microbiol 10:2074–2086

Salcher MM, Pernthaler J, Posch T (2010) Spatiotemporal distribution and activity patterns of bacteria from three phylogenetic groups in an oligomesotrophic lake. Limnol Oceanogr 55:846–856

Salcher MM, Pernthaler J, Posch T (2011a) Seasonal bloom dynamics and ecophysiology of the freshwater sister clade of SAR11 bacteria 'that rule the waves' (LD12). ISME J 5:1242–1252

Salcher MM, Pernthaler J, Frater N, Posch T (2011b) Vertical and longitudinal distribution patterns of different bacterioplankton populations in a canyon-shaped, deep prealpine lake. Limnol Oceanogr 56:2027–2039

Sander BC, Kalff J (1993) Factors controlling bacterial production in marine and freshwater sediments. Microb Ecol 26:79–99

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

Schauer M, Kamenik C, Hahn MW (2005) Ecological differentiation within a cosmopolitan group of planktonic freshwater bacteria (SOL cluster, Saprospiraceae, Bacteroidetes). Appl Environ Microbiol 71:5900–5907

Schauer M, Jiang J, Hahn MW (2006) Recurrent seasonal variations in abundance and composition of filamentous SOL cluster bacteria (Saprospiraceae, Bacteroidetes) in oligomesotrophic Lake Mondsee (Austria). Appl Environ Microbiol 72:4704–4712

Scheffer M, Hosper SH, Meijer ML, Moss B, Jeppesen E (1993) Alternative Equilibria in Shallow Lakes. Trends Ecol Evol 8:275–279

Schink B (1989) Microbial Communities in Sediments. Naturwissenschaften 76:364–372

Schubert CJ, Vazquez F, Losekann-Behrens T, Knittel K, Tonolla M, Boetius A (2011) Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di Cadagno). FEMS Microbiol Ecol 76:26–38

Schweitzer B, Huber I, Amann R, Ludwig W, Simon M (2001) Alpha- and beta-proteobacteria control the consumption and release of amino acids on lake snow aggregates. Appl Environ Microbiol 67:632–645

Shabarova T, Pernthaler J (2010) Karst pools in subsurface environments: collectors of microbial diversity or temporary residence between habitat types. Environ Microbiol 12:1061–1074

Shade A, Kent AD, Jones SE, Newton RJ, Triplett EW, McMahon KD (2007) Interannual dynamics and phenology of bacterial communities in a eutrophic lake. Limnol Oceanogr 52:487–494

Sharma AK, Sommerfeld K, Bullerjahn GS, Matteson AR, Wilhelm SW, Jezbera J et al (2009) Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater Actinobacteria. ISME J 3:726–737

Šimek K, Chrzanowski TH (1992) Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl Environ Microbiol 58:3715–3720

Šimek K, Pernthaler J, Weinbauer MG, Horňák K, Dolan JR, Nedoma J et al (2001) Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appl Environ Microbiol 67:2723–2733

Šimek K, Horňák K, Jezbera J, Masin M, Nedoma J, Gasol JM, Schauer M (2005) Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of beta-proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir. Appl Environ Microbiol 71:2381–2390

Šimek K, Horňák K, Jezbera J, Nedoma J, Vrba J, Straskrabova V et al (2006) Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir. Environ Microbiol 8:1613–1624

Šimek K, Weinbauer MG, Horňák K, Jezbera J, Nedoma J, Dolan JR (2007) Grazer and virus-induced mortality of bacterioplankton accelerates development of Flectobacillus populations in a freshwater community. Environ Microbiol 9:789–800

Šimek K, Kasalicky V, Jezbera J, Jezberova J, Hahn MW (2010a) Broad habitat range of the phylogenetically narrow R-BT065 cluster, representing a core group of the betaproteobacterial genus limnohabitans. Appl Environ Microbiol 76:3763

Šimek K, Kasalicky V, Horňák K, Hahn MW, Weinbauer MG (2010b) Assessing niche separation among coexisting *Limnohabitans* strains through interactions with a competitor, viruses, and a bacterivore. Appl Environ Microbiol 76:1406–1416

Šimek K, Kasalicky V, Zapomelova E, Horňák K (2011) Alga-derived substrates select for distinct betaproteobacterial lineages and contribute to niche separation in Limnohabitans strains. Appl Environ Microbiol 77:7307–7315

Simon M (1987) Biomass and production of small and large free-living bacteria in Lake Constance. Limnol Oceanogr 32:591

Singer G, Besemer K, Schmitt-Kopplin P, Hodl I, Battin TJ (2010) Physical heterogeneity Iincreases biofilm resource use and its molecular diversity in stream mesocosms. PLoS One 5:e9988

Sommaruga R (2001) The role of solar UV radiation in the ecology of alpine lakes. J Photochem Photobiol B-Biol 62:35–42

Sommaruga R, Casamayor EO (2009) Bacterial 'cosmopolitanism' and importance of local environmental factors for community composition in remote high-altitude lakes. Freshwat Biol 54:994–1005

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

Stepanauskas R, Moran MA, Bergamaschi BA, Hollibaugh JT (2003) Covariance of bacterioplankton composition and environmental variables in a temperate delta system. Aquat Microb Ecol 31:85–98

Stürmeyer H, Overmann J, Babenzien HD, Cypionka H (1998) Ecophysiological and phylogenetic studies of Nevskia ramosa in pure culture. Appl Environ Microbiol 64:1890–1894

Taipale S, Kankaala P, Hahn MW, Jones RI, Tiirola M (2011) Methane-oxidizing and photoautotrophic bacteria are major producers in a humic lake with a large anoxic hypolimnion. Aquat Microb Ecol 64:81–95

Tarao M, Jezbera J, Hahn MW (2009) Involvement of cell surface structures in size-independent grazing resistance of freshwater actinobacteria. Appl Environ Microbiol 75:4720–4726

Thingstad TF (2000) 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

Tonolla M, Demarta A, Peduzzi R, Hahn D (1999) In situ analysis of phototrophic sulfur bacteria in the chemocline of meromictic Lake Cadagno (Switzerland). Appl Environ Microbiol 65:1325–1330

Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ et al (2009) Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr 54:2298–2314

Urbach E, Vergin KL, Larson GL, Giovannoni SJ (2007) Bacterioplankton communities of Crater Lake, OR: dynamic changes with euphotic zone food web structure and stable deep water populations. Hydrobiologia 574:161–177

Van der Gucht K, Vandekerckhove T, Vloemans N, Cousin S, Muylaert K, Sabbe K et al (2005) Characterization of bacterial communities in four freshwater lakes differing in nutrient load and food web structure. FEMS Microbiol Ecol 53:205–220

Van der Gucht K, Cottenie K, Muylaert K, Vloemans N, Cousin S, Declerck S et al (2007) The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc Natl Acad Sci USA 104:20404–20409

van Hannen EJ, Zwart G, van Agterveld MP, Gons HJ, Ebert J, Laanbroek HJ (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl Environ Microbiol 65:795–801

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130

Warnecke F, Amann R, Pernthaler J (2004) Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol 6:242–253

Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J (2005) Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Appl Environ Microbiol 71:5551–5559

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

Wetzel RG (2000) Freshwater ecology: changes, requirements, and future demands. Limnology 1:3–9

Winter C, Hein T, Kavka G, Mach RL, Farnleitner AH (2007) Longitudinal changes in the bacterial community composition of the Danube river: a whole-river approach. Appl Environ Microbiol 73:421–431

Wobus A, Bleul C, Maassen S, Scheerer C, Schuppler M, Jacobs E, Roske I (2003) Microbial diversity and functional characterization of sediments from reservoirs of different trophic state. FEMS Microbiol Ecol 46:331–347

Wright RT, Hobbie JE (1965) The uptake of organic solutes in lake water. Limnol Oceanogr 10:22–28

Wu QL, Hahn MW (2006a) Differences in structure and dynamics of Polynucleobacter communities in a temperate and a subtropical lake, revealed at three phylogenetic levels. FEMS Microbiol Ecol 57:67–79

Wu QLL, Hahn MW (2006b) High predictability of the seasonal dynamics of a species-like Polynucleobacter population in a freshwater lake. Environ Microbiol 8:1660–1666

Wu OLL, Boenigk J, Hahn MW (2004) Successful predation of filamentous bacteria by a nanoflagellate challenges current models of flagellate bacterivory. Appl Environ Microbiol 70:332–339

Yannarell AC, Triplett EW (2004) Within- and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. Appl Environ Microbiol 70:214–223

Zeder M, Peter S, Shabarova T, Pernthaler J (2009) A small population of planktonic Flavobacteria with disproportionally high growth during the spring phytoplankton bloom in a prealpine lake. Environ Microbiol 11:2676–2686

Zwart G, Crump BC, Agterveld M, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28:141–155

7 Pelagic Oxygen Minimum Zone Microbial Communities

Osvaldo Ulloa[1] · *Jody J. Wright*[2] · *Lucy Belmar*[3] · *Steven J. Hallam*[2,4]
[1]Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile
[2]Department of Microbiology and Immunology, University of British Columbia, Life Sciences Institute, Vancouver, BC, Canada
[3]Departamento de Oceanografía and Programa de Postgrados en Oceanografía, Universidad de Concepción, Concepción, Chile
[4]Graduate Program in Bioinformatics, University of British Columbia, Life Sciences Institute, Vancouver, BC, Canada

Introduction

Oxygen minimum zones (OMZs) are regions of the global ocean in which dissolved oxygen in the water column is reduced or totally absent due to poor ventilation, sluggish circulation, and a high demand of oxygen by microbial aerobic respiration. Open-ocean OMZs are prominent in the eastern tropical and subarctic Pacific and the northern Indian Oceans (*Fig. 7.1*). The actual concentration of dissolved oxygen varies among recognized OMZs, and determining whether or not they reach total anoxia based exclusively on oxygen measurements has until recently been a problem due to technical limitations (Revsbech et al. 2009; Thamdrup et al. 2012). This is highly relevant because microbial-encoded enzymes mediating aerobic and anaerobic transformations of elements (e.g., nitrogen, sulfur, and carbon) manifest different oxygen sensitivities.

It has long been recognized that many OMZs are hotspots for oxygen-sensitive nitrogen transformations, where nitrate serves as the main terminal electron acceptor for the oxidation of organic matter (Lam and Kuypers 2011). In such cases, denitrification and anaerobic ammonium oxidation (anammox) contribute to the removal of fixed nitrogen as N_2, with resulting impacts on global nutrient cycles and the climate system (Codispoti et al. 2001). Geochemical signs of the functioning of these anaerobic processes include the presence of an inorganic fixed nitrogen deficit relative to phosphorus in addition to the accumulation of nitrite and excess N_2 in the oxygen-deficient regions of the water column. Processes occurring in the boundary regions of OMZs also contribute to the production of the potent greenhouse gas nitrous oxide (N_2O), due primarily to the activity of nitrifiers at low oxygen levels. Thus, continued OMZ expansion is an emerging environmental concern, as it will likely exacerbate the loss of fixed nitrogen from the ocean in addition to increasing N_2O production (Keeling et al. 2010; Codispoti 2010).

Certain coastal regions also develop periods of oxygen starvation during part of the year, either naturally or induced by anthropogenic eutrophication, affecting marine ecosystems and coastal economies (Diaz and Rosenberg 2009). Some examples of where this phenomenon occurs include the continental shelves off Namibia, western India, and in the Gulf of Mexico (*Fig. 7.1*). Moreover, in certain enclosed or semi-enclosed basins, such as inland seas (e.g., the Baltic Sea, the Black Sea), fjords (e.g., Saanich Inlet), and ocean basins with reduced ventilation (e.g., the Cariaco Basin), sulfate reduction becomes the main microbial respiratory process, and H_2S rather than nitrite accumulates (*Fig. 7.2*). Until recently, euxinic water bodies (where H_2S accumulates) were thought to have a different microbiology and biogeochemistry than marine non-euxinic OMZs, but recent studies, as discussed below, have shown that this is not entirely the case.

Culture-independent molecular studies have identified a diverse community of pelagic prokaryotes in OMZs that are not fundamentally different from those found in euxinic systems. The analyses have primarily been based on individual marker genes, but have recently incorporated community genomic and transcriptomic data sets. In the following sections we highlight specific trends resulting from this work and identify some of the key taxonomic players driving matter and energy transformations in OMZs.

Phylogenetic Diversity of OMZ Prokaryotes

Bacteria

In a recent study, Wright et al. (2012) reviewed the bacterial community composition in open-ocean and coastal OMZs

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_45,

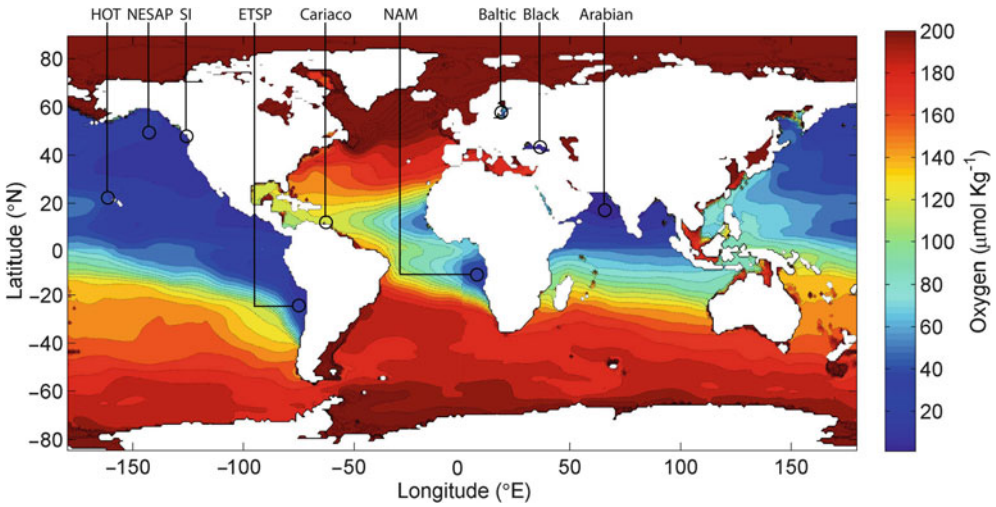


Fig. 7.1

Minimum dissolved oxygen concentrations for different regions of the global ocean. Locations mentioned in this chapter are indicated and comprise the Hawaii Ocean Time-series (*HOT*), the northeast subarctic Pacific (*NESAP*), Saanich Inlet (*SI*), the eastern tropical South Pacific (*ETSP*), the Cariaco Basin (*CB*), the Namibian upwelling (*NAM*; also known as the Benguela upwelling), and the Baltic, Black, and Arabian seas (Data are from the World Ocean Atlas 2009. Figure adapted from Wright et al. (2012) © MacMillan Publishers Ltd. All rights reserved)

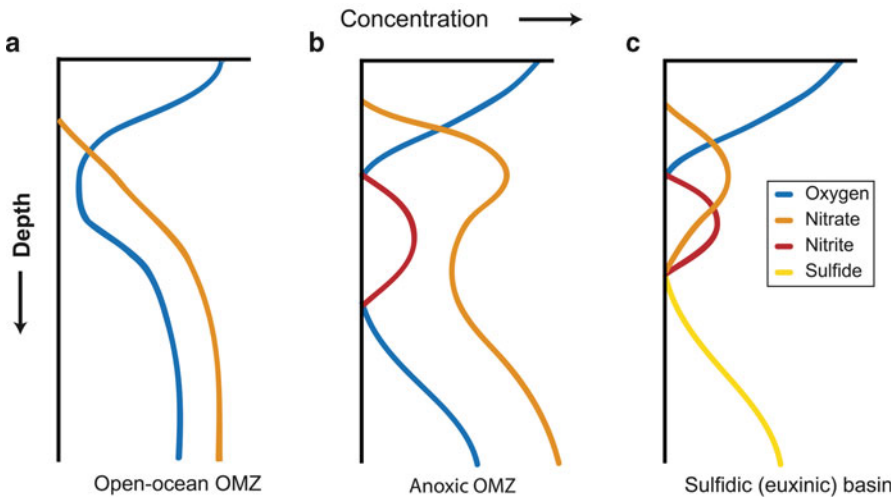


Fig. 7.2

Cartoon showing the characteristic geochemical profiles in different oxygen-deficient environments. (a) Open-ocean OMZs, with low oxygen concentrations and no nitrite accumulation (e.g., northeast subarctic Pacific); (b) Anoxic OMZs, with nitrite accumulation (e.g., eastern tropical North and South Pacific, Arabian Sea); (c) Euxinic basins showing H_2S accumulation (e.g., Saanich Inlet, Baltic Sea, Black Sea, Cariaco Basin)

and enclosed or semi-enclosed euxinic basins (including the Northeast subarctic Pacific (NESAP), the eastern tropical South Pacific (ETSP), the Namibian upwelling, and Saanich Inlet (SI)), based on taxonomic surveys of small subunit ribosomal rRNA (SSU rRNA) gene sequences (*Fig. 7.3*). Major groups in order of abundance include Proteobacteria, Bacteroidetes, candidate division Marine Group A, Actinobacteria, and Planctomycetes, while Cyanobacteria, Firmicutes, Verrucomicrobia, Gemmatimonadetes, Lentisphaerae, and Chloroflexi are also well represented (Wright et al. 2012). A number of candidate divisions are also present, including TM6, WS3, ZB2, ZB3, GN0, OP11, and OD1.

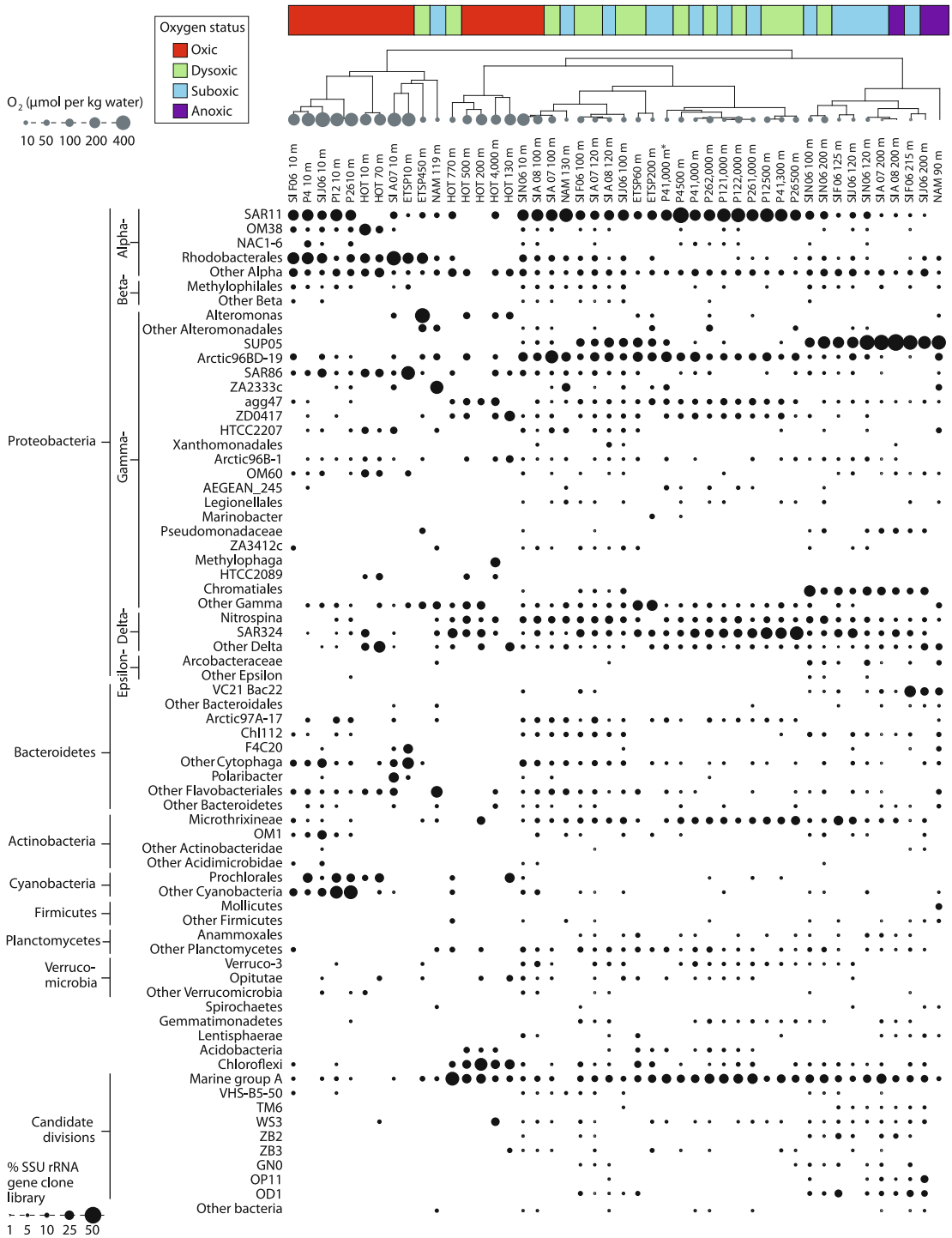


Fig. 7.3

Dot plot of the diversity of bacterial taxa at various sample points and depths in Saanich Inlet (SI), the northeastern subarctic Pacific (NESAP; labeled P4, P12, and P26), the Hawaii Ocean Time-series (HOT), the eastern tropical South Pacific (ETSP) and the Namibian upwelling (NAM), based on small-subunit ribosomal RNA (SSU rRNA) gene sequence profiles. ''*'' indicates a sample taken from P4 1,000 m in June 2008; all other NESAP samples were taken in 2009. Samples are organized according to the similarity of their community composition, as revealed by hierarchical clustering of the distribution of taxonomic groups across environmental samples. The dissolved oxygen concentration is shown for each oceanic sample, and the classification of the environment as oxic, dysoxic, suboxic, or anoxic is also indicated in the color bar (see text for definitions). Names for identifying bacterial groups were selected according to the taxonomic level at which the most relevant information was available (Data used to generate the dot plot were derived from sequences deposited in Genbank. Figure adapted from Wright et al. (2012)

The distribution of specific subdivisions of abundant taxa varies along the oxygen gradient in the different oxygen-deficient environments studied. Anoxic and sulfidic waters are often dominated by SUP05 (Sunamura et al. 2004) and ARCTIC96BD-19, groups of γ-proteobacteria related to sulfur-oxidizing symbionts of deep-sea bivalves (Fuchs et al. 2005; Woebken et al. 2007; Stevens and Ulloa 2008; Zaikova et al. 2010), with additional representation from the sulfur-oxidizing Arcobacteraceae (ε-proteobacteria) and the sulfate-reducing family Desulphobacteraceae (δ-proteobacteria). Suboxic (1–20 μmol O_2 per kg water) and dysoxic (20–90 μmol O_2 per kg water) waters contain high numbers of bacteria affiliated with the SAR11 cluster (α-proteobacteria), the agg47 cluster (γ-proteobacteria), the SAR324 cluster (δ-proteobacteria), the genus *Nitrospina* (δ-proteobacteria), and in some cases, bacterial groups affiliated with Cyanobacteria. Oxic surface waters above OMZs and euxinic basins are often dominated by sequences affiliated with SAR11 and the order Rhodobacterales (α-proteobacteria), the order Methylophilales (β-proteobacteria), and environmental clusters affiliated with SAR86 and Arctic96B-1 (γ-proteobacteria). Other abundant bacterial groups that appear to distribute differentially along the oxygen gradients of OMZs and euxinic basins include ZD0417 and ZA3412c (γ-proteobacteria), Flavobacteriales (Bacteroidetes), Microthrixineae (Actinobacteria), and Verruco-3 (Verrucomicrobia).

These results show recurring bacterial community composition patterns within OMZs and euxinic basins that are consistent with redox-driven niche partitioning, stressing the importance of oxygen concentration as an organizing principle in pelagic microbial communities.

Archaea

In contrast to the bacterial domain, less is known about archaeal community composition along the oxygen gradients of OMZs and euxinic basins. Here we use published SSU rRNA sequence data from the ETSP (Belmar et al. 2011), the Black Sea (Vetriani et al. 2003; Coolen et al. 2007), the Cariaco Basin (Madrid et al. 2001; Jeon et al. 2008), the Namibian upwelling (Woebken et al. 2007), and the Baltic Sea (Labrenz et al. 2010), as well as new data from the NESAP, Saanich Inlet (SI), and eastern subtropical South Pacific (ESP) to highlight the major groups present in these systems. *Figure 7.4* illustrates the distribution of the respective phylotypes within the general archaeal phylogenetic tree. Most phylotypes are affiliated with well-recognized pelagic marine clades such as Group I.1a (G-I.1a, DeLong 1998), pSL12-related group (Mincer et al. 2007), Marine Group II (MG II, DeLong 1992), and Marine Group III (MG III, Fuhrman and Davis 1997). However, a significant number of phylotypes cluster within clades originally found in sediments (Marine Benthic Group A and E; Vetriani et al. 1999) or deep-hydrothermal vent environments (DHVE-4 and DHVE-5, Takai and Horikoshi 1999).

A remarkably high proportion of archaeal sequences recovered from OMZs affiliates with the thaumarchaeotal G-I.1a, a group well represented in all of the considered systems (*Fig. 7.5*). This group, initially referred to as Marine Group I (DeLong 1992), is ubiquitous and abundant in the global ocean (Francis et al. 2005; Hallam et al. 2006). Group I.1a contains two statistically supported clusters, designated as A and B (Belmar et al. 2011), although some authors have divided this group into additional clusters (e.g., Massana et al. 2000). With the exception of *Cenarchaeum symbiosum*, which appears outside of the A and B subdivisions, the G-I.1a-A cluster comprises all marine thaumarchaeotal species that have been fully sequenced thus far (i.e., *Nitrosopumilus maritimus* and *Nitrosoarchaeum limnia*). The G-I.1a-A cluster also includes sequences retrieved from diverse terrestrial and marine environments including surface waters, deep-ocean sediments, and agricultural soils. In contrast, cluster G-I.1a-B includes very few phylotypes from oxic surface waters, and is mainly composed of sequences from deep waters, marine hydrothermal vents, and oxygen-deficient waters. Since many representatives of the G-I.1a archaeal group are known ammonium-oxidizers (AOA), and given the correlation between phylogenetic markers for AOA and the functional marker ammonia monooxygenase subunit alpha (*amoA*), OMZ representatives of this group are considered presumptive nitrifiers (Molina et al. 2010).

Some thaumarchaeal phylotypes found in anoxic or euxinic waters classify as being part of the major branch that includes the pSL12-related group (Mincer et al. 2007), the marine benthic group A (Vetriani et al. 1999), and the FFS cluster, which contains sequences retrieved from forest soil (Jurgens et al. 1997). This major branch is a sister group of the branch joining terrestrial Group I.1b and marine Group I.1a, and is related to the extremophile representative pSL12 (Mincer et al. 2007). Additional phylotypes recovered from below the chemocline in the Cariaco Basin (Jeon et al. 2008) appear at the base of the Thaumarchaeota. Interestingly, these sequences were generated using primers designed for eukaryotes from anoxic/euxinic waters in the Cariaco Basin and composed a group with phylotypes from freshwater sediments, rice roots and soil (Jurgens et al. 2000), sediments near deep hydrothermal vents (Takai et al. 2001), and sub-seafloor sediments (Inagaki et al. 2003; Sørensen and Teske 2006). This group has been named "Group I.3" (Jurgens et al. 2000) or Miscellanous Crenarchaeotic group (Inagaki et al. 2003) (*Fig. 7.4*).

Other groups prevalent in oxygen-deficient systems are the euryarchaeal MGII and MGIII (*Figs. 7.4* and *7.5*). MGII is a cosmopolitan group, and the majority of sequences observed in coastal and open-ocean OMZs are affiliated with the MGII-A cluster. MGIII is a less prominent group in the global ocean, but appears to be important in OMZs (Belmar et al. 2011). Finally, some euryarchaeal phylotypes from OMZs and euxinic waters associate with Marine Benthic Group E and DHVE-4 and DHVE-5 groups.

Archaeal community structure mirrors trends for bacterial denizens of OMZs, including close taxonomic affiliation with archaeal groups from diverse seafloor environments

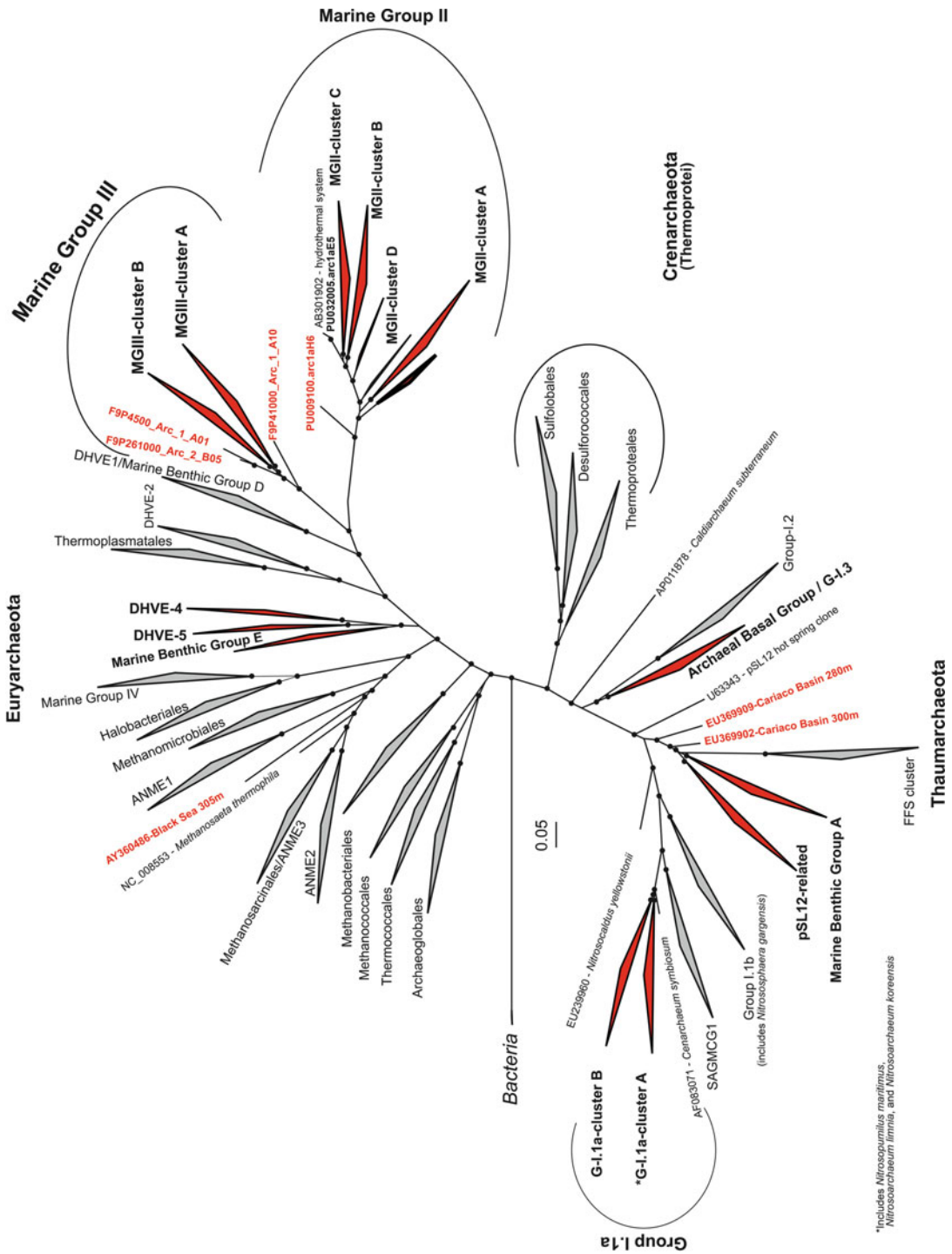


Fig. 7.4
Maximum-likelihood phylogenetic tree of archaeal SSU-rRNA gene sequences. Representative sequences of OMZ phylotypes (>97% of similarity, using UCLUST; Edgar 2010) together with other sequences from the Genbank database were aligned with Mafft (Katoh et al. 2002). The phylogenetic tree was built with the Bosque software (Ramírez-Flandes and Ulloa 2008), using FastTree (Price et al. 2010) and applying the general time-reversible DNA model. The selection of OMZ phylotypes included previously unpublished archaeal SSU-rRNA gene sequences from the northeastern subarctic Pacific (JQ220557 – JQ222567), Saanich Inlet (JQ222568 – JQ228228, except for a few sequences of apparent eukaryal origin), the eastern tropical and subtropical South Pacific (JX280966 – JX281688). Red boxes represent phylogenetic clusters containing OMZ phylotypes. Dots at nodes represent branches with support values of ≥70%. The scale bar indicates the expected changes per sequence position (Note: The scale only applies to the branches of the tree, the boxes are not scaled)

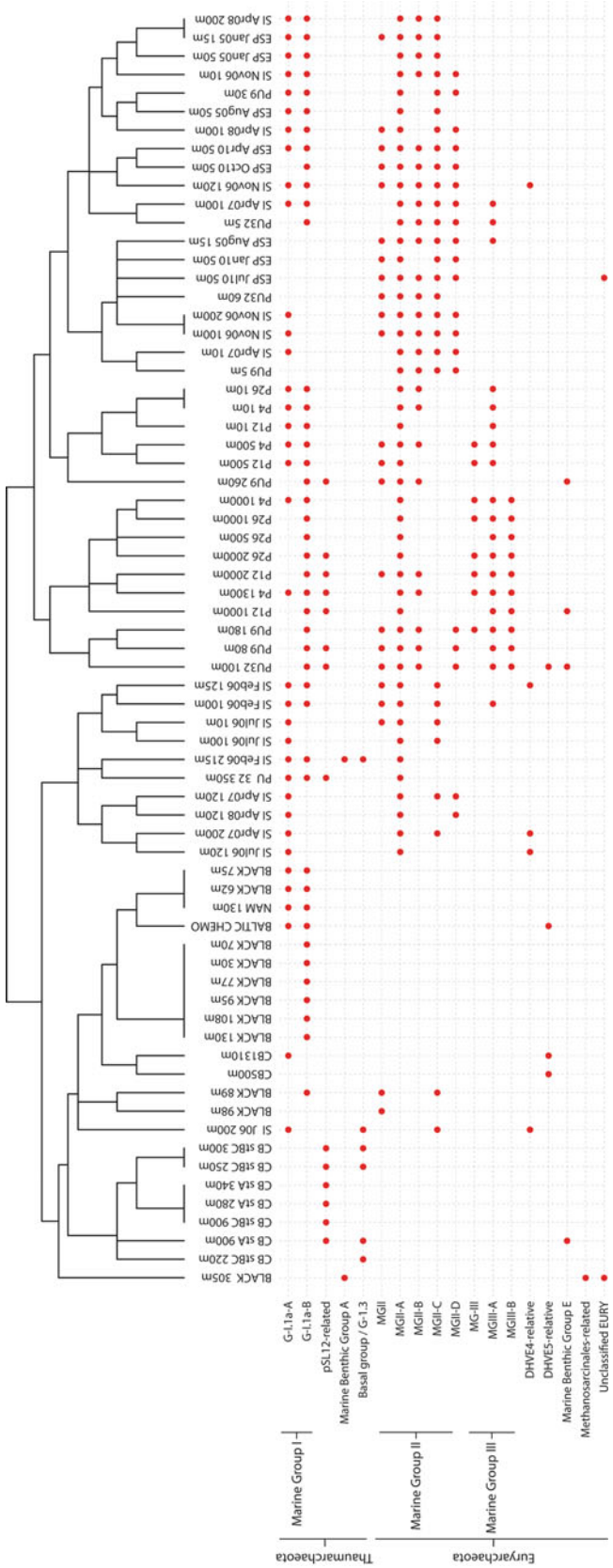


Fig. 7.5

Presence/absence dot plot of archaeal taxa at various sample points and depths in the northeastern subarctic Pacific (NESAP; labeled P4, P12, and P26), the eastern subtropical South Pacific (ESP), the Namibian upwelling (NAM), the Peru Upwelling (PU), Saanich Inlet (SI), the Black Sea (BLACK), the Baltic Sea (BALTIC), and the Cariaco Basin (CB), based on small-subunit ribosomal RNA (SSU rRNA) gene sequence profiles. Samples are organized according to the similarity of their community composition, as revealed by hierarchical clustering of the distribution of taxonomic groups across environmental samples. Names for identifying bacterial groups were selected according to the taxonomic level at which the most relevant information was available (Data used to generate the dot plot were derived from sequences deposited in Genbank)

(e.g., sub-seafloor sediments, deep-sea hydrothermal vents, and cold seeps). Although these similarities likely reflect recurring patterns of niche selection based on convergent environmental conditions (e.g., oxygen depletion), the precise ecological and biogeochemical roles of archaea in OMZs and other seafloor environments remain poorly constrained.

OMZ Prokaryotes as Biogeochemical Players

For almost a decade, OMZ gene surveys have focused extensively on microbes performing denitrification and anaerobic ammonia oxidation (anammox). Studies of the functional gene nitrite reductase (*nirS* and *nirK*) suggest that denitrification is mediated by a broad range of microorganisms from diverse taxonomic groups, which in turn vary among OMZ regions for reasons not yet clear (Jayakumar et al. 2004, 2009 Castro-González et al. 2005; Ward et al. 2009). In contrast, the OMZ anammox bacteria are much less diverse, with members clustering exclusively with the marine genus "*Candidatus* Scalindua" within the Planctomycetes (Hamersley et al. 2007; Woebken et al. 2008; Galán et al. 2009). While monophyletic, this sequence cluster contains high micro-diversity (Woebken et al. 2008; Galán et al. 2009) and the corresponding genomic and functional variations of micro-diverse clusters remain unknown. Analysis of microbial community gene expression from the anoxic core of the ETSP OMZ has revealed a dominance of transcripts matching the freshwater anammox species "*Candidatus* Kuenenia stuttgartiensis" (Strous et al. 2006), encompassing many of the anammox-specific functional gene repertoires (e.g., hydrazine oxidoreductase) (Stewart et al. 2012). The prevalence of sequences matching *Kuenenia* rather than *Scalindua* in early OMZ studies could reflect a lack of whole genome sequence information in public databases. During composition of this chapter, the draft genome sequence of the marine anammox bacteria "*Candidatus* Scalindua profunda" was reported (van de Vossenberg et al. 2012), expanding the range of fragment recruitment platforms for sequence analysis.

As indicated in the previous section, molecular surveys have recovered abundant and diverse sulfur-oxidizing microbial groups in the OMZ water column, with sequences affiliated with SUP05 and ARCTIC96BD-19. These results were unexpected, as an active sulfur cycle was not envisioned in nitrate- and nitrite-rich OMZ water columns (Canfield et al. 2010). Based on marker gene sequences (e.g., SSU rRNA gene, APS reductase gene *aprA*), sulfur-oxidizing bacteria from OMZs harbor many of the same functional properties as bacteria inhabiting other sulfidic marine habitats (*Sulfurimonas*-like ε-proteobacteria, green sulfur bacteria) (Stevens and Ulloa 2008; Lavik et al. 2009; Canfield et al. 2010; Stewart et al. 2012).

Metagenomic analysis of members of the SUP05 clade from Saanich Inlet revealed that they contain genes for carbon fixation, dissimilatory reduction of nitrate to nitrous oxide, and oxidation pathways for diverse reduced sulfur species such as sulfide, sulfite, elemental sulfur, and thiosulfate (Walsh et al. 2009). This sequence information revealed the genetic potential for chemolithoautotrophic oxidation of reduced sulfur with nitrate in the water column of marine OMZs. Though well described for bacteria in other anoxic marine environments, for example, sediments and sulfidic zones of anoxic marine basins (Fossing et al. 1995; Sunamura et al. 2004; Campbell et al. 2006), dissimilatory sulfur-oxidation coupled with nitrate reduction to N_2O and carbon fixation constitutes a form of autotrophic denitrification in OMZs. This process has been implicated in the sulfide detoxification of the shelf waters off the Namibian coast (Lavik et al. 2009) and has been observed to occur in waters of the ETSP OMZ (Canfield et al. 2010). In the latter study, metagenomic analysis showed that up to 16% of all protein-coding genes matched diverse sulfur-oxidizing taxa. SUP05 was particularly well represented, with over 80% of its genes present in this dataset at an average amino acid similarity of 70%. Metatranscriptome sequencing from this site confirmed that genes for diverse sulfur oxidation pathways are actively transcribed in situ along with symbiont-like nitrate reductases in the core of the anoxic OMZ (Stewart et al. 2012).

While the pelagic OMZ microbiota is dominated by symbiont-like sulfur-oxidizers, active sulfate-reducing assemblages are also indicated. Canfield et al. (2010) demonstrated surprisingly high rates of sulfate reduction to sulfide in experiments in the ETSP OMZ. Coupled metagenomic data for this community revealed sequences, including those encoding dissimilatory sulfur metabolism genes (*aprA, dsrB*), matching the genomes of known sulfate reducers of the δ-proteobacteria (e.g., *Desulfatibacillum*, *Desulfobacterium* sp.). Together, these results revealed a cryptic sulfur cycle in which sulfate reducers provide sulfide that is immediately consumed by a diverse oxidizer community. Moreover, sulfate reducers could also provide ammonium for anammox bacteria, another manifestation of the tight coupling between the sulfur and nitrogen cycles in OMZs.

Anoxic OMZs can also impinge on the photic zone, creating a unique environment for photoautotrophs, and particularly oxygenic ones adapted to low-oxygen tensions. The latter could provide a local source of oxygen to feed aerobic processes (e.g., nitrification) in a typically anoxic environment. Indeed, picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are frequent inhabitants of low-light oceanic OMZ waters of the Arabian Sea, ETNP and ETSP (Johnson et al. 1999; Goericke et al. 2000; Galán et al. 2009). A recent study in the eastern tropical Pacific showed that OMZ *Prochlorococcus* communities contain novel phylotypes (Lavin et al. 2010). The genomic characteristics of these OMZ photoautotrophs remain to be determined. They may provide new insights about the evolution of photosynthesis as the planet and the ocean became oxygenated.

While anaerobic microorganisms performing nitrogen and sulfur transformations characterize the core of the OMZ, the oxycline and low oxygen waters of the upper OMZ are critical zones for aerobic nitrifying microorganisms, particularly the AOA. Early studies pointed to a significant role for the process of ammonia oxidation in OMZs, particularly at the upper boundaries (e.g., Ward and Zafiriou 1988; Ward et al. 1989; Lipschultz et al. 1990). Catalyzed by the ammonia

monooxygenase (Amo) enzyme, the ability to oxidize ammonia was originally thought to be restricted to a few groups within the γ- and β-proteobacteria. However, metagenomic studies performed in the last decade revealed the existence of unique *amoA* genes derived from uncultivated, non-extremophilic Crenarchaeota (Venter et al. 2004; Hallam et al. 2006; Treusch et al. 2005), now recognized as a separate phylum, the Thaumarchaeota (➔ *Fig. 7.4*). In addition, an isolate of the marine thaumarchaeon *Nitrosopumilus maritimus* demonstrated a capacity for growth using ammonia oxidation as an energy source, resulting in stoichiometric production of nitrite (Könneke et al. 2005). Subsequently, high abundances of archaeal *amoA* genes have been detected in a variety of oxygen-deficient marine environments including the OMZs of the ETNP and ETSP, and the suboxic zones of the Black Sea, the Gulf of California, and the Baltic Sea (Francis et al. 2005; Coolen et al. 2007; Lam et al. 2007; Beman et al. 2008; Molina et al. 2010). Metatranscriptomic analysis in the ETSP showed that up to 20% of all protein-coding transcripts matched *N. maritimus* in the upper OMZ and that thaumarchaeotal *amo* genes were highly transcribed in this zone (Stewart et al. 2012). These results reinforce the emerging perspective that thaumarchaeotal ammonia-oxidation contributes substantially to nitrogen cycling in diverse marine environments (Wuchter et al. 2006; Prosser and Nicol 2008).

In addition to playing key roles in nitrogen and sulfur cycling, OMZ microorganisms may contribute a substantial proportion of fixed organic carbon. Sulfur-oxidizers like SUP05, for example, harbor genes for inorganic carbon fixation through the Calvin-Benson-Bassham cycle (Walsh et al. 2009), while anammox bacteria can make use of the acetyl-coenzyme A (CoA) pathway for carbon fixation (Strous et al. 2006). Isolation of the ammonia-oxidizing thaumarchaeon *N. maritimus* also revealed a capacity for chemolithoautotrophic growth on ammonia as a sole energy source and bicarbonate as a sole carbon source (Könneke et al. 2005). Subsequent sequencing of the *N. maritimus* genome confirmed that it contains genes for the 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) pathway of autotrophic carbon fixation (Walker et al. 2010). The actual contribution of these groups (and of others) to the carbon economy of OMZs remains to be determined.

Summary

OMZs were traditionally seen as regions dominated by heterotrophic denitrification fueled by the sinking of organic matter produced via photosynthesis in the sunlit surface ocean. They were also considered to have a fundamentally different microbiology and biogeochemistry than euxinic basins. The discovery of new microbial processes, such as anammox, and the recognition of an active but cryptic sulfur cycle in anoxic OMZs have significantly shifted the old paradigm. The recurring patterns of bacterial and archaeal community composition shared along the oxygen gradient of different pelagic ecosystems are consistent with fundamental organizing principles at work on different ecological scales. To identify and harness these principles, future studies are needed that explore the genomic information and physiological properties of isolates and whole communities from diverse OMZs.

Acknowledgments

This chapter was prepared while the lead author (O.Ulloa) was on sabbatical at the Department of Microbiology and Immunology, University of British Columbia, supported by the Life Sciences Institute Visiting Scholar Award, the Peter Wall Institute for Advanced Studies International Visiting Research Scholar Award, and a gift from the Agouron Institute.

This work was performed under the auspices of the Agouron Institute, the Gordon and Betty Moore Foundation, the FONDAP Program of the Chilean National Commission for Scientific and Technological Research (CONICYT), the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Foundation for Innovation, and the Canadian Institute for Advanced Research. J. J. Wright was supported by NSERC. L. Belmar was supported by CONICYT.

References

Belmar L, Molina V, Ulloa O (2011) Abundance and phylogenetic identity of archaeoplankton in the permanent oxygen minimum zone of the eastern tropical South Pacific. FEMS Microbiol Ecol 78:314–326

Beman JM, Popp BN, Francis CA (2008) Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. ISME J 2:429–441

Campbell BJ, Engel AS, Porter ML, Takai K (2006) The versatile ε-proteobacteria: key players in sulphidic habitats. Nat Rev Microbiol 4:458–468

Canfield DE, Stewart FJ, Thamdrup B, De Brabandere L, Dalsgaard T, Delong EF, Revsbech NP, Ulloa O (2010) A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast. Science 330:1375–1378

Castro-González M, Braker G, Farías L, Ulloa O (2005) Communities of *nirS*-type denitrifiers in the water column of the oxygen minimum zone in the eastern South Pacific. Environ Microbiol 7:1298–1306

Codispoti LA (2010) Interesting times for marine N_2O. Science 327:1339–1340

Codispoti LA, Brandes JAYA, Christensen JP, Devol AH, Naqvi SWA, Paerl HW, Yoshinari T (2001) The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene? Sci Mar 65:85–105

Coolen MJ, Abbas B, van Bleijswijk J, Hopmans EC, Kuypers MMM, Wakeham SG, Sinninghe Damsté JS (2007) Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16 S ribosomal and functional genes and membrane lipids. Environ Microbiol 9:1001–1016

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689

DeLong EF (1998) Everything in moderation: archaea as 'non-extremophiles'. Curr Opin Genet Dev 8:649–654

Diaz RJ, Rosenberg R (2009) Spreading dead zones and consequences for marine ecosystems. Science 926:926–929

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461

Fossing H, Gallardo VA, Jørgensen BB, Hüttel M, Nielsen LP, Schulz H, Canfield DE, Forster S, Glud RN, Gundersen JK, Küver J, Ramsing NB, Teske A,

Thamdrup B, Ulloa O (1995) Concentration and transport of nitrate by the mat-forming sulfur bacterium *Thioploca*. Nature 374:713–715

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci USA 102:14683–14688

Fuchs BM, Woebken D, Zubkov MV, Burkill P, Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. Aquat Microb Ecol 39:145–157

Fuhrman JA, Davis AA (1997) Widespread archaea and novel bacteria from the deep sea as shown by 16 S rRNA gene sequences. Mar Ecol Prog Ser 150:275–285

Galán A, Molina V, Thamdrup B, Woebken D, Lavik G, Kuypers MMM, Ulloa O (2009) Anammox bacteria and the anaerobic oxidation of ammonium in the oxygen minimum zone off northern Chile. Deep-Sea Res II 56:1021–1031

Goericke R, Olson RJ, Shalapyonok A (2000) A novel niche for *Prochlorococcus* sp. in low-light suboxic environments in the Arabian Sea and the eastern tropical North Pacific. Deep-Sea Res I 47:1183–1205

Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM, DeLong EF (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4:e95

Hamersley MR, Lavik G, Woebken D, Rattray JE, Lam P, Hopmans EC, Damste JSS, Kruger S, Graco M, Gutierrez D, Kuypers MMM (2007) Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. Limnol Oceanogr 52:923–933

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Ohkotsk. Appl Environ Microbiol 69:7224–7235

Jayakumar DA, Francis CA, Naqvi SWA, Ward BB (2004) Diversity of nitrite reductase genes (*nirS*) in the denitrifying water column of the coastal Arabian Sea. Aquat Microb Ecol 34:69–78

Jayakumar A, O'Mullan GD, Naqvi SWA, Ward BB (2009) Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. Microb Ecol 58:350–362

Jeon SO, Ahn TS, Hong SH (2008) A novel archaeal group in the phylum Crenarchaeota found unexpectedly in an eukaryotic survey in the Cariaco Basin. J Microbiol 46:34–39

Johnson Z, Landry ML, Bidigare RR, Brown SL, Campbell L, Gunderson J, Marra J, Trees C (1999) Energetics and growth kinetics of a deep *Prochlorococcus* spp. population in the Arabian Sea. Deep-Sea Res II 46:1719–1743

Jurgens G, Lindström K, Saano A (1997) Novel group within the kingdom Crenarchaeota from boreal forest soil. Appl Environ Microbiol 63:803–805

Jurgens G, Glöckner FO, Amann R, Saano A, Montonen L, Likolammi M, Munster U (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization. FEMS Microbiol Ecol 34:45–56

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066

Keeling RF, Körtzinger A, Gruber N (2010) Ocean deoxygenation in a warming world. Ann Rev Mar Sci 2:199–229

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546

Labrenz M, Sintes E, Toetzke F, Zumsteg A, Herndl GJ, Seidler M, Jürgens K (2010) Relevance of a crenarchaeotal subcluster related to Candidatus *Nitrosopumilus maritimus* to ammonia oxidation in the suboxic zone of the central Baltic Sea. ISME J 4:1496–1508

Lam P, Kuypers MMM (2011) Microbial nitrogen cycling processes in oxygen minimum zones. Ann Rev Mar Sci 3:317–345

Lam P, Jensen MM, Lavik G, McGinnis DF, Müller B, Schubert CJ, Amann R, Thamdrup B, Kuypers MMM (2007) Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. Proc Natl Acad Sci USA 104:7104–7109

Lavik G, Stuhrmann T, Bruchert V, Van der Plas A, Mohrholz V, Lam P, Mussmann M, Fuchs BM, Amann R, Lass U, Kuypers MMM (2009) Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. Nature 457:581–584

Lavin P, González B, Santibánez JF, Scanlan DJ, Ulloa O (2010) Novel lineages of *Prochlorococcus* thrive within the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol Rep 2:728–738

Lipschultz F, Wofsy SC, Ward BB, Codispoti LA, Friedrich G, Elkins JW (1990) Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical South-Pacific ocean. Deep-Sea Res 37:1513–1541

Madrid VM, Taylor GT, Scranton MI, Chistoserdov AY (2001) Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the Cariaco Basin. Appl Environ Microbiol 67:1663–1674

Massana R, DeLong EF, Pedrós-Alió C (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl Environ Microbiol 66:1777–1787

Mincer TJ, Church MJ, Taylor LT, Preston C, Karl DM, DeLong EF (2007) Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ Microbiol 9:1162–1175

Molina V, Belmar L, Ulloa O (2010) High diversity of ammonia-oxidizing archaea in permanent and seasonal oxygen-deficient waters of the eastern South Pacific. Environ Microbiol 12:2450–2465

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490

Prosser JI, Nicol GW (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ Microbiol 10:2931–2941

Ramírez-Flandes S, Ulloa O (2008) Bosque: integrated phylogenetic analysis software. Bioinformatics 24:2539–2541

Revsbech NP, Larsen LH, Gundersen J, Dalsgaard T, Ulloa O, Thamdrup B (2009) Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. Limnol Oceanogr Methods 7:371–381

Sørensen KB, Teske A (2006) Stratified communities of active archaea in deep marine subsurface sediments. Appl Environ Microbiol 72:4596–4603

Stevens H, Ulloa O (2008) Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol 10:1244–1259

Stewart FJ, Ulloa O, DeLong EF (2012) Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ Microbiol 14:23–40

Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P, Barbe V, Fonknechten N, Vallenet D, Segurens B, Schenowitz-Truong C, Medigue C, Collingro A, Snel B, Dutilh BE, Op den Camp HJM, van der Drift C, Cirpus I, van de Pas-Schoonen KT, Harhangi HR, van Niftrik L, Schmid M, Keltjens J, van de Vossenberg J, Kartal B, Meier H, Frishman D, Huynen MA, Mewes HW, Weissenbach J, Jetten MSM, Wagner M, Le Paslier D (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440:790–794

Sunamura M, Higashi Y, Miyako C, Ishibashi J, Maruyama A (2004) Two bacteria phylotypes are predominant in the Suiyo Seamount hydrothermal plume. Appl Environ Microbiol 70:1190–1198

Takai K, Horikoshi K (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. Genetics 152:1285–1297

Takai K, Komatsu T, Inagaki F, Horikoshi K (2001) Distribution of archaea in a black smoker chimney structure. Appl Environ Microbiol 67:3618–3629

Thamdrup B, Dalsgaard T, Revsbech NP (2012) Widespread functional anoxia in the oxygen minimum zone of the eastern South Pacific. Deep-Sea Res I 65:36–45

Treusch AH, Leiningger S, Kletzin A, Schuster SC, Klenk HP, Schleper C (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7:1985–1995

van de Vossenberg J, Woebken D, Maalcke WJ, Wessels HJCT, Dutilh BE, Kartal B, Janssen-Megens EM, Roeselers G, Yan J, Speth D, Gloerich J, Geerts W, van der Biezen E, Pluk W, Francoijs K-J, Russ L, Lam P, Malfatti SA, Tringe SG, Haaijer SCM, Op den Camp HJM, Stunnenberg HG, Amann R, Kuypers

MMM, Jetten MSM (2012) The metagenome of the marine anammox bacterium '*Candidatus* Scalindua profunda' illustrates the versatility of this globally important nitrogen cycle bacterium. Environ Microbiol. doi:10.1111/j.1462-2920.2012.02774.x

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu D, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers YH, Smith HO (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74

Vetriani C, Jannasch HW, MacGregor BJ, Stahl DA, Reysenbach AL (1999) Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. Appl Environ Microbiol 65:4375–4384

Vetriani C, Traan HV, Kerkof L (2003) Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea. Appl Environ Microbiol 69:6481–6488

Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, Arp DJ, Brochier-Armanet C, Chain PSG, Chan PP, Gollabgir A, Hemp J, Hügler M, Karr EA, Könneke M, Shin M, Lawton TJ, Lowe T, Martens-Habbena W, Sayavedra-Soto LA, Lang D, Sievert SM, Rosenzweig AC, Manning G, Stahl DA (2010) *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proc Natl Acad Sci USA 107:8818–8823

Walsh DA, Zaikova E, Howes CG, Song YC, Wright JJ, Tringe SG, Tortell PD, Hallam SJ (2009) Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. Science 326:578–582

Ward BB, Zafiriou O (1988) Nitrification and nitric oxide in the oxygen minimum of the eastern tropical North Pacific. Deep-Sea Res 35: 1127–1142

Ward BB, Glover HE, Lipschultz F (1989) Chemoautotrophic activity and nitrification in the oxygen minimum zone off Peru. Deep-Sea Res 36:1031–1051

Ward BB, Devol AH, Rich JJ, Chang BX, Bulow SE, Naik H, Pratihary A, Jayakumar A (2009) Denitrification as the dominant nitrogen loss process in the Arabian Sea. Nature 461:78–81

Woebken D, Fuchs BA, Kuypers MAA, Amann R (2007) Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl Environ Microbiol 73: 4648–4657

Woebken D, Lam P, Kuypers MMM, Naqvi SWA, Kartal B, Strous M, Jetten MSM, Fuchs BM, Amann R (2008) A microdiversity study of anammox bacteria reveals a novel Candidatus Scalindua phylotype in marine oxygen minimum zones. Environ Microbiol 10:3106–3119

Wright JJ, Konwar KM, Hallam SJ (2012) Microbial ecology of expanding oxygen minimum zones. Nat Rev Microbiol 10:381–394

Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Damste JSS (2006) Archaeal nitrification in the ocean. Proc Natl Acad Sci USA 103:12317–12322

Zaikova E, Walsh DA, Stilwell CP, Mohn WW, Tortell PD, Hallam SJ (2010) Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. Environ Microbiol 12:172–191

8 Marine Deep Sediment Microbial Communities

Andreas Teske
Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Introduction

A new biosphere of bacteria, archaea, and eukaryotes in deep marine subsurface sediments, characterized by unexpected evolutionary depth and metabolic diversity, is currently being explored by gene sequencing surveys, cell counts, molecular quantification studies, and cultivations. Although cultivable bacteria have been detected and quantified in deep marine sediments decades ago (Morita and Zobell 1955), the pioneering work by John R. Parkes and colleagues provided comprehensive proof of the deep microbial biosphere in subsurface marine sediments, by integrating microbial cell counts, Most-Probable-Number counts, characterizations of subsurface bacterial isolates, geochemical porewater profiles, and measurements of microbial process rates (Parkes et al. 1994). In initial quantifications of total cell numbers and biomass of this subsurface biosphere account for a major portion of all living biomass (Whitman et al. 1998; Parkes et al. 2000). Specific phylum-level lineages of bacteria and archaea occur consistently in marine subsurface sediments, often in distinctly structured communities that reflect specific subsurface habitats and geochemical settings, and that are distinct from the surface biosphere. Investigating the activity, metabolism, biogeochemical role, and energy and carbon sources of subsurface bacteria, archaea, and eukaryotes remains an ongoing challenge of deep subsurface microbiology. Most deep sediment samples for deep subsurface microbial research were recovered through the Ocean Drilling Program (ODP) and its successor, the Integrated Ocean Drilling Program (IODP). Deep sediment sampling expeditions have to a very large extent provided the sediment samples, subsurface habitat characterizations, and biogeochemical process studies that have nurtured, and continue to develop, the field of deep subsurface microbiology (Jørgensen et al. 2006; D'Hondt et al. 2007).

Deep marine subsurface sediments are one of the most extensive microbial habitats on Earth, by areal extent and by volume. Marine sediments cover more than two-thirds of the Earth's surface; they begin to accumulate on newly generated seafloor at the flanks of mid-ocean ridges, accumulate in layers of several hundred to several thousand meters thickness toward continental margins, and reach maximal thickness of more than 10 km at locations with highly active sedimentation, fluvial and aerial erosion—such as the Gulf of Mexico, the Bay of Bengal, and the Atlantic continental margins of North America and western Africa (Divins 2003). These sediments are permeated with microbial life, as far as temperature gradients and pore space availability permit. The published depth range for living microbial cells reaches 1,626 mbsf (meters below seafloor) on the Newfoundland Margin, where archaeal hyperthermophiles (*Thermococcus*/*Pyrococcus*) were found at in situ temperatures of 60–100°C (Roussel et al. 2008). At typical geothermal gradients of 20–50°C/km in deep marine sediments, microbial life could persist in sediments of several kilometers depth. Geothermal heating is likely to increase the bioavailability of deeply buried, recalcitrant organic matter, and might, therefore, provide energy and carbon sources for a very deep and hot subsurface biosphere (Wellsbury et al. 1997) below the relatively cool subsurface sediments within a few hundred meters depth that have been generally sampled. Integrated microbiological and geochemical characterizations of deep, geothermally heated hydrocarbon reservoirs have shown that long-term microbial degradation of petroleum hydrocarbons to dense, heavy oil peters out at

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_42,

approx. 80°C; this limit might represent a practical temperature barrier at least for hydrocarbon-degrading microbial communities in the subsurface (Head et al. 2003). Continued investigation of the deep and hot subsurface biosphere will require increasingly deep subsurface drilling integrated with microbiological and geochemical habitat characterization. In this regard, the frequently used term "deep, hot biosphere" as coined by Gold (1992) carries the connotation of abiogenic syntheses of organic compounds under high heat and pressure, and therefore implies subsurface life that is entirely independent of buried organic matter and fossil hydrocarbons – tantalizing but elusive.

In general, microbial cell counts in subsurface sediments decrease exponentially with depth, most likely as a result of decreasing organic carbon quality and availability in aged, deeply buried sediments (Parkes et al. 2000). Cell concentrations start in the range of 10^9 cells per ml at the surface of organic-rich, continental margin sediments, and decrease toward 10^6 cells per ml near 1,000 m depth, given some extrapolation due to scarce data at the deep end (Parkes et al. 2000). Recent counts in organic-lean sediments have used newly developed cell separation methods to concentrate countable cells (Kallmeyer et al. 2008) and to push the statistical limits of direct microscopic quantification. Cell count profiles in the ultraoligotrophic sediments of the South Pacific Gyre started at ca. 10^6 cells per ml near the sediment surface and decreased toward 10^3–10^4 cells near 10 m sediment depth (D'Hondt et al. 2009), an offset by three orders of magnitude in comparison with the previously assembled dataset dominated by continental margin sediments (Parkes et al. 2000). Cell counts from moderately oligotrophic open ocean locations, for example, northwest of New Zealand outside the South Pacific Subtropical Gyre, represent intermediate values between the ultraoligotrophic and the continental margin endmembers (D'Hondt et al. 2009). These integrated global cell counts in the marine sedimentary subsurface account for ca. 0.6% of Earth's living biomass (Kallmeyer et al. 2012).

Currently, epifluorescence cell count techniques in deep subsurface sediments are being improved by automation (Morono et al. 2009; Morono and Inagaki 2010), with the goal of reducing this time-consuming procedure to a more manageable task that frees up shipboard research time and science crew. Removing or reducing background fluorescence, for example, in hydrocarbon- and organic-rich sediments, is also a crucial development (Lappe and Kallmeyer 2012).

Are these deep subsurface cells to some extent metabolically active? Bacterial and archaeal cells were detected and quantified by qPCR, FISH, and CARD-FISH, methods that rely on intact ribosomal RNA genes and intact ribosomal RNA maintained by living cells (Schippers et al. 2005; Biddle et al. 2006), and also by structural and ^{13}C-isotopic analysis of intact polar membrane lipids of bacteria and archaea (Sturt et al. 2004; Biddle et al. 2006; Lipp et al. 2008) which are chemically labile and therefore indicate active, living microbial populations. These lines of evidence indicate that subsurface microbes maintain essential macromolecules and cell structures, and gain the minimum of metabolic energy that is required for this purpose. However, comparisons of anaerobic respiration rates in deep marine sediments with cell numbers in the same environments repeatedly indicated extraordinarily low cell-specific metabolic rates (D'Hondt et al. 2002); the results questioned whether the majority of microbial cells in the deep subsurface were alive, in the sense that they were capable of metabolic activity and growth. Recently, it was demonstrated that additions of carbon and nitrogen substrates resuscitate the majority of apparently dormant or minimally active bacterial and archaeal cells in the subsurface; substrate uptake and cell growth were monitored by stable isotope tracer incubation combined with nanometer-scale secondary ion mass spectrometry (Nano-SIMS) (Morono et al. 2011). If these results from a continental margin sediment can be generalized, they indicate that most microbial cells in deep subsurface sediments are alive and can be resuscitated by substrate pulses and added energy sources, but their slow metabolism in situ merely sustains very slow biomass turnover on time frames ranging from years to millennia (Jørgensen and Boetius 2007; Jørgensen 2011). Quantifications for the turnover times of biomass (vegetative microbial cells and endospores) in Peru Margin and Peru Trench deep sediments are in the range of thousands of years, whereas necromass (buried bulk organic carbon) turns over two orders of magnitude more slowly (Lomstein et al. 2012). Under these conditions, deeply buried microbial cells could plausibly represent the descendants of microbial communities that were deposited thousands or millions of years ago, and have since then persisted by slowly degrading and assimilating of organic carbon that has been buried at the same time. In this regard, buried microbial communities could represent living fossils that are descended from, and thus provide a record of, past microbial input into the sediment column (Inagaki et al. 2005). High-resolution biogeographic studies of deep sediment columns that rely on more sensitive genetic markers than 16S rRNA genes might address this question in the future.

General Biogeochemical Controls

The gradual downcore depletion of electron acceptors places energetic constraints on deep subsurface microbial communities. Sulfate, the most highly concentrated electron acceptor in seawater and consequently the dominant electron acceptor for anaerobic metabolism in marine sediments (Jørgensen 1982), penetrates organic-lean deep marine subsurface sediments on a scale of tens to hundreds of meters, whereas in organic-rich coastal and continental margin sediments, heterotrophic, sulfate-reducing microbial populations deplete sulfate within a few meters of the sediment–water interface (D'Hondt et al. 2002, 2004). Other anaerobic electron acceptors occur in lower concentrations in the sediment column, and penetrate the sediment column to a lesser extent before they are used up. Downcore electron acceptor depletion is ultimately controlled by the amount of buried organic carbon in the sediment column, which varies substantially between continental margin and open ocean sites (Meister et al. 2006). Most microbial community studies have been performed in continental margin locations with reducing, organic-carbon replete, and electron

acceptor-limited marine subsurface sediments (see Parkes et al. 1994; Reed et al. 2002; D'Hondt et al. 2004; Parkes et al. 2005; Sørensen and Teske 2006; Biddle et al. 2006; Inagaki et al. 2006; Nunoura et al. 2008; Webster et al. 2006). Yet, most of the seafloor is covered by organic-poor sediments on deep continental slopes and in abyssal basins; almost 90% of the ocean floor lies deeper than 2,000 m (Dunne et al. 2007). In contrast to margin or coastal sediments, these sediments are characterized by low organic carbon content (<<1%), slow rates of deposition (Seiter et al. 2004), and slow depletion of electron acceptors that can penetrate deeply into the sediment column. Abyssal sediments of the extremely oligotrophic subtropical gyres are permeated by dissolved porewater nitrate and oxygen over tens of meters or down to basement basalt, indicating minimal microbial electron acceptor demand within the sediment column (Gieskes and Boulègue 1986; D'Hondt et al. 2004, 2009; Ziebis et al. 2012; Røy et al. 2012). Interestingly, the penetration depth of oxygen in these sediments depends not only on net organic carbon input controlled by primary productivity of the overlying water column and by microbial degradation during passage through the water column (Wakeham et al. 1997), but also on sedimentation rate and carbon burial depth. Very slow sedimentation concentrates organic carbon input at the sediment surface and counteracts carbon burial, which limits carbon remineralization rates and oxygen demand within the subsurface sediment, and results in very deep oxygen penetration (Røy et al. 2012).

Oxidants permeate the sediment column not only from the seawater/sediment interface, but also from the underlying basaltic crust, where oxidized seawater circulates through deep aquifers and conduits and creates a second oxidation interface. In contrast to the classical downcore redox sequence of marine sediments (Froelich et al. 1979), the reduced (or, less oxidized) center of the sediment column then appears sandwiched between somewhat symmetrical profiles of porewater oxidants in the top and bottom sediment layers (DeLong 2004; D'Hondt et al. 2004; Ziebis et al. 2012). In another deviation from the standard redox sequence, different anaerobic respiration processes can have similar in-situ energy yields and therefore coexist in the sediment column, as demonstrated for iron and sulfate reduction in oligotrophic sediments of the Pacific Ocean (Wang et al. 2008, 2010).

The low microbial activities and respiration rates in these oligotrophic sediments (Wellsbury et al. 2002; D'Hondt et al. 2009; Picard and Ferdelman 2012; Røy et al. 2012) nevertheless reveal persistent microbial life that, in principle, remains accessible with cultivation and gene-based approaches. These bacterial and archaeal communities change in composition according to electron acceptor availability and redox stratification of the sediment (Sørensen et al. 2004; Durbin and Teske 2011); occasionally DNA detection becomes limiting (Durbin and Teske 2010) and can distort the sequence-based microbial community census (Webster et al. 2003).

With decreasing numbers of microbial cells, microbial detection assays, especially PCR assays or selective enrichment cultures, become increasingly prone to microbial contamination from entrained seawater, drilling fluid and other circulating fluids, ship sewage, or laboratory reagents and equipment. Sewage released from a drill ship can impact microbial surveys directly or indirectly by triggering microbial blooms around the drill ship (Santelli et al. 2010). Conservative chemical tracers that are continuously mixed into the drilling lubricating fluid reveal the extent of seawater and drilling fluid penetration into the cored sediment (Smith et al. 2000; House et al. 2003; Lever et al. 2006). The extent of bacterial contamination can be monitored by adding live tracer organisms, for example, *Xanthomonas* spp., to the recirculating drilling fluid (Masui et al. 2008). Even if external contamination is ruled out, sample storage for post-cruise cultivation and activity measurements remains a concern, as microbial communities in live sediments change significantly over time—almost certainly as a consequence of gradual sample oxidation—and lead to unintended enrichments and microbial community shifts (Lin et al. 2010; Mills et al. 2012a).

Bacterial 16S rRNA Lineages

The bacterial subsurface biosphere contains multiple phylum-level lineages characterized by diverging cultivation success. Numerous subsurface strains and species of the Proteobacteria, Firmicutes, and Actinobacteria have been brought into culture, whereas most other phylum-level lineages have resisted cultivation. The extent of microbial diversity in the deep subsurface is rapidly expanded and redefined by sequencing surveys, whereas cultivation-based studies advance at a slower pace but provide essential links between microbial community structure and function. The disciplinary boundaries between molecular and cultivation-based microbiology begin to blur in many innovative in situ microbial activity assays, for example, Nano-SIMS detection of microbial carbon assimilation and growth in deep subsurface cells (Morono et al. 2011). This overview on subsurface microbial communities proceeds from entirely or largely uncultured bacterial phyla to those that are also reasonably well represented by cultures, and then, for contrast, discusses the subsurface archaea that—with the exception of the methanogens—have been highly recalcitrant to cultivation so far. It is hoped that new developments in cultivation of deep subsurface microorganisms, for example, enrichment and isolation under in situ pressure (Parkes et al. 2009; Sauer et al. 2012), long-term enrichment strategies (Imachi et al. 2011), separation of cells into microspheres (Zengler 2010; Zengler et al. 2005), and in situ enrichment in the subsurface (Orcutt et al. 2010) will lead to a more representative evolutionary and physiological range of subsurface isolates.

Microbial community analyses of marine subsurface sediments have impacted the evolving census of the microbial world, as several novel phylum-level lineages of bacteria and archaea were first discovered in deep marine sediments, or dominate 16S rRNA clone libraries from these habitats. The tally of originally twelve bacterial phyla (the highest-order lineages within the bacterial domain) before the onset of environmental rRNA sequencing (Woese 1987) grew quickly to 36 (Hugenholtz et al. 1998a,b) before increasing further to 52

(Rappé and Giovannoni 2003); more recently, 15 additional new bacterial phyla were added from deep rRNA sequencing of a single marine microbial mat (Rey et al. 2006). Currently (May 2012), the ARB-Silva database recognizes a total of 57 bacterial phylum-level lineages; of these, 27 phyla have cultured members and 30 phylum-level candidate lineages remain without cultured representatives (http://www.arb-silva.de). Cloning and sequencing of bacterial 16S rRNA genes from marine subsurface sediments consistently detected several bacterial phylum-level lineages, notably the Chloroflexi, the Japan Sea 1 group, the Planctomycetes, Proteobacteria, Deferribacteres, and several uncultured lineages (OP1, OP3, OP8, OP10, OP11, WS1, WS3) initially found in terrestrial hot springs (Hugenholtz et al. 1998a) and in hydrocarbon-contaminated soil (Dojka et al. 1998). These phyla have become extensively populated with bacterial rRNA sequences, phylotypes, and sequence clusters from marine subsurface sediments (Kormas et al. 2003; Newberry et al. 2004; Inagaki et al. 2006; Teske 2006; Teske and Sørensen 2008; Fry et al. 2008; Roussel et al. 2009; Blazejak and Schippers 2010; Durbin and Teske 2011; Orcutt et al. 2011).

Japan Sea Group 1

The first subsurface lineage of bacteria discovered during the first PCR-based 16S rRNA analysis of marine subsurface sediments (Rochelle et al. 1994) was designated Japan Sea Group I (JS-1), after the location of its discovery during ODP Leg 128 in the Japan Sea (Webster et al. 2004). The JS-1 group was also reported under different names from deep subsurface sediments in the Nankai Trough, southeast of Japan (Kormas et al. 2003; Newberry et al. 2004) and hydrothermal sediments at Guaymas Basin (Teske et al. 2002), and is common in organic-rich marine sediments (Webster et al. 2004). In a study of shallow intertidal subsurface sediments of the German Wadden Sea, JS-1 bacteria were frequently found in organic-rich, sulfate-depleted sediment layers, and appeared only rarely in clone libraries from organic-poor intertidal sediments (Webster et al. 2007). JS-1 represented the most frequently found bacterial group in 16S rRNA clone library surveys of organic-rich, highly reducing sediments of the Peru Trench and the Cascadia Margin (Inagaki et al. 2006). Members of the JS-1 group and of the Chloroflexi subphylum I have been enriched from marine sediments under anaerobic long-term incubations, using artificial seawater media amended with glucose, acetate, and sulfate (Webster et al. 2011). These results are compatible with a heterotrophic, anaerobic metabolism for JS-1 bacteria.

Chloroflexi

Sequencing surveys of 16S rRNA gene clone libraries from shallow subsurface sediments have shown that the Chloroflexi become a dominant bacterial phylum within a few meters downcore where they replace other bacterial phyla, especially the Proteobacteria, that are most frequently found in the surficial sediment layers. This trend has been documented in such divergent locales as German Wadden Sea intertidal sediments (Wilms et al. 2006) and South Pacific abyssal sediments (Durbin and Teske 2011). The physiological rationale for this Proteobacterial/Chloroflexi shift remains poorly known, although the cultivation of some Chloroflexi bacteria has provided some clues on their metabolism. Among the cultured members of the Chloroflexi, species and strains of the genera *Dehalogenimonas* and *Dehalococcoides* are anaerobic H_2-oxidizers that respire with halogenated hydrocarbons (Moe et al. 2009). Growth on organohalides in the subsurface is supported by the detection of reductive dehalogenase homologous (rdhA) genes in a wide range of marine subsurface sediments (Futagami et al. 2009), including ODP Site 1226 in the eastern equatorial Pacific, Site 1227 on the Peru Margin, Site 1230 in the Peru Trench, IODP Site 1301 on the Juan de Fuca Ridge Flank, and sites C9001 off Shimokita and C0002 in the Nankai Trough Forearc Basin (Futagami et al. 2009). Although the successful *rdhA* primer pair was originally designed to target *Dehalococcoides* (Krajmalnik-Brown et al. 2004), and indeed detected sequences closely related to terrestrial *Dehalococcoides*, the Subseafloor *rdhA* clusters I and II diverged from previously detected *Dehalococcoides* sequences (Futagami et al. 2009); thus, deep subsurface sediments harbor novel types of halorespiring Chloroflexi. Most likely, additional metabolisms occur within the subsurface Chloroflexi. Several new genera and species of filamentous, mostly anaerobic and fermentative bacteria within the Chloroflexi have been isolated from organic-rich, reducing habitats such as anaerobic bioreactors and waste-water processing plants (Sekiguchi et al. 2001, 2003; Yamada et al. 2006, 2007). In molecular assays combining microsphere adhesion to cells with enzyme-labeled fluorescence, several uncultured Chloroflexi populations were shown to express chitinase, esterase, galactosidase, and glucuronidase activity, although under aerobic conditions (Kragelund et al. 2007). Hydrolysis of polysaccharides as a metabolic strategy would be consistent with the dominance of Chloroflexi in bacterial 16S rRNA gene clone libraries from organic-rich marine sapropel layers where elevated hydrolysis rates of monomeric, fluorescently labeled sugars have been observed (Coolen et al. 2002). Fermentative or polymer-degrading metabolisms may extend to many subsurface representatives of the Chloroflexi. For example, Chloroflexi were successfully enriched from Indian continental margin subsurface sediments under pressurized conditions in anaerobic, heterotrophic media (Parkes et al. 2009). Recently, the first Chloroflexi strains that fall into the subsurface clone groups (Chloroflexi subphylum I) were isolated after long-term incubation of subseafloor sediments under methanogenic conditions; they turned out to be anaerobic heterotrophs that can be grown on glucose and yeast media (Imachi et al. 2011).

Planktomycetes

The phylum Planktomycetes shows a wide divergence of metabolisms among its cultured members. Most cultured genera

within this phylum isolated from soil, freshwater, and peat bogs are chemoheterotrophic, and are capable of degrading various complex polysaccharides, whereas a distinct, autotrophic lineage harbors bacteria capable of anaerobic ammonia oxidation (anammox) (Fuerst and Sagulenko 2011). All currently cultured members of the Planctomycetes are characterized by unusual intracellular compartmentalization and peculiar cell walls lacking peptidoglycane (Fuerst and Sagulenko 2011). Most Planktomycetes clones from the marine subsurface sediments fall into phylogenetic lineages that are distinct from the cultured heterotrophic or ammonium-oxidizing representatives of this phylum (Durbin and Teske 2011). One of their preferred habitats appears to be within and below the nitrate-reducing zone in organic-lean marine subsurface sediments (Durbin and Teske 2011) and in stratified marine water columns (Kirkpatrick et al. 2006).

Proteobacteria

The Proteobacteria in marine subsurface sediments defy succinct summaries; in contrast to the sparsely cultured JS-1 bacteria, Chloroflexi, and Planktomycetes, they harbor the majority of cultivated bacterial species that are known today, with a wide range of heterotrophic, autotrophic, aerobic, and anaerobic metabolisms. Regarding their phylogenetic division into at least six subphyla (Alpha-, Beta-, Gamma-, Delta-, Epsilon-, and Zetaproteobacteria), they resemble the "superphyla" that have been proposed for other major bacterial phyla that appear to be linked by shared common ancestry (Wagner and Horn 2006). Interestingly, extensive cultivation surveys of deep subsurface bacteria have succeeded in isolating predominantly Proteobacteria, together with Firmicutes, Actinobacteria, and Bacteroidetes (D'Hondt et al. 2004; Toffin et al. 2004a,b, 2005; Lee et al. 2005; Biddle et al. 2005a,b; Batzke et al. 2007; Parkes et al. 2009). In general, this spectrum of cultured subsurface isolates is narrower than the diverse lineages of bacteria and archaea that were detected using DNA-based molecular methods. However, within this window of cultured strains there is good evidence that these isolates are actually native inhabitants of the sedimentary subsurface. The best example of a cultured subsurface bacterium that contributes substantially to the molecular tally of in situ subsurface populations, the Alphaproteobacterium *Rhizobium radiodurans*, has been found in mediterranean sapropel sediments where it constitutes up to 5% of the prokaryotic communities (Suess et al. 2004, 2006). This species also represented the most frequently isolated Proteobacterium from Pacific Ocean, Peru Margin, and Peru Trench sediments (D'Hondt et al. 2004; Batzke et al. 2007). A second proteobacterial "hot spot" of frequently isolated subsurface bacteria can be found within the Gammaproteobacteria. Here, several independent studies have found members of the genera *Marinobacter*, *Psychrobacter*, *Photobacterium*, and *Vibrio* in the Peru Margin and Peru Trench sediments (Batzke et al. 2007); *Photobacterium*, *Vibrio*, *Shewanella*, and *Halomonas* in the Peru Trench (Biddle et al. 2005a,b); *Shewanella* and *Pseudomonas* in Nakai Trough sediments (Toffin et al. 2004b), *Vibrio* in Mediterranean sapropels (Suess et al. 2004), and *Pseudomonas* in Indian continental margin sediments (Parkes et al. 2009). A third area of proteobacterial cultivation success can be found within the Deltaproteobacteria. Here, incompletely oxidizing sulfate-reducing bacteria of the genera *Desulfovibrio* (Bale et al. 1997; Barnes et al. 1998; Fichtel et al. 2012) and *Desulfomicrobium* (Batzke et al. 2007) have been isolated from subsurface sediments of the Peru Margin, the open Pacific Ocean and the Juan de Fuca Ridge Flank. A *Desulfofrigus* strain has been cultured from Nankai Trough sediments (Toffin et al. 2004b). With the exception of a single *Desulfotignum* isolate (Fichtel et al. 2012), sulfate-reducing bacteria of the Desulfobacteriales that remineralize a wide range of low-molecular-weight organic substrates completely to DIC are missing from the subsurface culture collection, although they generally dominate molecular surveys of surficial marine and estuarine sediments (Bahr et al. 2005).

At present, it is difficult to say why exactly this mixed assemblage of heterotrophic, aerobic, facultatively, and obligately anaerobic Alpha-, Gamma-, and Deltaproteobacteria is preferentially recovered from subsurface sediments. Many of these bacteria belong to genera that are typically recovered in cultivation surveys of marine heterotrophs in the water column and in surficial sediments. Although these specific Proteobacteria do not appear to be conspicuous or abundant in most molecular surveys of the marine subsurface, they might be among the most enthusiastic responders to in situ substrate and energy stimulation (Morono et al. 2011).

Molecular Detection of Sulfate-Reducing Bacteria in Subsurface Sediments

The sporadic isolation and spotty 16S rRNA detection of deltaproteobacterial sulfate reducers does not mean that they occur rarely in the deep subsurface. Extensive downward depletion of porewater sulfate, downward accumulation of sulfide under sufficiently reducing conditions (D'Hondt et al. 2002, 2004), sulfate reduction activities modeled on sulfate profiles (Canfield 1991), and biogenic sulfur-isotope fractionation of sulfates and sulfides (Böttcher et al. 2006), reveal pervasive microbial sulfur cycling in deep subsurface sediments. Subsurface sulfate-reducing bacteria and archaea are targeted with functional gene surveys based on PCR, cloning, and sequencing of two functionally conserved and phylogenetically informative key genes, dissimilatory sulfite reductase alpha and beta subunit (*dsrA* and *dsrB*) and adenosine-5′-phosphosulfate reductase alpha subunit (*aprA*). These genes allow the selective detection and phylogenetic identification of sulfate-reducing and sulfite-reducing prokaryotes against considerably more abundant background populations (Wagner et al. 1998; Klein et al. 2001; Friedrich 2002; Wagner et al. 2005; Zverlov et al. 2005). The *dsrA* gene provided the basis for a qPCR assay (Kondo et al. 2004) that was subsequently used for quantitative assessments of deep subsurface sulfate reducers. In qPCR quantifications of

Peru Margin site 1227, high *dsrA* and *aprA* gene copy numbers in the range of 10^7–10^8 gene copies per gram sediment were found in near surface sediments; these numbers decrease toward 10^5–10^6 gene copies per gram sediment in the shallow subsurface near 1 m depth and gradually toward 10^3–10^4 gene copies per gram sediment at 10 m sediment depth, before sinking below detection threshold at 121 and 42 mbsf, respectively (Blazejak and Schippers 2012). A similar decline from 10^7–10^8 gene copies per gram sediment near the surface to below 10^3 gene copies per gram sediment was observed over the top 6 m of sediments from the central basin of the Black Sea (Blazejak and Schippers 2012; Schippers et al. 2012). In sediments from the highly productive Benguela upwelling area offshore Namibia, the initially high *dsrA* and *aprA* gene copy numbers at the sediment surface (quite variable, 10^6–10^9) stabilized at higher values (10^4–10^7 down to 5 m depth) than in the Black Sea and the Peru Margin; in these sediments, the *aprA* genes were unexpectedly considerably more abundant than the *dsrA* genes (Schippers et al. 2012). The *dsrA* and *aprA* gene copy numbers remained mostly two to three orders of magnitude below 16S-rRNA gene-based qPCR quantifications at the Peru Margin, in the Black Sea, and (disregarding the high *aprA* counts) offshore Namibia. Thus, sulfate reducers generally represent on the order of 1% or less of the overall bacterial population in reducing subsurface sediments of the Black Sea and the Peru Margin (Schippers and Neretin 2006; Blazejak and Schippers 2012). Similar trends were found independently in Eastern North Atlantic sediments of the Porcupine Basin, where relatively low *dsrA* gene copy numbers in the range of 10^3–10^5 gene copies/ml remain mostly three orders of magnitude below bacterial 16S rRNA gene counts (Webster et al. 2009). In organic-poor deep-water turbidite sediments, *dsrA* gene detection becomes spotty, and quantification remains in the low range of 10^2–10^4 gene copies per ml (Nunoura et al. 2009). In coastal, organic-rich sediments with high sulfate-reducing activity (Arhus Bay, Denmark), the proportion of sulfate reducers determined by *dsrA* gene qPCR increased to over 10% of the total bacterial count (Leloup et al. 2009). Viewed together, the *dsrA* and *aprA* gene copy trends are consistent with the geochemical prognosis for abundance and activity of sulfate reducers: The highest numbers are found in surficial sediments where fresh organic matter from the water column is supplied; gene copy numbers decrease downcore within each site, and also decrease when moving from organic-rich sediments in shallow coastal waters, to organic-lean or recalcitrant sediments in deep-water locations.

The *dsrA* and *aprA* genes recovered from the Peru Margin and Black Sea subsurface sediments belonged to diverse clusters and lineages of Deltaproteobacteria (families *Desulfobacteriaceae*, *Desulfovibrionaceae*, *Desulfobulbaceae*, *Desulfoarculaceae*, *Synthrophobacteraceae*), Firmicutes (genus *Desulfotomaculum*), and clusters without cultured representatives (dsrA clusters A and B) (Blazejak and Schippers 2012). Some of the *dsrA* gene phylotypes recovered from deep subsurface sediments were nearly identical to those of cultured sulfate-reducing bacteria (*Desulfovibrio acrylicus*, Peru Margin) and in addition to each other; for example, *dsrA* phyloytypes of *Desulfobacterium autotrophicum* were recovered both from Peru Margin and Black Sea sediments (Blazejak and Schippers 2012). Independent *dsrAB* clone library surveys of sediment cores from the Peru Margin, the Black Sea, and the coastal Baltic Sea have recovered multiple phylogenetic lineages mostly within the uncultured Group IV (Webster et al. 2006), the Desulfobacteraceae, the Firmicutes, and numerous uncultured groups (Leloup et al. 2007, 2009). Thus, the marine sedimentary subsurface is not dominated by a single group or by a phylogenetically restricted subgroup of sulfate reducers.

Firmicutes and Actinobacteria

Although members of the Firmicutes, the low G + C gram-positive bacteria do not dominate 16S rRNA clone library surveys, they are repeatedly isolated from deep subsurface sediments and constitute perhaps the most consistently isolated group of cultivable subsurface bacteria, under atmospheric pressure and also under in situ pressurized conditions. Two new species of the genera *Acetobacterium* and *Marinilactobacillus* were isolated from sediments of Nankai Trough, a subduction trench southeast of Japan (ODP Leg 190, site 1173) (Toffin et al. 2005). A similar assemblage of Firmicutes, containing strains of *Acetobacterium*, *Marinilactobacillus*, *Clostridium*, and *Carnobacterium*, were isolated under in situ hydrostatic pressure and anaerobic, heterotrophic conditions from the Indian continental Margin (Parkes et al. 2009). Two species of the newly described thermophilic genus *Thermosediminibacter* were isolated from the upper 10 m of the sediment column at ODP Sites 1227, 1228, and 1230 at the Peru Margin and the Peru Trench (Lee et al. 2005). Numerous strains related to the genera *Alkaliphilus*, *Paenibacillus*, and *Bacillus* within the Firmicutes, and strains of the genera *Kocuria*, *Brachybacterium*, *Janibacter*, and *Oerskovia* within the Actinobacteria were isolated from a wide range of organic-poor to organic-rich sediments in the Equatorial Pacific Ocean, Peru Margin, Peru Trench, and Peru Basin sediments (Batzke et al. 2007). Their habitat range exceeded the range of gammaproteobacterial isolates from the same survey. A similar assemblage of spore formers, with strains of the genera *Brachybacterium*, *Micrococcus*, and *Bacillus*, was isolated from Mediterranean sapropels (Süß et al. 2004). The isolation of acetogenic sporeformers closely related to *Acetobacterium psammolithicum* in Juan de Fuca sediment indicates the existence of acetogenic bacteria in subsurface sediments (Fichtel et al. 2012). Sulfate-reducing Firmicutes of the genera *Desulfotomaculum* and *Desulfosporosinus* were isolated from Cascadia Margin (Barnes et al. 1998) and from Juan de Fuca Ridge flank sediments (Fichtel et al. 2012).

The wide distribution of gram-positive, spore-forming bacteria most likely represents a consequence of broad endospore dispersal. Spores are resistant against nutrient depletion, desiccation, salinity and temperature fluctuations, radiation, and changing redox conditions, and they can be resuscitated after

long dormancy. A historical bacterial culture of known age has been revised after 34 years (Braun et al. 1981); spores from archaeological samples and ancient lake sediments have survived for several 1,000 s of years (Gest and Mandelstam 1987); finally, spore-forming bacteria were revived from the guts of a bee encased in fossil amber for 25–40 Ma (Cano and Borucki 1995). Thus, endospore-forming gram-positive bacteria may remain dormant for longtime periods in deep subsurface sediments until sample retrieval, resuscitation, and isolation. The deep, anoxic, geothermally heated subsurface has been suggested as the source habitat of thermophilic spore formers related to the genera *Desulfotomaculum*, *Chlostridium*, and *Eubacterium* that constantly reinoculate marine surficial sediments of the world's oceans, and accumulate to high abundances in marine surface sediments that are too cold for them to grow (Isaksen et al. 1994; Hubert et al. 2009). However, this source habitat does not necessarily have to be the marine subsurface. Consistent with the working hypothesis of a terrestrial source, many species of the genus *Desulfotomaculum* have been originally isolated from geothermally heated oil fields, geothermal springs, and deep terrestrial sediments, as summarized in Amend and Teske (2005). In marine sediments at Juan de Fuca, *Desulfotomaculum* and *Desulfosporosinus* strains have been isolated from the top 10 m of the sediment column, but not from geothermally heated deeper sediments near basement basalt (Fichtel et al. 2012). The Peru margin strains and species of the thermophilic genus *Thermosediminibacter* were isolated from the top 1 or 2 meters of the sediment column, whereas enrichment and isolation attempts from deeper sediment layers remained unsuccessful (Lee et al. 2005). Terrestrial soils that either heat up geothermally or under solar irradiation could provide a source habitat for at least some of these thermophilic firmicutes cultured from marine subsurface sediments. However, it is very likely that the downward extent of gram-positive bacteria and other endospore formers in the sediment column exceeds the range of successful cultivations and resuscitations. Endospore quantifications in Peru Margin sediments based on muramic acid and dipicolinic acid determination (the latter a unique cell wall component of endospores) yielded endospore counts very close to acridine orange counts of prokaryotic cells throughout the sediment column (Lomstein et al. 2012); these results suggest a steady input rate of spore-forming cells throughout the depositional history of the Peru Margin and Peru Trench sediments.

Acetogenic Bacteria

The acetogens are taxonomically diverse bacteria capable of generating acetate autotrophically from CO_2 and H_2, or by heterotrophically using C1-compounds derived from complex substrates and polymers. Acetogens are among the most consistently cultured bacteria in the sedimentary subsurface. For example, autotrophic and heterotrophic acetogens were cultured throughout deep oligotrophic sediments of the Woodlark Basin, near Papua New Guinea (Wellsbury et al. 2002). More than half of all currently described strains and species with acetogenic capability belong to the heterotrophic category (Lever et al. 2010). The wide substrate range utilized as C1-source by heterotrophic acetogens means that acetogenic microbial populations can access and utilize a wider range of substrates than sulfate-reducing and methanogenic communities with more restricted substrate ranges (Lever et al. 2010). Acetogenesis is energetically feasible and competitive with sulfate reduction and methanogenesis in the subsurface; one of the reasons is the use of the acetyl-CoA pathway for both energy generation and biosynthetic carbon assimilation, tapping into a wide range of energy-rich carbon substrates (Lever 2012; Oren 2012). Recent δ^{13}C-isotopic analyses of porewater acetate have indicated a significant acetogenic contribution to total acetate turnover in subsurface sediments at Juan de Fuca on the Cascadia Margin (Heuer et al. 2009; Lever et al. 2010); these results prompted a functional gene investigation at the same location (Lever et al. 2010) based on a PCR assay for the gene for formyl tetrahydrofolate synthetase (*fhs*), an enzyme that catalyzes the ATP-dependent activation of formate in acetogenesis (Leaphart et al. 2003). The original, highly degenerate PCR primers (Leaphart et al. 2003) were complemented with a new, less degenerate primer pair that targets a smaller gene fragment for the successful detection of *fhs* genes in deep subsurface sediments of the Juan de Fuca Ridge (Lever et al. 2010). Most *fhs* gene sequences were related to homologs from cultured Deltaproteobacteria, or the non-acetogenic sulfate-reducing firmicute *Desulfotomaculum reducens*. Classical gram-positive acetogens within the genera *Clostridium*, *Acetobacterium*, or *Thermoanaerobacterium* were not found (Lever et al. 2010), although these acetogens within the Firmicutes are quite frequently isolated from diverse subsurface sediments (Parkes et al. 2009; Fichtel et al. 2012). The likely importance of acetogenic metabolisms in the sedimentary subsurface justifies further development of molecular tools for acetogen detection (Gagen et al. 2010), and expansion of the reference sequence database especially for heterotrophic acetogens.

Archaeal 16S rRNA Lineages

The domain Archaea accounts for a large portion, perhaps the majority, of deep subsurface prokaryotic cells and biomass (Biddle et al. 2006; Lipp et al. 2008). Within the archaeal domain, two subdomain-level branches were initially recognized, the crenarchaeota represented by hyperthermophilic, sulfur-dependent archaea from hot springs and hydrothermal vents and the euryarcheota containing the methanogens and extreme halophiles (Woese 1987). Gradually, new archaeal discoveries superseded the traditional crenarchaeotal/euryarchaeal divide (Hugenholtz 2002; Schleper et al. 2005; Guy and Ettema 2011), and new lineages, in part from the marine subsurface, contributed to this ongoing revision of archaeal diversity: the Korarchaeota discovered in hot terrestrial springs (Barns et al. 1996), the Ancient Archaeal Group and the Marine Hydrothermal Vent Group lineages that appear in hydrothermal environments and

in cold subsurface sediments (Takai and Horikoshi 1999; Nunoura et al. 2009), the Thaumarcheota (Brochier-Armanet et al. 2008), the Aigarchaeota (Nunoura et al. 2010), the obligate symbiont *Nanoarchaeum equitans* (Huber et al. 2002), and the Marine Benthic Group B (Vetriani et al. 1999) that is ubiquitous in anoxic subsurface marine sediments.

Within the archaeal domain, the most commonly encountered subsurface phylum-level lineages are the Miscellaneous Crenarchaeotal Group (MCG), discovered in deep marine sediments (Inagaki et al. 2003) and hydrothermal vents (Takai and Horikoshi 1999); the Marine Benthic Groups A to E (MBG-A to MBG-E) first found in open ocean sediments (Vetriani et al. 1999); the Deep-Sea Hydrothermal Vent Euryarchaeotal groups 1–7 (DHVG-1–7), originally detected in hydrothermal vent habitats (Takai and Horikoshi 1999); and the South African Goldmine Euryarchaeotal Group (SAGMEG) first found in terrestrial deep goldmines (Takai et al. 2001) but also widespread in marine subsurface sediments (*Fig. 8.1*).

Continued surveys of deep subsurface sediments, surficial marine sediments, and deep-sea hydrothermal vents have uncovered additional, not-yet cultured archaeal lineages (*Fig. 8.1*). The most frequently found lineages remain the MCG, MBG-B, MBG-D, and SAGMEG clades, but other lineages are found in oligotrophic marine sediments (Deep-Sea Euryarchaeotal Groups 1–4, DSEG 1–4; Durbin and Teske 2011); some sediment clones appear also within the DHVEG clades that were originally found at hydrothermal vents (Takai and Horikoshi 1999). Thus, the 16S rRNA phylogeny provides the taxonomic skeleton for a complex archaeal subsurface biosphere, and suggests linkages between the sedimentary and the hydrothermal biospheres. The names of these archaeal lineages reflect the absence or the problematic ambiguity of ecophysiological clues and hypothesis-generating phylogenetic affiliations; genomic and cultivation approaches will be required to make progress.

The Miscellaneous Crenarchaeotal Group (MCG), one of the most frequently found and widespread archaeal subsurface lineages, is currently being investigated by fosmid sequencing (Meng et al. 2009; Li et al. 2012), molecular quantification in multiple environments (Kubo et al. 2012), and single-cell genome sequencing (Karen Lloyd et al. 2012; Lloyd et al. 2012 Goldschmidt abstract). Members of the MCG archaea have been enriched from marine sediments in stable isotope probing experiments with ^{13}C-labeled acetate (Webster et al. 2010). Interestingly, the MCG archaea were so far most abundantly detected in shallow pushcore sediments from a coastal estuary in North Carolina, the White Oak River, and showed unchanging downcore abundance profiles that do not respond to sulfate/methane gradients in these organic-rich sediments (Kubo et al. 2012). These results are broadly consistent with the working hypothesis that MCG archaea can use low-molecular-weight organic acids as well as complex biopolymers as carbon source for heterotrophic, anaerobic, and most likely fermentative metabolism; they are unlikely to drive the methane and sulfur cycles as sulfate reducers, methanogens, or sulfate-dependent methanotrophs.

Cultured archaea from marine subsurface sediments are at present mostly limited to the methanogens; the new species *Methanoculleus submarinus* (Mikucki et al. 2003), *Methanococcus aeolicus* (Kendall et al. 2006), *Methanosarcina baltica* (von Klein et al. 2002), and new strains of the genera *Methanosarcina*, *Methanobacterium*, *Methanococcoides*, and *Methanobrevibacter* (Imachi et al. 2011) have been isolated, in part after extensive preincubation and enrichment of methanogens from marine subsurface sediments under methanogenic conditions (Imachi et al. 2011). Long-term incubation and enrichment could be an essential strategy to increase the success of methanogenic enrichments, as demonstrated with a 2-year methanogenic incubation experiment with sediments from Hydrate Ridge (Kendall and Boone 2006). In addition to these methanogenic archaea from deep sediments, a hyperthermophilic sulfate-reducing archaeon of the genus *Archaeoglobus* was isolated from thermal deposits inside an in situ borehole monitoring station at Juan de Fuca (Nakagawa et al. 2006; Steinsbu et al. 2010), possibly reflecting contrasting sulfate availability in hydrothermally influenced boreholes where basalt-hosted formation fluids derived from entrained seawater provide sulfate, and in deep sediments where sulfate is ultimately depleted and methane accumulates. Sulfate availability is a crucial geochemical control on the outcome of enrichments and cultivations. Increasing sulfate availability in slowly oxidizing subsurface sediment samples that have been kept in live storage at 4 °C probably favored the accidental long-term enrichment of sulfate-dependent methane-oxidizing archaea of the ANME-2 group (Lin et al. 2010). To the best of the author's knowledge, this is the first successful enrichment of ANME archaea from deep subsurface sediments, where these sulfate-dependent methane oxidizers are essential catalysts of subsurface methane cycling.

Functional gene assays for archaea have focused on the methanogens and sulfate-dependent anaerobic methane oxidizers. Both physiological groups require the gene coding for methyl-coenzyme M reductase alpha subunit (*mcrA*), essential for the reduction of coenzyme M-bound methyl-carbon and its concomitant release as methane, and also for the reverse reaction of methane activation and oxidation to methyl-carbon (Hallam et al. 2004; Scheller et al. 2010). The *mcrA* genes are phylogenetically conserved and largely congruent with 16S rRNA phylogeny, and have been used extensively for the detection and phylogenetic identification of methanogenic and anaerobic methane-oxidizing archaea (Springer et al. 1995; Luton et al. 2002; Friedrich 2005). Interestingly, the *mcrA* phylotypes that have been recovered from deep subsurface sediments are often closely affiliated with cultured genera and families of methanogens. For example, the *mcrA* phylotypes in organic-rich sediments of the Peru Margin (ODP Site 1229) were closely related to members of the methanogenic genera *Methanobrevibacter* and *Methanosarcina* (Parkes et al. 2005; Webster et al. 2006); the same phylogenetic affiliation was found for *mcrA* phylotypes from the Western Pacific in the Nankai Trough offshore Japan (Site 1173; Newberry et al. 2004) and in subseafloor sediments offshore the Shimokita peninsula, Japan

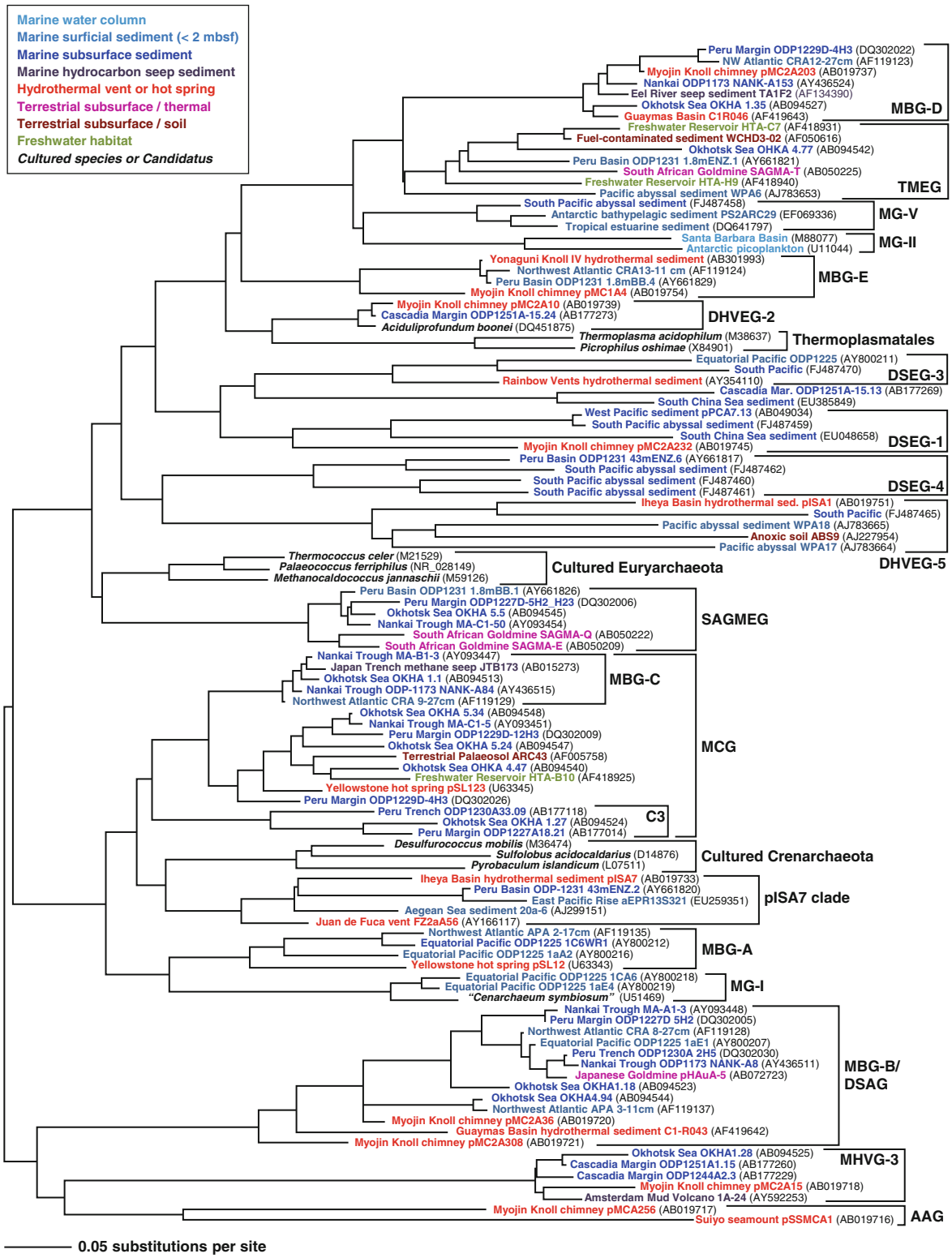


Fig. 8.1

Archaeal 16S rRNA phylogeny for sedimentary subsurface lineages based on HKY85 distance analysis of 16S rRNA nucleotide positions 24–906. References for archaeal lineages: Marine Benthic Group A to E, Vetriani et al. 1999; Marine Groups I and II; DeLong 1992; Deep-Sea Hydrothermal Vent Euryarchaeotal Groups (DHVEG), Takai and Horikoshi 1999; Miscellaneous Crenarchaeotal Group (MCG), Inagaki et al. 2003; South African Goldmine Euryarchaeotal Group (SAGMEG), Takai et al. 2001; Deep-Sea Euryarchaeotal Groups (DSEG) and pSIA17 clade, Durbin and Teske 2011; C3 archaea, DeLong and Pace 2001, and Inagaki et al. 2006; Ancient Archaeal Group (AAG), Takai and Horikoshi 1999; Marine Hydrothermal Vent Group (MHVG), Takai and Horikoshi 1999, updated as MHVG-3 by Durbin and Teske 2012

(Imachi et al. 2011). *Methanobacterium*-related phylotypes were found in two independent surveys on opposite sides of the Pacific, at the Cascadia Margin and offshore Shimokita (Yoshioka et al. 2010; Imachi et al. 2011). Such coincidences were also noted for sulfate-reducing bacteria as detected by *dsrA* genes (Schippers et al. 2012); the repeated detection of mutually near-identical phylotypes that are close to cultured strains and species poses the question of their source and dispersal (Teske and Biddle 2008). Phylotypes that are more distant to cultured species and genera of methanogens were found in deep sediments of the Peru Trench, where *Methanosaeta*-related phylotypes were detected (Inagaki et al. 2006).

Eukaryotic 18S rRNA Lineages

The third domain of life, the eukaryotes, was initially neglected in small subunit rRNA sequencing surveys. In qPCR assays of 18S rRNA gene abundance, eukaryotes remained one order of magnitude below bacterial 16S rRNA gene counts in Black Sea sediments, two to three orders of magnitude below bacterial counts in Benguela sediments (Schippers et al. 2012), and three to four orders of magnitude below bacterial counts in Peru Margin sediments (Schippers and Neretin 2006). These intriguing data show that eukaryotes, or at least their 18S rRNA genes, exist in subsurface sediments, but leave their identity open. The first comprehensive cloning and sequencing survey focused on the Peru Margin and Peru Trench (ODP sites 1227, 1229, and 1230) and detected mostly fungi in the subsurface (Edgcomb et al. 2011). The subsurface fungal phylotypes were derived from rDNA and also from rRNA sequences; most of these subsurface fungal phylotypes were related to single-celled yeasts within the Basidiomycota (Edgcomb et al. 2011). Metagenome sequence reads associated with Ascomycota and Basidiomycota (Biddle et al. 2008) and live counts of fungal colonies per gram sediment (Biddle et al. 2005a,b), both datasets from Peru Margin site 1229, further support the notion of a eukaryotic subsurface biosphere dominated by small, single-cell fungi that assimilate buried organic compounds (Edgcomb and Biddle 2011).

Biogeographic Patterns of Subsurface Microbial Communities

The 16S rRNA sequences of different bacterial and archaeal lineages are not randomly distributed in the marine subsurface, but show evidence of biogeographical structure most likely controlled by in situ chemical regime. For example, sediment-hosted methane hydrates (Inagaki et al. 2006), volcanic ash layers embedded in marine sediments (Inagaki et al. 2003), methane/sulfate transition zones (Sørensen and Teske 2006), oxygen/nitrate porewater gradients (Durbin and Teske 2011), and organic carbon content and redox status of subsurface sediments (Durbin and Teske 2012) appear to select in favor of phylogenetically distinct bacterial and archaeal lineages.

Of all subsurface geochemical interfaces, the methane/sulfate transition zone (SMTZ) has received the most sustained attention; archaeal/bacterial consortia that oxidize methane with sulfate as the electron donor were predicted to be a major component of the subsurface biosphere (D'Hondt et al. 2002). Interestingly, 16S rRNA surveys of subsurface sediment found fairly consistent archaeal community signatures within and around the SMTZ sediment horizons. Within the archaea, MCG, MBG-B, SAGMEG, and MBG-D were repeatedly detected based on reverse-transcribed 16S rRNA (Biddle et al. 2006; Sørensen and Teske 2006) and also based on sequencing of 16S rRNA genes (Parkes et al. 2005; Webster et al. 2006; Nunoura et al. 2009). Diverse Beta-, Gamma-, Deltaproteobacteria, Planctomycetales, and Chloroflexi dominate the bacterial community of SMTZ sediments in changing proportions (Parkes et al. 2005; Harrison et al. 2009; Mills et al. 2012b). Anaerobic, sulfate-dependent methanotrophic archaea (ANME) and their sulfate-reducing syntrophic partners within the Desulfobacteriaceae are conspicuous within and also below the SMTZ in shallow benthic marine sediments, estuarine sediments, and hydrocarbon seep sediments (Harrison et al. 2009; Knittel and Boetius 2009; Webster et al. 2011; Lloyd et al. 2006, 2010, 2011), but they are hard to detect in deep subsurface surveys and remain commonly below detection limit with general archaeal and bacterial 16S rRNA primers. So far, ANME communities required group-specific 16S rRNA and functional gene primers for successful detection in deep subsurface sediments (Lever 2008). Apparently, most bacteria and archaea in deep subsurface sulfate/methane transition zones are not sustained by sulfate-dependent methane oxidation, and rely on presumably heterotrophic metabolisms and assimilation of buried organic matter (Biddle et al. 2006). In contrast, seep-associated fractures that intersect the SMTZ in subsurface sediments can sustain ANME-dominated microbial communities and biofilms, most likely due to channelized methane flux in the fracture space (Briggs et al. 2011).

Subseafloor sediments in the open ocean are generally oxidized and do not harbor distinct methane/sulfate transition zones. Recent sequencing surveys are examining drilling sites and marine sedimentary environments representing different trophic regimes, from thoroughly oxidized, extremely organic-lean sediments that accumulate with very slow sedimentation rates in the ultraoligotrophic South Pacific Gyre (D'Hondt et al. 2009), to reduced, organic-rich, sulfidic, or methanogenic sediments on highly productive continental margins, such as the Peru Margin or the Cascadia Margin (D'Hondt et al. 2004). This ultraoligotrophic to eutrophic spectrum should influence the composition and activity of subsurface microbial communities, in response to different redox regime, organic carbon content, and available substrate spectra. A comparison of contrasting sites shows that archaeal lineages change systematically over a spectrum of organic-lean, oxidized marine sediments in abyssal plains to organic-rich, reduced sediments on continental margins (Durbin and Teske 2012).

The visible impact of organic carbon availability and redox status on microbial community structure in marine subsurface sediments, as reflected in 16S rRNA gene diversity, indicates

a basic correspondence between geochemical in situ regime and 16S rRNA gene-based microbial community structure. The empirically observed correspondences between subsurface habitat and microbial community 16S rRNA signature support the working hypothesis that microbial community structure and in situ geochemistry are coupled, even in the metabolically slow sedimentary subsurface. Decoupling mechanisms, for example, accumulations of fossil and/or inactive microbial cells, remnant populations from past geological and geochemical regimes, or dispersed cells from distant source habitats (Inagaki et al. 2001) have to be carefully considered in individual case studies and specific localities, but are unlikely to overwrite and invalidate global distribution and abundance patterns of active in situ microbial populations, or living cells with intact rRNA and rRNA genes. By the same token, comprehensive meta-analyses of microbial community composition and abundance in the deep subsurface biosphere, and their correlations to geochemical habitat characteristics and controls should be a high research priority for the immediate future.

So far, small subunit rRNA sequencing surveys in all three domains of life are consistent with the notion of a heterotrophic subsurface biosphere that ultimately persists on buried organic matter of planktonic, photosynthetic origin as carbon and energy source (Biddle et al. 2006). Sequence signatures of predominantly chemolithotrophic, autotrophic subsurface microbial ecosystems comparable to terrestrial subsurface examples (Chivian et al. 2008) have not been found, or at least not been identified with certainty. A possible exception might be sequences of the Marine Group I archaea within the Thaumarchaeota, whose few cultured representatives are autotrophic ammonia oxidizers (Pester et al. 2011). Marine Group I archaea are usually abundant in the marine water column, but occur in oxidized marine sediments as long as oxygen or nitrate are present (Durbin and Teske 2010, 2011). The working hypothesis of a (mostly) heterotrophic subsurface biosphere has to be qualified by the persistent sampling bias toward organic-rich, reducing continental margin sediments where at least some amount of buried organic biomass is omnipresent, and by the general paucity of physiological knowledge on uncultured microorganisms in the subsurface. As long as cultivation problems persist, inferring the physiological potential of deep subsurface microorganisms requires different approaches, such as functional gene sequencing and metagenomic surveys.

Deep Subsurface Omics

Clone libraries of specific functional genes and rRNA genes are limited in resolution by the number of individual clones that can be processed and sequenced; more fundamentally, they are limited by the requirement for conserved primers that rule out discoveries of novel genes (Teske and Sørensen 2008). For example, established PCR and qPCR assays for functional genes of sulfate reduction, methanogenesis, and sulfate-dependent methane oxidation (*dsrAB*, *aprA*, *mcrA*) have shown that only a minority of deep subsurface cells carries these genes; the metabolic and biogeochemical functionality of the majority of subsurface microorganisms remains unknown.

As an initial step to address these shortcomings, the phylogenetic composition of deep subsurface communities can be analyzed in high resolution by high-throughput 16S rRNA-tagged pyrosequencing (Sogin et al. 2006) using multiple bacterial and archaeal 16S-targeted primer pairs simultaneously. For example, in a sequencing survey of organic-lean sediments and deeply buried coral carbonates (IODP Expedition 307), approx. 15,000–25,000 bacterial and archaeal 16S-tag sequences per sample yielded up to approx. 300 archaeal and 3,000 bacterial OTUs per sample, sufficient for a census that included the majority populations (Chloroflexi, Alphaproteobacteria, bacterial candidate divisions OD1, OP1, OP11, and MCG, SAGMEG, and MBG-B archaea) and also low-frequency microbial groups that would have been missed in classical clone libraries (Hoshino et al. 2011). Some recalcitrant sediments do not yield sufficient DNA for PCR; in these cases, multiple displacement amplification of the small amounts of extracted DNA before the PCR step can overcome this barrier and generate sufficient DNA for cloning and sequencing (Teske and Biddle 2008), as demonstrated for recalcitrant Arctic Ocean subsurface sediments (Forschner et al. 2009). These methods can be adapted to RNA instead of DNA monitoring; pyrosequencing of reverse-transcribed 16S rRNAs monitored bacterial 16S rRNA gene expression patterns in marine subsurface sediments that changed during sample storage (Mills et al. 2012a) and with sediment depth around a sulfate/methane transition zone (Mills et al. 2012b).

Metagenomic surveys in deep subsurface sediments avoid PCR amplification, and have to rely on multiple displacement amplification of extracted DNA. Among the Peru Margin and Gulf of Mexico subsurface microbial communities that have been examined by the metagenomic approach, nearly all samples (with the exception of a single near-surface sediment sample) required whole-genome amplification in order to provide sufficient amounts of DNA (Biddle et al. 2008, 2011). High-throughput pyrosequencing of extracted metagenomic DNA avoids the PCR selection for 16S rRNA genes, and produces a comprehensive sample of DNA sequences and gene fragments that can be screened for phylogenetic affiliation and functional identity of the in situ microbial community and its metagenome. The subsurface metagenomes turned out to be functionally stratified. For example, genes affiliated with cell motility decreased rapidly in frequency with sediment depth, suggesting that cells in the deep subsurface stay put in their pore space and subsist on local substrates and energy sources that can be accessed without movement (Biddle et al. 2008). Physiological gene categories can show unexpected correlations with geochemical habitat characteristics. For example, strong negative and positive correlations to increasing ammonia porewater concentration were found for genes of cell motility and posttranslatational modification and for genes of membrane biogenesis and energy production/conversion, respectively (Biddle et al. 2008, 2011).

The narrow detection window for conserved PCR primers targeting specific functional genes can be opened up significantly toward a de-facto metagenome survey with a new

strategy: Random amplification metagenomic PCR (RAMP) is essentially a PCR-based method using highly degenerate primers for whole-genome amplification using 454 pyrosequencing amplicon primers with an added degenerate 3′-terminal region (Martino et al. 2012). In comparative tests against previously sequenced Peru Margin sediments from ODP site 1229, the RAMP metagenome contained gene fragments of the same phyla that were previously found in unamplified metagenomic DNA and whole-genome-amplified DNA, although in changing proportions. These fluctuations in phylum-level identity and proportion become stronger once specific genes (16S, rpoB) are filtered out from the metagenome and analyzed separately (Martino et al. 2012). At present, WGA- and RAMP-amplified subsurface metagenomes from the same sites and sediment depths show compositional differences that are most likely rooted in the poorly constrained amplification biases during metagenome sample processing. Of course, these poorly constrained amplification biases and contamination-prone outcomes of multiple displacement amplification call for healthy skepticism and vigilance in sequence data quality control and interpretation (Binga et al. 2008).

References

Amend JP, Teske A (2005) Expanding frontiers in deep subsurface microbiology. Palaeogeography, Palaeoclimatology, Palaeoecology 219:131–155

Bahr M, Crump BC, Klepac-Ceraj V, Teske A, Sogin ML, Hobbie JE (2005) Molecular characterization of sulfate-reducing bacteria in a New England salt marsh. Environ Microbiol 7:1175–1185

Bale SJ, Goodman K, Rochelle PA, Marchesi JR, Fry JC, Weightman AJ, Parkes RJ (1997) *Desulfovobrio profundus* sp. nov., a novel barophilic sulphate-reducing bacterium from deep sediment layers in the Japan Sea. Int J Syst Bacteriol 47:515–521

Barnes SP, Bradbrook SD, Cragg BA, Marchesi JR, Weightman AJ, Fry JC, Parkes RJ (1998) Isolation of sulphate reducing bacteria from deep sediment layers of the Pacific Ocean. Geomicrobiol J 15:67–83

Barns SM, Delwiche CF, Palmer JD, Pace NR (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. Proc Natl Acad Sci USA 93:9188–9193

Batzke A, Engelen B, Sass H, Cypionka H (2007) Phylogenetic and physiological diversity of cultured deep-biosphere bacteria from equatorial Pacific Ocean and Peru margin sediments. Geomicrobiol J 24:261–273

Biddle JF, House CH, Brenchley JE (2005a) Enrichment and cultivation of microorganisms from sediment from the slope of the Peru Trench (ODP site 1230). In: Jørgensen BB, D'Hondt SL, Miller DJ (eds) Proceedings of ODP, science results, 201 [online]. Texas A&M University, College Station, TX. Available from World Wide Web: http://www-odp.tamu.edu/publications201_SR/107107.htm

Biddle JF, House CH, Brenchley JE (2005b) Microbial stratification in deeply-buried marine sediment reflects changes in sulfate/methane profiles. Geobiology 3:287–295

Biddle JF, Fitz-Gibbon S, Schuster SC, Brenchley JE, House CH (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. Proc Natl Acad Sci USA 105:10583–10588

Biddle JF, Lipp JS, Lever MA, Lloyd KG, Sørensen KB, Anderson R, Fredricks HF, Elvert M, Kelly TJ, Schrag DP, Sogin ML, Brenchley JE, Teske A, House CH, Hinrichs K-U (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proc Natl Acad Sci USA 103:3846–3851

Biddle JF, White JR, Teske A, House CH (2011) Metagenomics of the subsurface Brazos-Trinity Basin (IODP Site 1320): comparison with other sediment and pyrosequenced metagenomes. ISME J 5:1038–1047

Binga EK, Lasken RS, Neufeld JD (2008) Something from (almost) nothing: the impact of multiple displacement amplification on microbial ecology. ISME J 2:233–241

Blazejak A, Schippers A (2010) High abundance of JS-1- and Chloroflexi-related bacteria in deeply buried marine sediments revealed by quantitative, real-time PCR. FEMS Microbiol Ecol 72:198–207

Blazejak A, Schippers A (2012) Real-time quantification and diversity analysis of the functional genes *aprA* and *dsrA* of sulfate-reducing prokaryotes in marine sediments of the Peru continental Margin and the Black Sea. Front Microbiol 2:253. doi:10.3389/jmicb.2011.00253

Böttcher ME, Ferdelman TG, Jørgensen BB, Blake RE, Surkov AV, Claypool GE (2006) Sulfur isotope fractionation by the deep biosphere within sediments of the eastern equatorial Pacific and Peru Margin. In: Jørgensen BB, D'Hondt SL, Miller DJ (eds) Proceedings of ODP, science results, 201 [online]. Texas A&M University, College Station, TX. Available from World Wide Web: http://www-odp.tamu.edu/publications/201_SR/109/109.htm

Braun M, Mayer F, Gottschalk G (1981) *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Arch Microbiol 128:288–293

Briggs BR, Pohlman JW, Torres M, Riedel M, Brodie EL, Colwell FS (2011) Macroscopic biofilms in fracture-dominated sediment that anaerobically oxidize methane. Appl Environ Microbiol 77:6780–6787

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat Rev Microbiol 6:245–252

Canfield DE (1991) Sulfate reduction in deep-sea sediments. Am J Sci 291:177–188

Cano RJ, Borucki MK (1995) Revival and identification of bacterial spores in 25 to 40 million year-old Dominican amber. Science 268:1060–1064

Chivian D, Brodie EL, Alm AJ, Culley DE, Dehal PS, DeSantis TZ, Gihring TM, Lapidus A, Lin L-H, Lowry SR, Moser DP, Richardson PM, Southam G, Wanger G, Pratt LM, Andersen GL, Hazen TC, Brockman FJ, Arkin AP, Onstott TC (2008) Environmental genomics reveals a single-species ecosystem within Earth. Science 322:275–278

Coolen MJL, Cypionka H, Sass AM, Sass H, Overmann J (2002) Ongoing modification of Mediterranean Pleistocene sapropels mediated by prokaryotes. Science 296:2407–2419

D'Hondt S, Inagaki F, Ferdelman T, Jørgensen BB, Kato K, Kemp P, Sobecky P, Sogin M, Takai K (2007) Exploring subseafloor life with the integrated ocean drilling program. Sci Drill 5:26–37

D'Hondt S, Jørgensen BB, Miller DJ et al (2004) Distribution of microbial activities in deep subseafloor sediments. Science 306:2216–2221

D'Hondt S, Rutherford S, Spivack AJ (2002) Metabolic activity of subsurface life in deep-sea sediments. Science 295:2067–2070

D'Hondt S, Spivack AJ, Pockalny R, Ferdelman TG, Fischer JP, Kallmeyer J, Abrams LJ, Smith DC, Graham D, Hasiuk F, Schrum H, Stancin AM (2009) Subseafloor sedimentary life in the South Pacific Gyre. Proc Natl Acad Sci USA 106:11651–11656

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689

DeLong EF (2004) Microbial life breaths deep. Science 306:2198–2200

DeLong F, Pace N (2001) Environmental diversity of bacteria and archaea. Syst Biol 50:470–478

Divins DL (2003) Total Sediment Thickness of the World's Oceans & Marginal Seas. NOAA National Geophysical Data Center, Boulder, CO

Dojka MA, Hugenholtz P, Haack SK, Pace NR (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Appl Environ Microbiol 64:3869–3877

Dunne JP, Sarmiento JL, Gnanadesikan A (2007) A synthesis of global particle export from the surface ocean and cycling through the ocean interior and on the seafloor. Global Biogeochem Cycles 21:GB4006

Durbin AM, Teske A (2010) Sediment-associated microdiversity within the marine group I Crenarchaeota. Environ Microbiol Rep 2:693–703

Durbin AM, Teske A (2011) Microbial diversity and stratification of South Pacific abyssal marine sediments. Environ Microbiol 13:3219–3234

Durbin AM, Teske A (2012) Archaea in organic-lean and organic-rich marine subsurface sediments: an environmental gradient reflected in distinct phylogenetic lineages. Front Microbiol 3:168

Edgcomb VP, Biddle JF (2011) Microbial eukaryotes in the marine subsurface. In: Altenbach AV et al (eds) Anoxia: evidence for eukaryote survival and paleontological strategies, vol 21, Cellular origin, life in extreme habitats and astrobiology. Springer, New York, pp 479–493. doi:10.1007/978-94-007-1896-8_25

Edgcomb VP, Beaudoin D, Gast R, Biddle JF, Teske A (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 13:172–183

Fichtel K, Mathes F, Könneke M, Cypionka H, Engelen B (2012) Isolation of sulfate-reducing bacteria from sediments above the deep-subseafloor aquifer. Front Microbiol 3:65. doi:10.3389/fmicb.2012.00065

Forschner SR, Sheffer R, Rowley DC, Smith DC (2009) Microbial diversity in Cenozoic sediments from the Lomonosov Ridge in the central Arctic Ocean. Environ Microbiol 11:630–639

Friedrich M (2005) Methyl-coenzyme M reductase genes: unique functional markers for methanogenic and anaerobic methane-oxidizing Archaea. Meth Enzymol 26:428–442

Friedrich MW (2002) Phylogenetic analysis reveals multiple lateral transfers of adenosine-5 ′-phosphosulfate reductase genes among sulfate-reducing microorganisms. J Bacteriol 184:278–289

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P, Hammond D, Hartman B, Maynard V (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic suboxic diagenesis. Geochim Cosmochim Acta 43:1075–1090

Fry JC, Parkes RJ, Cragg BA, Weightman AJ, Webster G (2008) Prokaryotic biodiversity and activity in the deep subseafloor biosphere. FEMS Microbiol Ecol 66:181–196

Fuerst JA, Sagulenko E (2011) Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. Nat Rev Microbiol 9:403–413

Futagami T, Morono Y, Terada T, Kaksonen AH, Inagaki F (2009) Dehalogenation activities and distribution of reductive dehalogenase homologous genes in marine subsurface sediments. Appl Environ Microbiol 75:6905–6909

Gagen EM, Denman SE, Padmanabha J, Zadbuke S, Jassim RA, Morrison M, McSweeney CS (2010) Functional gene analysis suggests different acetogen populations in the bovine rumen and Tammar Wallaby forestomach. Appl Environ Microbiol 76:7785–7795

Gest H, Mandelstam J (1987) Longevity of microorganisms in natural environments. Microbiol Sci 4:69–71

Gieskes JM, Boulègue J (1986) Interstitial water studies, Leg 92. In: Leinen M, Rea DK, Anderson RN, Becker K, Boulègue JJ, Erzinger J, Gieskes JM, Goldberg D, Goldfarb M, Goldsborough R, Hobart MA, Kastner M, Knuettel S, Lyle MW, Moos D, Newmark R, Nishatani T, Owen RM, Pearce JA, Romine K, Stephan RA (eds) Initial reports DSPD, 92. US Govt Printing Office, Washington, pp 423–429

Gold T (1992) The deep, hot biosphere. Proc Natl Acad Sci USA 89:6045–6049

Guy L, Ettema TJG (2011) The archaeal TACK superphylum and the origin of eukaryotes. Trends Microbiol 19:580–587

Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM, DeLong EF (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305:1457–1462

Harrison BK, Zhang H, Berelson W, Orphan VJ (2009) Variations in archaeal and bacterial diversity associated with the sulfate-methane transition zone in continental margin sediments (Santa Barbara Basin, California). Appl Environ Microbiol 75:1487–1409

Head IM, Joes DM, Larter SR (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nature 426:344–352

Heuer VB, Pohlman JW, Torres ME, Elvert M, Hinrichs K-U (2009) The stable carbon isotope biogeochemistry of acetate and other dissolved carbon species in deep subseafloor sediments at the northern Cascadia Margin. Geochim Cosmochim Acta 73:3323–3336

Hoshino T, Morono Y, Terada T, Imachi H, Ferdelman TG, Inagaki F (2011) Comparative study of sub-seafloor microbial community structures in deeply buried coral fossils and sediment matrices from the Challenger Mound in the Porcupine Seabight. Front Microbiol 2:231. doi:10.3389/fmicb.2011.00231

House C, Cragg B, Teske A, the Leg 201 Scientific Party (2003) Drilling contamination tests on ODP Leg 201 using chemical and particulate tracers. In: D'Hondt SL, Jørgensen BB, Miller DJ et al (eds) Proceedings of ODP, initial reports, 201 [online]. Texas A&M University, College Station, TX. Available from the World Wide Web: http://www-odp.tamu.edu/publications/201_IR/chap_02/chap_02.htm

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

Hubert C, Loy A, Nickel M, Arnosti C, Baranyi C, Bruechert V, Ferdelman T, Finster K, Christensen FM, de Rezende JR, Vandieken V, Jørgensen BB (2009) A constant flux of diverse thermophilic bacteria into the cold arctic seabed. Science 325:1541–1544

Hugenholtz P (2002) Exploring prokaryotic diversity in the genomic era. http://genomebiology. com/2002/3/2/reviews/0003. Accessed May 26, 2012

Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998a) Novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180: 366–376

Hugenholtz P, Goebel BM, Pace NR (1998b) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774

Imachi H, Aoi K, Tasumi E, Saito Y, Yamanaka Y, Saito Y, Yamaguchi T, Tomaru H, Takeuchi R, Morono Y, Inagaki F, Takai K (2011) Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor. ISME J 5:1913–1925

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. Appl Environ Microbiol 69:7224–7235

Inagaki F, Takai K, Komatsu T, Kanamatsu T, Fujiioka K, Horikoshi K (2001) Archaeology of archaea: geomicrobiological record of pleistocene thermal events concealed in a deep-sea subseafloor environment. Extremophiles 5:385–392

Inagaki F, Nunoura T, Nakagawa S, Teske A, Lever MA, Lauer A, Suzuki M, Takai K, Delwiche M, Colwell FS, Nealson KH, Horikoshi K, D'Hondt SL, Jørgensen BB (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. Proc Natl Acad Sci USA 103:2815–2820

Inagaki F, Okada H, Tsapin AI, Nealson KH (2005) The Paleome: a sedimentary genetic record of past microbial communities. Astrobiology 5:141–153

Isaksen MF, Bak F, Jørgensen BB (1994) Thermophilic sulfate-reducing bacteria in cold marine sediment. FEMS Microbiol Ecol 14:1–8

Jørgensen BB (1982) Mineralization of organic matter in the sea bed – the role of sulphate reduction. Nature 296:643–645

Jørgensen BB (2011) Deep subseafloor microbial cells on physiological standby. Proc Natl Acad Sci USA 108:18193–18194

Jørgensen BB, Boetius A (2007) Feast and famine—microbial life in the deep-sea bed. Nat Rev Microbiol 5:770–781

Jørgensen BB, D'Hondt SL, Miller DJ (2006) Leg 201 synthesis: controls on microbial communities in deeply buried sediments. In: Jørgensen BB, D'Hondt SL, Miller DJ (eds) Proceedings of ODP, science result, 201. ODP, College Station, pp 1–45

Kallmeyer J, Smith DC, Spivack AJ, D'Hondt S (2008) New cell extraction procedure applied to deep subsurface sediments. Limnol Oceanogr Methods 6:236–245

Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D'Hondt S (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. Proc Natl Acad Sci USA, early edition. doi:10.1073/pnas.1203849109

Karen Lloyd, KG, et al (2012) Single cell genomics of uncultured archaea. Abstract, 22nd Goldschmidt Conference, Montreal, Canada, June 24-29, 2012

Kendall MM, Boone DR (2006) Cultivation of methanogens from shallow marine sediments at hydrate ridge, oregon. Archaea 2:31–38

Kendall MM, Liu Y, Sieprawska-Lupa M, Stetter KO, Whitman WB, Boone DR (2006) *Methanococcus aeolicus* sp. nov., a mesophilic, methanogenic archaeon from shallow and deep marine sediments. Int J Syst Evol Microbiol 56:1525–1529

Kirkpatrick J, Oakley B, Fuchsman C, Srinivasan S, Staley JT, Murray JW (2006) Diversity and distribution of *Planktomycetes* and related bacteria in the suboxic zone of the Black Sea. Appl Environ Microbiol 72:3079–3083

Klein M, Friedrich M, Roger AJ, Hugenholtz P, Fishbain S, Abicht H, Blackall LL, Stahl DA, Wagner M (2001) Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. J Bacteriol 183:6028–6035

Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. Annu Rev Microbiol 63:311–334

Kondo R, Nedwell DB, Purdy KJ, de Queiroz Silva S (2004) Detection and enumeration of sulphate-reducing bacteria in estuarine sediments by competitive PCR. Geomicrobiol J 21:145–157

Kormas AK, Smith DC, Edgcomb V, Teske A (2003) Molecular analysis of deep subsurface microbial communities in Nankai Trough sediments (ODP Leg 190, Site 1176). FEMS Microbiol Ecol 45:115–125

Kragelund C, Levantesi C, Borger A, Thelen K, Eikelboom D, Tandoi V, Kong Y, van der Waarde V, Krooneman J, Rosetti S, Thomson TR, Nielsen PH (2007) Identity, abundance and ecophysiology of filamentous *Chloroflexi* species present in activated sludge treatment plants. FEMS Microbiol Ecol 59:671–682

Krajmalnik-Brown R, Hölscher T, Thomson IN, Saunders FM, Ritalahti KM, Löffler FE (2004) Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. Appl Environ Microbiol 70:6347–6351

Kubo K, Lloyd KG, Biddle JF, Amann R, Teske A, Knittel K (2012) Archaea of the Miscellaneous Crenarchaeotal Group (MCG) are abundant, diverse and widespread in marine sediments. ISME J. doi:10.1038/ismej.2012.37doi. Advance online publication, 3 May 2012

Lappe M, Kallmeyer J (2012) A cell extraction method for oily sediments. Front Microbiol 2:233. doi:10.3389/fmicb.2011.00233

Leaphart AB, Friez MJ, Lovell CR (2003) Formyltetrahydrofolate synthetase sequences from salt marsh plant roots reveal a diversity of acetogenic Bacteria and other bacterial functional groups. Appl Environ Microbiol 69:693–696

Lee Y, Wagner I, Brice M-E, Kevbrin VV, Mills G, Romanek CS, Wiegel J (2005) *Thermosediminibacter oceani* gen. nov., sp. nov. and *Thermosediminibacter litoriperuensis* sp. nov., new anaerobic thermophilic bacteria isolated from Peru Margin. Extremophiles 9:375–383

Leloup J, Fossing H, Kohls K, Holmkvist L, Borowski C, Jørgensen BB (2009) Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. Environ Microbiol 11:1278–1291

Leloup J, Loy A, Knab NJ, Borowski C, Wagner M, Jørgensen BB (2007) Diversity and abundance of sulfate-reducing microorgsnisms in the sulfate and methane zones of a marine sediment, Black Sea. Environ Microbiol 9:131–142

Lever MA (2012) Acetogenesis in the energy-starved deep biosphere – a paradox? Front Microbiol 2:294. doi:10.3389/fmicb.2011.00284

Lever MA, Alperin MJ, Engelen B, Inagaki F, Nakagawa S, Steinsbu B, Teske A (2006) Trends in basalt and sediment core contamination during IODP Expedition 301. Geomicrobiol J 23:517–530

Lever MA, Heuer VB, Morono Y, Masui N, Schmidt F, Alperin MJ, Inagaki F, Hinrichs K-U, Teske A (2010) Acetogenesis in deep subseafloor sediments of the Juan de Fuca Ridge Flank: a synthesis of geochemical, thermodynamic, and gene-based evidence. Geomicrobiol J 27:183–211

Lipp JS, Morono Y, Inagaki F, Hinrichs K-U (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454:991–994

Li P-Y, Xie B-B, Zhang X-Y, Qin Q-L, Dang H-Y, Wang X-M, Chen X-L, Yu J, Zhang Y-Z (2012) Genetic structure of three fosmid fragments encoding 16S rRNA genes of the Miscellaneous Crenarchaeotic Group (MCG): implications for physiology and evolution of marine sedimentary archaea. Environ Microbiol 14:467–479

Lin Y-S, Biddle JF, Lipp JS, Orcutt B, Holler T, Teske A, Hinrichs K-U (2010) Effect of storage conditions on archaeal and bacterial communities in subsurface marine sediments. Geomicrobiol J 27:261–272

Lloyd KG, Lapham L, Teske, A (2006) An anaerobic methane-oxidizing community of ANME-1 archaea in hypersaline Gulf of Mexico sediments. Appl Environ Microbiol 72:7218–7230

Lloyd KG, Albert D, Biddle JF, Chanton L, Pizarro O, Teske A (2010) Spatial structure and activity of sedimentary microbial communities underlying a *Beggiatoa* spp. mat in a Gulf of Mexico hydrocarbon seep. PLoS ONE 5(1):e8738. doi:10.1371/journal.pone.0008738

Lloyd KG, Alperin M, Teske A (2011) Environmental evidence for net methane production and oxidation in putative Anaerobic MEthanotrophic (ANME) archaea. Environ Microbiol 13:2548–2564

Lloyd KG, Schreiber L, Petersen DG, Richter M, Kjeldsen K, Lever M, Lenk S, Kleindienst S, Schramm A, Jørgensen BB (2012) Single cell genomics of uncultured subsurface archaea. Abstract at 22nd V.M. Goldschmidt conference, Montreal, Canada, June 24–29, 2012

Lomstein BA, Langerhuus AT, D'Hondt S, Jørgensen BB, Spivack AJ (2012) Endospore abundance, microbial growth and necromass turnover in deep subseafloor sediment. Nature 484:101–104

Luton PE, Wayne JM, Sharp RJ, Riley PW (2002) The *mcrA* gene as an alternative to 16 S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148:3521–3530

Martino AJ, Rhodes ME, Biddle JF, Brandt LD, Tomsho LP, House CH (2012) Novel degenerate PCR method for whole-denome amplification applied to Peru Margin (ODP Leg 201) subsurface samples. Front Microbiol 3:17. doi:10.3389/fmicb.2012.00017

Masui N, Morono Y, Inagaki F (2008) Microbiological assessment of circulation mud fluids during the first operation of riser drilling by the deep-earth research vessel *Chikyu*. Geomicrobiol J 25:274–282

Meister P, Prokopenko M, Skilbeck CG, Watson M, McKenzie J (2006) Data report: compilation of total organic and inorganic carbon data from Peru Margin and eastern Equatorial Pacific drill sites (ODP Legs 112, 138, and 201). In: Jørgensen BB, D'Hondt SL, Miller DJ (eds) Proceedings of ODP, science results, 201, pp 1–19 [online]. Texas A&M University, College Station, TX. Available from World Wide Web: http://www-odp.tamu.edu/publiactions/201_SR/VOLUME/CHAPTERS/105.PDF

Meng J, Wang F, Wang F, Zheng Y, Peng X, Zhou H et al (2009) An uncultivated crenarchaeota contains functional bacteriochlorophyll a synthase. ISME J 3:106–116

Mikucki JA, Liu Y, Delwiche M, Colwell FS, Boone DR (2003) Isolation of a methanogen from deep marine sediments that contain methane hydrates, and description of *Methanoculleus submarinus* sp.nov. Appl Environ Microbiol 69:3311–3316

Mills HJ, Reese BK, Peter CS (2012a) Characterization of Microbial Population Shifts during Sample Storage. Frontiers in Microbiology 3:49. doi:10.3389/fmicb.2012.00049

Mills HJ, Reese BK, Shepard AK, Riedinger N, Dowd SE, Morono Y, Inagaki F (2012b) Characterization of metabolically active bacterial populations in subseafloor nankai trough sediments above, within, and below the sulfate–methane transition zone. Frontiers in Microbiology 3:113. doi:10.3389/fmicb.2012.00113

Moe, WMJ Yan, Fernanda Nobre M, da Costa MS, Rainey FA (2009) *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. Int J Syst Evol Microbiol 59:2692–2697

Morita RY, Zobell CE (1955) Occurrence of bacteria in pelagic sediments collected during the Mid-Pacific Expedition. Deep-Sea Research 3:66–73

Morono Y, Inagaki F (2010) Automatic slide-loader fluorescence microscope for discriminative enumeration of subseafloor life. Sci Drill 9:32–36

Morono Y, Terada T, Masui N, Inagaki F (2009) Discriminative detection and enumeration of microbial life in marine subsurface sediments. ISME J 3:503–511

Morono Y, Terada T, Nishizawa M, Ito M, Hillon F, Takahata N, Sano Y, Inagaki F (2011) Carbon and nitrogen assimilation in deep subseafloor microbial cells. Proc Natl Acad Sci USA 108:18295–18300

Nakagawa S, Inagaki F, Suzuki Y, Steinsbu BO, Lever MA, Takai K, Engelen B, Sako Y, Wheat CG, Horikoshi K, Integrated Ocean Drilling Program Expedition 301 Scientists (2006) Microbial community in black rust exposed to hot ridge flank crustal fluids. Appl Environ Microbiol 72:6789–6799

Newberry CJ, Webster G, Weightman AJ, Fry JC (2004) Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the nankai trough, ocean drilling program Leg 190. Environ Microbiol 6:274–287

Nunoura T, Inagaki F, Delwiche ME, Colwell FS, Takai K (2008) Subseafloor microbial communities in methane-hydrate bearing sediments at two

distinct locations (ODP Leg 204) in the Cascadia Margin. Microbes Environ 23:317–325

Nunoura T, Soffientino B, Blazejak A, Kakuta J, Oida H, Schippers A, Takai K (2009) Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308). FEMS Microbiol Ecol 69:410–424

Nunoura T, Takai Y, Kakuta J, Nishi S, Sugahara J, Kazama H, Chee GJ, Hattori M, Kanai A, Atomi H, Takai K, Takami H (2010) Insights into the evolution of archaea and eukarytic protein modifier systems revealed by the genome of a novel archaeal group. Nucl Acids Res, advance online publication. doi:10.1093/nar/gkq1228

Orcutt BN, Bach W, Becker K, Fisher AT, Hentscher M, Toner BM, Wheat CG, Edwards KJ (2010) Colonization of subsurface microbial observatories deployed in young ocean crust. ISME J 5:692–703

Orcutt BN, Sylvan JB, Knab NJ, Edwards KJ (2011) Microbial ecology of the dark ocean above, at, and below the seafloor. Microbiol Mol Biol Rev 75:361–422

Oren A (2012) There must be an acetogen somewhere. Front Microbiol 3:22. doi:10.3389/fmicb.2012.00022

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ, Harvey SM (1994) Deep bacterial biosphere in Pacific Ocean sediments. Nature 371:410–413

Parkes R, Cragg B, Wellsbury P (2000) Recent studies on bacterial populations and processes in subseafloor sediments: a review. Hydrogeol J 8:11–28

Parkes RJ, Sellek G, Webster G, Martin D, Anders E, Weightman AJ, Sass H (2009) Culturable prokaryotic diversity of deep, gas hydrate sediments: first use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). Environ Microbiol 11: 3140–3153

Parkes RJ, Webster G, Cragg BA, Weightman AJ, Newberry CJ, Ferdelman TG, Kallmeyer J, Jørgensen BB, Aiello IW, Fry JC (2005) Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature 436: 390–394

Pester M, Schleper C, Wagner M (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr Opin Microbiol 14:300–306

Picard A, Ferdelman TG (2012) Linking microbial heterotrophic activity and sediment lithology in oxic, oligotrophic sub-seafloor sediments of the North Atlantic Ocean. Front Microbiol 2:263. doi:10.3389/fmicb.2011.00263

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

Reed DW, Fujita Y, Delwiche ME, Blackwelder DB, Sheridan PP, Uchida T, Colwell FS (2002) Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. Appl Environ Microbiol 68:3759–3770

Rey RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72:3685–3695

Rochelle PA, Cragg BA, Fry JC, Parkes RJ, Weightman AJ (1994) Effect of sample handling on estimation of bacterial diversity in marine sediments by 16 S rRNA gene sequence analysis. FEMS Microbiol Ecol 19:215–226

Roussel EG, Bonavita M-AC, Querellou J, Cragg BA, Webster G, Prieur D, Parkes RJ (2008) Extending the sub-sea-floor biosphere. Science 320:1046

Roussel EG, Sauvadet A-L, Chaduteau C, Fouquet Y, Charlou J-L, Prieur D, Cambon Bonavita M-A (2009) Archaeal communities associated with shallow to deep subseafloor sediments of New Caledonia Basin. Environ Microbiol 11:2446–2462

Røy H, Kallmeyer J, Adhikari RR, Pockalny R, Jørgensen BB, D'Hondt S (2012) Aerobic microbial respiration in 86-million-year-old deep-sea red clay. Science 336:922–925

Santelli CM, Banerjee N, Bach W, Edwards KJ (2010) Tapping the subsurface ocean crust biosphere: low biomass and drilling-related contamination calls for improved quality controls. Geomicrobiol J 27:158–169

Sauer P, Glombitza C, Kallmeyer J (2012) A system for incubations at high gas partial pressure. Front Microbiol 3:25. doi:10.3389/fmicb.2012.00025

Scheller S, Goenrich M, Boecher R, Thauer R, Jaun B (2010) The key nickel enzyme of methanogenesis catalyzes the anaerobic oxidation of methane. Nature 465:606–609

Schippers A, Kock D, Höft C, Köweker G, Siegert M (2012) Quantification of microbial communities in subsurface sediments of the Black Sea and off Namibia. Front Microbiol 3:16. doi:10.3389/fmicb.2012.00016

Schippers A, Neretin LN (2006) Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. Environ Microbiol 8:1251–1260

Schippers A, Neretin LN, Kallmeyer J, Ferdelman TG, Cragg BA, Parkes JR, Jørgensen BB (2005) Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature 433:861–864

Schleper C, Jurgens G, Jonuscheit M (2005) Genomic studies of uncultivated archaea. Nat Rev Microbiol 3:479–488

Seiter K, Hensen C, Schroter J, Zabel M (2004) Organic carbon content in surface sediments-defining regional provinces. Deep-Sea Res I 51:2001–2026

Sekiguchi Y, Takahashi H, Kamagata Y, Ohashi A, Harada H (2001) In situ detection, isolation, and physiological properties of a thin filamentous microorganism abundant in methanogenic granular sludges: a novel isolate affiliated with a clone cluster, the green non-sulfur bacteria, subdivision I. Appl Environ Microbiol 67:5740–5749

Sekiguchi Y, Yamada T, Hanada S, Ohashi A, Harada H, Kamagata Y (2003) *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. Int J Syst Evol Microbiol 53:1843–1851

Smith DC, Spivack AJ, Fisk MR, Haveman SA, Staudigel H (2000) Tracer-based estimates of drilling-induced microbial contamination of deep-sea crust. Geomicrobiol J 17:207–219

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci USA 103:12115–12120

Sørensen KB, Lauer A, Teske A (2004) Archaeal phylotypes in a metal-rich, low-activity deep subsurface sediment of the Peru Basin, ODP Leg 201, Site 1231. Geobiology 2:151–161

Sørensen KB, Teske A (2006) Stratified communities of active archaea in deep marine subsurface sediments. Appl Environ Microbiol 72:4596–4603

Springer E, Sachs MS, Woese CR, Boone DR (1995) Partial gene sequences for the A subunit of methyl-coenzyme M reductase (*mcrI*) as a phylogenetic tool for the family Methanosarcinaceae. Int J Syst Bacteriol 45:554–559

Steinsbu BO, Thorseth IH, Nakagawa S, Inagaki F, Lever MA, Engelen B, Øvreås L, Pedersen RB (2010) *Archaeoglobus sulfaticallidus* sp. nov., a novel thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids. Int J Syst Evol Microbiol 60:2745–2752

Sturt HF, Summons RE, Smith KJ, Elvert M, Hinrichs K-U (2004) Intact polar lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry – new biomarkers for biogeochemistry and microbial ecology. Rapid Commun Mass Spectrom 18:617–628

Süß J, Engelen B, Cypionka H, Sass H (2004) Quantitative analysis of bacterial communities from Mediterranean sapropels based on cultivation-dependent methods. FEMS Microbiol Ecol 51:109–121

Süß J, Schubert K, Sass H, Cypionka H, Overmann J, Engelen B (2006) Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. Environ Microbiol 8:1753–1763

Takai K, Horikoshi K (1999) Genetic diversity of Archaea in deep-sea hydrothermal vent environments. Genetics 152:1284–1297

Takai K, Moser DP, DeFlaun M, Onstott TC, Fredrickson JK (2001) Archaeal diversity in waters from deep South African gold mines. Appl Environ Microbiol 67:5750–5760

Teske AP (2006) Microbial communities of deep marine subsurface sediments: molecular and cultivation surveys. Geomicrobiol J 23:357–368

Teske A, Biddle JF (2008) Analysis of deep subsurface microbial communities by functional genes and genomics. In: Dilek Y, Furnes H, Muehlenbachs K (eds) Links between geological processes, microbial activities & evolution of life, vol 4, Modern approaches in solid earth sciences. Springer, Berlin, pp 159–176

Teske A, Hinrichs K-U, Edgcomb V, Gomez AD, Kysela D, Sylva SP, Sogin ML, Jannasch HW (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl Environ Microbiol 68:1994–2007

Teske A, Sørensen KB (2008) Uncultured Archaea in deep marine subsurface sediments: have we caught them all? ISME J 2:3–18

Toffin L, Bidault A, Pignet P, Tindall BJ, Slobodkin A, Kato C, Prieur D (2004a) *Shewanella profunda* sp.nov., isolated from deep marine sediment of the Nankai Trough. Int J Syst Evol Microbiol 54:1943–1949

Toffin L, Webster G, Weightman AL, Fry JC, Prieur D (2004b) Molecular monitoring of culturable bacteria from deep-sea sediment of the Nankai Trough, Leg 190 Ocean Drilling Program. FEMS Microbiol Ecol 48:357–367

Toffin L, Zink K, Kato C, Pignet P, Bidault A, Bienvenu N, Birrien JL, Prieur D (2005) *Marinilactibacillus piezotolerans* sp. nov., a novel marine lactic acid bacterium isolated from deep sub-seafloor sediment of the Nankai trough. Int J Syst Evol Microbiol 55:345–351

Vetriani C, Jannasch HW, MacGregor BJ, Stahl DA, Reysenbach AL (1999) Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. Appl Environ Microbiol 65:4375–4384

von Klein D, Arab H, Völker H, Thomm M (2002) *Methanosarcina baltica*, sp. nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. Extremo-philes 6:103–110

Wagner M, Horn M (2006) The *Planctomycetes, Verrucomicrobia, Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr Opin Biotechnol 17:241–249

Wagner M, Loy A, Klein M, Lee N, Ramsing NB, Stahl DA, Friedrich MW (2005) Functional marker genes for identification of sulfate-reducing prokaryotes. Methods Enzymol 397:469–489

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J Bacteriol 180:2975–2982

Wang G, Spivack AJ, Rutherford S, Manor U, D'Hondt S (2008) Quantification of co-occurring reaction rates in deep subseafloor sediments. Geochim Cosmochim Acta 72:3479–3488

Wang G, Spivack AJ, D'Hondt S (2010) Gibbs energies of reaction and microbial mutualism in anaerobic deep subseafloor sediments of ODP Site 1226. Geochim Cosmochim Acta 74:3938–3947

Wakeham SG, Lee C, Hedges JI, Hernes PJ, Peterson ML (1997) Molecular indicators of diagenetic status in marine organic matter. Geochim Cosmochim Acta 61:5363–5369

Webster G, Newberry CJ, Fry JC, Weightman AJ (2003) Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16 S rDNA-based techniques: a cautionary tale. J Microbiol Methods 55:155–164

Webster G, Parkes RJ, Fry JC, Weightman AJ (2004) Widespread occurrence of a novel division of bacteria identified by 16 S rRNA gene sequences originally found in deep marine sediments. Appl Environ Microbiol 70:5708–5713

Webster G, Parkes RJ, Cragg BA, Newberry CJ, Weightman AJ, Fry JC (2006) Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. FEMS Microbiol Ecol 58:65–85

Webster G, Yarram L, Freese E, Köster J, Sass H, Parkes RJ, Weightman AJ (2007) Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16 S rRNA gene PCR-DGGE. FEMS Microbiol Ecol 62:78–89

Webster G, Blazejak A, Cragg BA, Schippers A, Sass H, Rinna J, Tang X, Mathes F, Ferdelman TG, Fry JC, Weightman AJ, Parkes RJ (2009) Subsurface microbiology and biogeochemistry of a deep, cold-water carbonate mound from the Porcupine Seabight (IODP Expedition 307). Environ Microbiol 11:239–257

Webster G, Rinna J, Roussel EG, Fry JC, Weightman AJ, Parkes RJ (2010) Prokaryotic functional diversity in different biogeochemical depth zones in todal sediments of the Severn Estuary, UK, revealed by stable-isotope probing. FEMS Microbiol Ecol 72:179–197

Webster G, Sass H, Cragg BA, Gorra R, Knab NJ, Green CJ, Mathes F, Fry JC, Weightman AJ, Parkes RJ (2011) Enrichment and cultivation of prokaryotes associated with the sulphate-methane transition zone of diffusion-controlled sediments of Aarhus Bay, Denmark, under heterotrophic conditions. FEMS Microbiol Ecol 77:248–263

Wellsbury P, Goodman K, Barth T, Cragg BA, Barnes SP, Parkes RJ (1997) Deep marine biosphere fuelled by increasing organic matter availability during burial and reheating. Nature 388:573–576

Wellsbury P, Mather I, Parkes RJ (2002) Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. FEMS Microbiol Ecol 42:59–70

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

Wilms R, Köpke B, Sass H, Chang TS, Cypionka H, Engelen B (2006) Deep biosphere-related bacteria within the subsurface of tidal flat sediments. Environ Microbiol 8:709–719

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

Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, Kamagata Y (2006) *Anaerolinea thermolimosa* sp. nov., *Levilinea saccarolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldineae* classis nov. in the bacterial phylum Chloroflexi. Int J Syst Evol Microbiol 56:1331–1340

Yamada T, Imachi H, Ohashi A, Harada H, Hanada S, Kamagata Y, Sekiguchi Y (2007) *Bellilinea caldifistulae* gen. nov., sp. nov. and *Longilinea arvoryzae* gen. nov., sp. nov., strictly anaerobic, filamentous bacteria of the phylum Chloroflexi isolated from methanogenic propionate-degrading consortia. Int J Syst Evol Microbiol 57:2299–2306

Yoshioka H, Maruyama A, Nakamura T, Higashi Y, Fuse H, Sakata S, Bartlett DH (2010) Activities and distribution of methanogenic and methane-oxidizing microbes in marine sediments from the Cascadia Margin. Geobiology 8:223–233

Zengler K (2010) Central role of the cell in microbial ecology. Microl Mol Biol Rev 73:712–729

Zengler K, Walcher M, Clark G, Haller I, Toledo G, Holland T, Mathur EJ, Woodnutt G, Short JM, Keller M (2005) High-throughput cultivation of microorganisms using microcapsules. Methods Enzymol 397:124–130

Ziebis W, McManus J, Ferdelman TG, Schmidt-Schierhorn F, Bach W, Muratli J, Edwards KJ, Villinger H (2012) Interstitial fluid chemistry of sediments underlying the North Pond Atlantic Gyre and the influence of subsurface fluid flow. Earth Planet Sci Lett 323–324:79–91

Zverlov V, Klein M, Lücker S, Friedrich MW, Kellermann J, Stahl DA, Loy A, Wagner M (2005) Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited. J Bacteriol 187:2203–2208

9 Sea-Ice Microbial Communities

John P. Bowman
Tasmanian Institute of Agriculture, University of Tasmania, Hobart, TAS, Australia

Introduction

Sea ice microbial communities (SIMCO) contributes substantially to the total biological productivity within polar oceans and strongly influences global energy budgets via atmospheric-oceanic interactions (Zwally et al. 1983). SIMCO also has a major role in trophic food webs (Lizotte 2003) and occurs on the surface of sea ice, within ice floes, and within assemblages concentrated near the sea ice: seawater interface (Palmisano and Garrison 1993). Sea ice is both highly variable temporally and spatially exhibiting enormous seasonally transience and is constantly being broken up and reshaped by wind and ocean currents. The sea-ice ecosystem is an extreme, low temperature environment (Thomas and Dieckmann 2002; Mock and Thomas 2005) with internal temperatures perpetually subzero, ranging from -1°C to $< -20^{\circ}$C in winter. Furthermore, channels and cracks within the sea-ice ice matrix formed when sea salt concentrates during freezing can rise as high as 150 psu. The development, diversity, and stability of the SIMCO are thus predicated largely by physical forces (Ackley and Sullivan 1994). Despite this sea ice is a biologically productive, albeit with a rather heterogeneous, patchy distribution. On a purely volume-to-volume basis, cell biomass and growth rates are larger than the underlying pelagic zone likely due to the ice matrix providing surfaces for inhabitation (Grossmann and Diekmann 1994). The assemblages of organisms that make up SIMCO are rather complex and constitute a well-developed microbial loop that includes, besides algae and bacteria, ice-preferring (i.e., sympagic) and less habitat-specific allochthonous protists, zooplankton, and small metazoa (Palmisano and Garrison 1993). SIMCO biomass is typically dominated by sea-ice algae, which are capable of adapting to low light levels. Bacterial activity and populations has been found to be in part tightly coupled to and dependent upon algal primary productivity; thus, most bacteria present are heterotrophs and play an important role in secondary mineralization of organic matter within sea ice (Kottmeier and Sullivan 1987). During winter as light levels decline, bacteria become more important and eventually dominate the activity in sea ice (Kottmeier and Sullivan 1987). SIMCO and the bacterial population it includes clearly play an integral role in the food web in polar oceans.

Sea-Ice Formation and Thaw

Sea-ice occurs in the Arctic Ocean, the Southern Ocean, which is essentially the polar extensions of the Pacific, Atlantic, and Indian Oceans below the 60th parallel (as unofficially ratified by the International Hydrographic Organization), and during winter in the North Pacific and Atlantic, and the Baltic Sea. The high latitudes of these regions means they receive markedly asymmetric radiation regimes over the course of the year with winter insolation dramatically lower than that of summer

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_46,

months. The heat input to the ocean is thus so minimal that heat is lost from the unfrozen surface water, resulting in temperatures just above the freezing point of seawater (~ − 1.8 °C; Priddle et al. 1996). Turbulent mixing of surface water leads to loss of heat (supercooling) by an additional ~0.1 °C or less, leading to ice formation (Wadhams 1994; Maykut 1985). Small ice crystals that form during supercooling appear as 1–4-mm disk-like objects and are referred to as frazil ice. Frazil ice that concentrate eventually form a suspended layer of ice slush, called "grease ice." Wave-induced compression forces the frazil ice crystals together, and these grow in size to visible disk-shaped edifices that have a roughly pancake shape as shown in *Fig. 9.1*. Eventually pancake ice further compresses and coalesces to form a consolidated ice sheet, and individual ice floes over a period of several weeks, the timing of which varies from year to year. Another feature of young (or first year) ice is the formation of frost flowers via sublimation on the ice floe during its consolidation. Frost flowers are characterized by high salinity levels (~100 psu) due to concentration of brine during freezing (Style and Worster 2009).

Fig. 9.1
A photograph taken in the Southern Ocean ice pack showing grease ice slush containing floating pancake ice, indicative of newly forming sea ice as well as an ice floe containing a well-developed algal band assemblage (Image care of M. Carver, University of Tasmania)

In areas where the sea surface is completely ice covered, congelation ice eventually forms due to additional heat loss occurring from the ice surface, which can reach <−20 °C, as well as freezing at the ice-sea water interface. Ice crystals within congelation ice become organized with the lattice developing a uniform orientation. Growth occurs perpendicular to this axis, producing interlocking columns about 1–2 cm in diameter (Lewis and Weeks 1971). During freezing, salts and other solutes are rejected out of the ice matrix into brine channels (Gianelli et al. 2001; Thomas et al. 2001), leading to the congelation ice itself being rather free of organic matter. Rejected solutes increase the density of underlying seawater, which in the process of sinking helps carry more heat away, further aiding consolidation of the sea-ice layer. Typically most sea ice occurs as pack ice but when attached to the shoreline is called fast ice (*Fig. 9.2*).

Owing to climate change, this last fact has invited great interest (e.g., Wong et al. 1999). Growth of the ice sheet is affected by localized oceanographic regimes, resulting in high spatial heterogeneity (Palmisano and Garrison 1993; Eicken et al. 1995; Cavalieri et al. 1997; Fritsen et al. 2011). Indeed, most sea-ice formation occurs in particular regions, called polynyas, where wind, currents, and waves act to maintain areas of open water. Polynyas continually produce frazil and pancake ice that consolidates into ice floes and are then pushed outward (Clarke and Ackley 1984; Vincent 1988; Wadhams 1994). Due to the sinking of oxygen-rich denser seawater, polynyas around Antarctic also generate Antarctic Bottom Water, which ventilates the deep ocean and thus can impact atmospheric CO_2 levels (Skinner et al. 2010).

Below sheets of sea-ice further thickening of the layer is due to ice platelet-like structures (10–15 × 0.2–0.3 cm) forming in the water column that rise and consolidate at the seawater-ice interface and eventually form a porous layer approximately 5-cm thick (see *Fig. 9.3*) (Horner 1985; Lange 1988). In addition to this under-ice formation, accumulation of precipitated snow can cause the ice to slightly bend, thus allowing infiltration of seawater that pools and then freezes as surface layers. Furthermore, variations in ice structure can occur in which ice sheets collide and raft over each other leading to, for example, ridging of the pack ice (*Fig. 9.2*, top image). Due to dynamic processes, such as drifting, divergence, and rafting, high levels of heterogeneity within the physical structure of ice can occur that subsequently affects the types of life that may flourish there (Horner 1985).

Sea-ice extent around Antarctic is tremendously seasonal due to its overall lower latitude (between 60 and 75°S). At its maximum extent in September, the ice extends to cover about 20 million km^2 encircling the Antarctic continent in a 400–1,900-km wide ring of ice (Zwally et al. 1983) (*Fig. 9.4*). By comparison in the Arctic Ocean, sea ice forms a polar cap (mostly north of 75°N) adjacent to the Canadian Archipelago, Greenland, extending to Svalbard, Franz Josef Land, and Svetnaya Zemyla ("Northern Land") in winter (*Fig. 9.4*). Until fairly recently, the Arctic ice cap has been stable until the thinning and reduction of recent decades. Antarctic sea-ice thaw in the spring and summer is due to increased solar irradiance. Melt water that is low in salt (<10 psu) tends to sit above the denser waters, forming zones of water column stability along the retreating ice edge (referred to as the marginal ice zone or MIZ). The release of nutrients from sea ice likely contributes to the observation that south of the Antarctic Convergence, the Southern Ocean is overall relatively nutrient (nitrate, phosphate, silica) rich (El-Sayed 1971) though nutrient levels can be affected by deep vertical mixing (Mitchell and Holm-Hansen 1991).

Phytoplankton Blooms and Their Associated Bacteria in the Polar Oceans

Most of the primary producers in sea ice are diatoms, including pennate, centric, and chain-forming species of the genera

Fig. 9.2
The top photograph shows the consolidated Southern Ocean ice pack possessing an accumulated snow layer and substantial ridging caused by collisions and rafting of individual ice floes. The bottom photograph shows fast ice attached to the Antarctic coast of the Vestfold Hills region (Images care of M. Carver, University of Tasmania)

Fragilariopsis, *Nitzschia*, *Eucampia*, and *Melosira* as well as the prymnesiophyte *Phaeocystis*. During most of the year, phytoplankton cells are mixed to depths where the photon flux is low, resulting in populations which can be highly adapted to low light levels. The stabilization of the water column down to 20–40 m if sufficiently prolonged can thus facilitate phytoplankton blooms where primary production exceeds the mean total loss rate through respiration, excretion of dissolved organic compounds, grazing, and sedimentary export (Holm-Hansen and Vernet 1990; Gleitz et al. 1994). The blooms that occur are highly productive, similar to temperate areas, and may quickly result in large standing stocks of organic matter, i.e., $>10\ \mu gL^{-1}$ chlorophyll *a* (chl*a*). By comparison, various studies have reported a distinct absence of phytoplankton blooms in the MIZ possibly due to climactic variability (e.g., Bathmann et al. 1997). Giesenhagen et al. (1999) using microcosm experiments suggested phytoplankton blooms were stimulated by the seeding of the water column by sea-ice-derived cells that survived grazing. When grazers were present, high levels of DOM released by the "sloppy feeding" of grazers stimulated pelagic bacterial populations but at the expense of algae. This suggests MIZ phytoplanktons are controlled by grazing activity and also by potentially the lower salinity.

Regeneration of nutrients occurs via a microbial loop, involving energy flow through microheterotrophic and zooplanktonic assemblages. Bacteria utilize high and low molecular weight dissolved organic material mainly produced by phytoplankton, regenerating nitrogen, and through grazing, death, and autolysis, returning carbon, energy, and trace elements to the nutrient pool. Bacterial growth and production appears to be coupled with phytoplankton over the course of the year (Delille et al. 1995). However, a lag in bacterial growth occurs during and immediately after the spring phytoplankton blooms that uncouples bacterioplankton and phytoplankton production (e.g., Karl et al. 1991). Karl (1993) discussed this in relation to the Pomeroy-Deibel hypothesis (Pomeroy and Wiebe 2001), which suggests this phenomenon could be due to

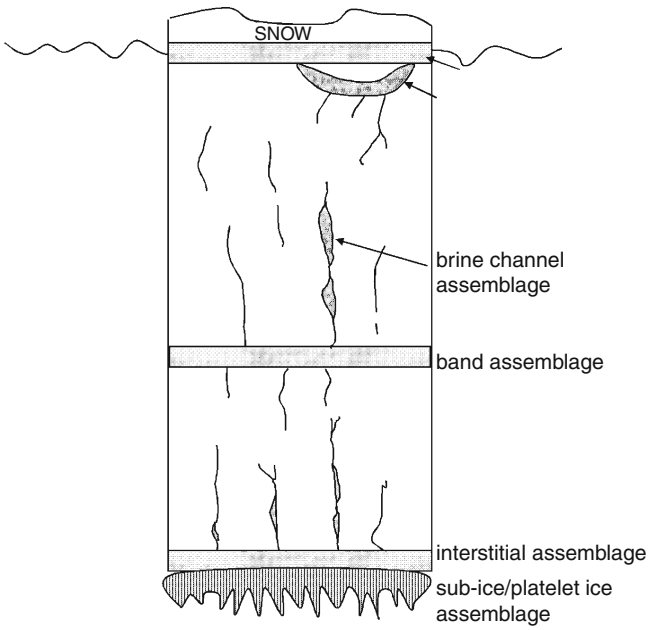


Fig. 9.3
A schematic diagram of the types and positions of various biological assemblages which may occur in sea ice (Redrawn from Horner et al. 1988)

a differential dependency of phytoplankton and bacterial production to temperature. The thermal budget of the top 100 m of the water column is dominated by ice formation and ice melt (Priddle et al. 1996) and as a consequence, the surface waters is always near 0°C (Delille 1996). Low temperature is probably the ultimate rate limiting factor in potential phytoplankton primary production. The phytoplankton inhabiting the Southern Ocean are typically obligate psychrophiles, with maximal growth rates at low temperatures (El-Sayed and Fryxell 1993). However, even at the greatest recorded values, when other potentially limiting factors are optimal, growth rates fail to reach predicted

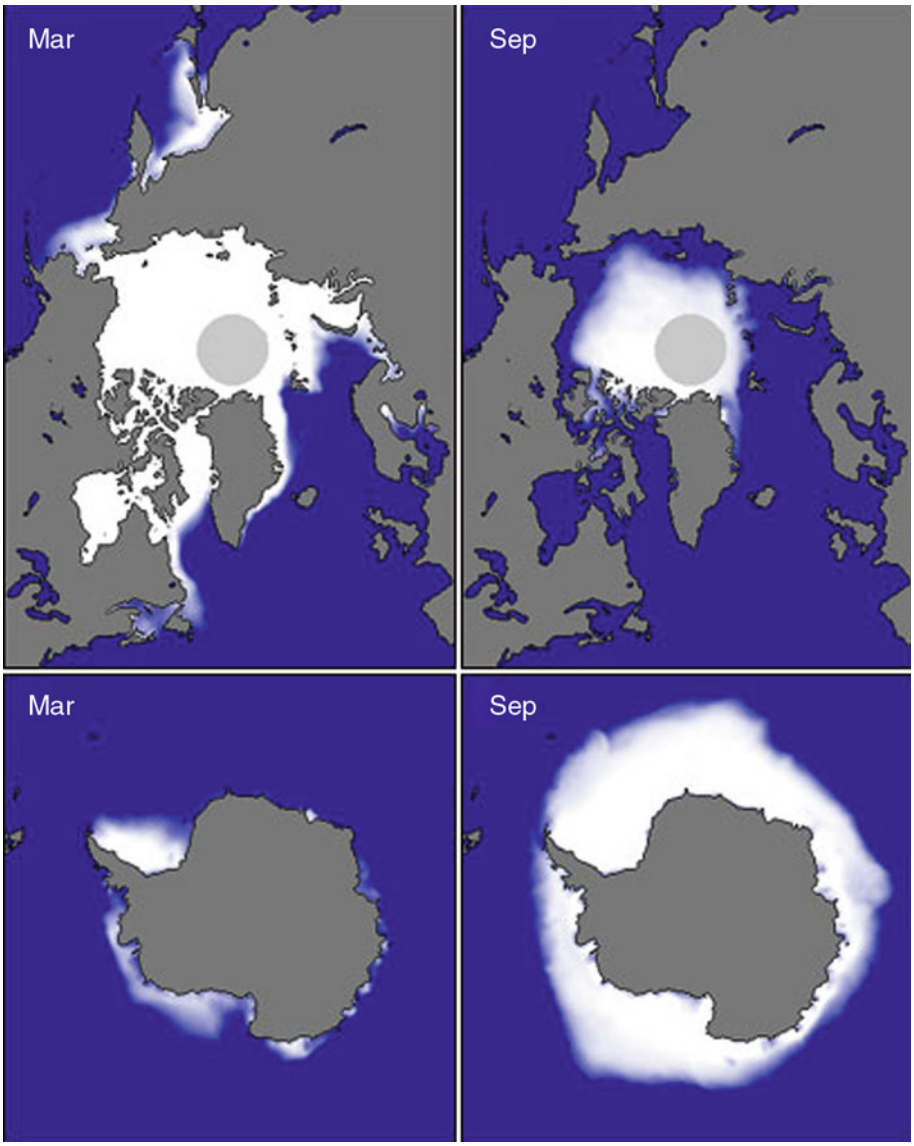


Fig. 9.4
Maximal seasonal changes in the surface area of sea ice in the Arctic and around Antarctica during the vernal (spring) and autumnal equinoxes (The image is reproduced with permission from the National Ice and Snow Data Center, University of Colorado, Boulder, CO, USA)

maximum levels as calculated by Ratkowsky's biokinetic growth model (Ratkowsky et al. 1983). Cold-adapted bacteria may be grouped into two categories. Psychrophiles comprise those organisms whose minimum, optimum, and maximum (cardinal) growth temperatures are <0 °C, <15 °C, and <20 °C respectively, while "psychrotrophs" comprise those organisms whose cardinal temperatures are typically 0–5 °C, >15 °C, and >20 °C (Morita 1975). It however should be remembered that temperature optima for growth represent a continuum (Russell 1998). The bacterial assemblages of polar oceans seem to be relatively psychrophilic (Connelly et al. 2006) though most bacteria that can be grown are not especially so (Delille et al. 1995), which would tend to agree with the observation that prevailing temperature of the pelagic zone does not observably restrict bacterioplankton population growth (e.g., Karl 1993; Delille and Rosiers 1996).

During the initial phase of a bloom, phytoplankton standing stocks develop quickly while grazing rates and bacterial consumption of DOM and dead or moribund cells tends to lag behind until the bloom goes past its peak. Bacterial consumption and grazing is then stimulated during the bloom demise typically during late summer and early autumn. At the time of new ice formation, standing stocks of organic matter tend then to be generally low and the scavenging of this remaining matter during sea-ice formation effectively ends the seasonal production of the water column (Gleitz et al. 1994).

The Development of SIMCO

The physical mechanisms involved in the formation of sea ice provide not only varied environments for microbial habitation but are also an avenue for the incorporation and enrichment of microorganisms into sea ice. The surface properties of frazil ice crystals facilitate the adhesion of particles from the water column. As the crystals form and move in the water column, inorganic and organic material can accumulate on and between them, so that the ice matrix incorporates exogenous matter (Garrison et al. 1983). Incorporation of silt and clay particles, sand grains, diatom and foraminiferal detritus, living algal and bacteria cells is an active scavenging-like process (Garrison et al. 1983; Reimnitz et al. 1993). Physical matter and solutes of the sea water, including nutritious substances, are considerably enriched in the forming ice, and content and concentration reflects what is found in the water column at the time of ice formation (Gleitz and Thomas 1993; Grossmann and Gleitz 1993; Garrison and Buck 1989; Gianelli et al. 2001; Thomas et al. 2001) This emphasizes that this early stage of scavenging introduces a level of stochasticity to subsequent biological activity within the ice matrix (Mock and Thomas 2005). Scavenging is a size-dependent process (Grossmann and Diekmann 1994; Weissenberger and Grossmann 1998), and small bacterioplankton of $< \sim 1\ \mu m^3$ appear to be less likely to be scavenged. It was observed that bacterial concentrations in newly formed ice are much greater than can be accounted for by biological growth over the short time span involved. Co-incorporation of bacteria with algae is now considered the predominant mechanism of bacterial enrichment into sea ice (Sullivan and Palmisano 1984; Grossi et al. 1984; Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Diekmann 1994; Weissenberger and Grossmann 1998). In support of this, strong correlations have been reported between the numbers of bacterial cells incorporated into sea ice and the concentration of phytoplankton in the sea ice and water column. In experimental sea-ice formation, a number of studies (Grossmann 1994; Grossmann and Diekmann 1994; Weissenberger and Grossmann 1998) found that physical enrichment of bacteria within new ice was negligible and bacterial biomass was only enhanced when the concentration of algae was high. This co-incorporation may result in selective enrichment of epiphytic bacteria as well as cells attached to particulates (Helmke and Weyland 1995). Another possible mechanism of cell enrichment in sea ice is through percolation pumping. This occurs in the outer regions of the pack ice where ocean swells still influence the environmental conditions. Heavy swells cause bending of the ice sheet and percolation by seawater, leading to the enrichment of adhesive particles that are retained preferentially in the pore system (Ackley et al. 1987). The topography of the sea ice: seawater boundary is also very important with the flow over the uneven boundary surface leading to localized algal accumulations (Krembs et al. 2002). In a study in Arctic multiyear ice during winter analysis of microbial communities indicated that the harsh winter conditions do not act to select community members through taxon-specific mortality and that pelagic bacteria and archaea retained in ice remain unaffected in terms of community richness or diversity but rather slowly decline in abundance (Collins et al. 2010).

The physicochemical stresses experienced by pelagic biota during the transition from a pelagic to a sympagic habitat may result in the development of a characteristic SIMCO (see "Bacterial Diversity and Its Selection Within SIMCO" section below for more specific information on selection). Algae and their associated bacteria receive reduced PAR (photosynthetically available radiation) due to the ice layer and can be restricted to small brine channels which have sea salts concentrations 2–4 or more times that of normal seawater in tandem with freezing temperatures $< -10\ ^\circ C$. The ability to acclimate to these conditions likely determines which components of the initial sea-ice community become dominant. Gleitz and Thomas (1993) suggest that size could be important for algae with smaller polar diatom species favored ($<40\ \mu m$) due to the spatial confinement. These conditions appear to at least initially impair photosynthesis during the early ice formation (Palmisano et al. 1997; Kottmeier and Sullivan 1988). However, algae appear to compensate by increasing cellular chl*a* and other accessory pigment levels as PAR declines with the increasing ice thickness (Palmisano et al. 1997; Watanabe and Satoh 1997; Gleitz and Thomas 1993). Bacterial metabolism and growth rates are also repressed during new ice formation as suggested by incorporation rates of [^{3}H] leucine and [^{3}H] thymidine, indicators of heterotrophic activity and turnover, respectively (Grossmann and Gleitz 1993; Grossmann 1994; Grossmann and Diekmann 1994).

Since algal cells are generally attached to the sea-ice crystal matrix, they are retained and so are not dispersed back into the underlying seawater by brine ejection. Indeed phytoplankton standing stocks have been observed at concentrations far in excess of the maximum predicted that can be supported by the amount of nutrients initially present (Gunther et al. 1999). An upward flux of nutrients into the boundary layer (Gosselin et al. 1985; Knox 1994) does occur which could replenish nutrients. This process appears active when the boundary layer beneath the ice is destabilized, which frequently occurs due to brine rejection and unidirectional water movement (Knox 1994). Nutrients may also be regenerated in situ by heterotrophic remineralization by bacteria and protistan flagellates (Griffiths et al. 1982). In pack ice areas which make up most of the polar ice cover, hydraulic pumping by oceanic wave action leads to perfusion of the ice by seawater, resulting in an influx of both nutrients and microbial biomass. This, along with the continued scavenging of matter during ice formation, leads to the presence of substantial microbial communities throughout the ice column (Kottmeier and Sullivan 1987) and also at the ice surface (Garrison and Buck 1989). Pack ice constitutes regions of substantial heterotrophic potential, with both bacterial biomass and production exceeding significantly that of the water column (Grossmann 1994). Helmke and Weyland (1995) found bacterial counts during their investigations ranged over four orders of magnitude with frazil and mixed frazil/congelation ice having the highest bacterial counts ($\sim 10^7$ cells ml^{-1}) while predominantly congelation ice had lower counts (10^3–10^4 cells.ml^{-1}).

By comparison where sea-ice attaches to land (i.e., fast ice) (Fig. 9.2), congelation ice predominates and the upper ice layers brine channels are very small, isolated systems, effectively closed off from both the surface and from the water column (see Fig. 9.3). As a result nutrient influx tends to become restricted, and without a source of nutrient input, scavenged algae and bacteria recede quickly into unproductive layers of the ice matrix. The continued downward growth of ice is mirrored by a downward shift in chl*a* maxima, as new productive bottom layers develop, while older layers are internalized, resulting ultimately in a dispersed interior assemblage of low to negligible productivity and a thin basal assemblage characterized by high productivity (Clarke and Ackley 1984; Sullivan and Palmisano 1984; Grossmann and Diekmann 1994). An example internal algal assemblage in an ice flow is shown in Fig. 9.1, while position of basal and internal assemblages is shown diagrammatically in Fig. 9.3. In the basal assemblage, the temperature is fairly stable at around −2 °C and does not drop to the severe levels experienced closer to the ice-air interface (Vincent 1988). Fast ice in general does not accumulate snow as readily as pack ice; thus, PAR is greater for active photosynthesis (Ackley and Sullivan 1994). Also in the platelet layer, numerous, spacious brine channels and interstitial spaces occur (lowest layer of the ice core as shown in Fig. 9.3.), thus allowing active recharging of nutrients and oxygen due to the convective flushing of seawater (Mock et al. 2003). In McMurdo Sound, Sullivan and Palmisano (1981, 1984) found a sharp gradient in the distribution and production of biological matter within the fast sea-ice matrix with the lower 20 cm of the sea ice containing virtually all of the chl*a* content and bacterial biomass. The bacteria present were morphologically diverse and were at least 10 times larger in volume than those found in the upper ice layers (Grossi et al. 1984). Bacteria present in the upper layers appeared similar to those from the water column and were usually singular, reminiscent of starved cells.

Where seawater flushing can recharge nutrients, production can continue until it is self-limiting due to the shading created by dense concentration of algal pigments (Gunther et al. 1999). Seawater can be restricted due to stabilization of the boundary layer restricted water movement into the ice matrix, and this can be induced by SIMCO, developing to a high density. In fast ice areas, calm water of coastal areas impedes nutrient recharging to only the basal layer. When nutrient exchange becomes diminished, SIMCO growth must rely on rates of in situ nutrient regeneration occurring by virtue of the manifested microbial loop (Sullivan and Palmisano 1984; Kivi and Kuosa 1994; Lizotte 2003). A number of reports have described significant numbers of bactivorous and algae grazing protozoans in SIMCO (Garrison and Buck 1986; Kottmeier et al. 1987; Kivi and Kuosa 1994). Krembs et al. (1999) reported that large predators seem to be generally excluded from brine channels; however, more elastic predators, such as turbellaria, are able to penetrate the brine channel network. Nutrients may also be released by algal lysis, owing to senescence and sloppy feeding by grazers. This release of DOC may be large enough to accumulate and thus be accessible to active secondary remineralization (Gunther et al. 1999). The upper sea-ice layer, close to the ice-air interface, is particularly depleted of nutrients (McMinn et al. 1999). This is due to restriction of either recharging or remineralization processes. This effectively limits SIMCO growth, leading to distinct banded assemblages. This is clearly most obvious in fast ice based on reasons outlined above. Since well-developed SIMCO is readily observed in multiyear pack ice (Kottmeier and Sullivan 1990), the processes of nutrient regeneration are generally well maintained due to the turbulence of the underlying oceanic water mass. Analysis of Baltic Sea-ice SIMCO indicated that bacteria community structure change, analyzed via temperature gradient gel electrophoresis fingerprinting, was more strongly temporal than spatial. However, there was still substantial similarity in the patterns between bottom ice and underlying seawater communities during SIMCO development. This indicates the maturity of the ice is a critical factor to be considered when considering details of SIMCO community structure in relation to ice formation and structure (Kaartokallio et al. 2008).

Bacterial Interactions and Secondary Production Within Sea Ice

Bacteria in sea ice are primarily either attached to algae (epiphytic) (see Fig. 9.5), detritus, and other surfaces or freely motile, or planktonic. Studies suggest that the epiphytic and free-living populations are approximately equivalent (Sullivan and Palmisano 1984; Grossi et al. 1984) and bacterial populations correlate with chl*a* levels (e.g., Kivi and Kuosa 1994; Sullivan and Palmisano 1984; Staley et al. 1989). The larger cell volumes of epiphytic bacteria suggest a mutualism exists with algae. The fact that PAR controls both algal and bacterial growth (Grossi et al. 1984) emphasizes the coupled nature of these primary SIMCO components. Increased PAR also promotes the growth of epiphytic bacteria along with their algal hosts such that with no limitation in PAR, growth rates double from 0.05 d^{-1} to approximately 0.1 d^{-1}. Bacterial production during spring in the newly formed ice was shown to lag behind the algal bloom by 1–2 weeks. An experiment in which the microbial loop was shocked by cutting out ice cores and adding them back to the ice in the reverse direction, thus affecting light, temperature, and salinity levels, suddenly also showed a similar lag in response to the availability of dissolved organic carbon (DOC) while algae were much more sensitive to the perturbation (Martin et al. 2011). Once the bloom has sufficiently progressed, the bacterial populations increase exponentially (Kottmeier et al. 1987). This could be due to increased availability of living algal surfaces for colonization as well as DOC. Some suggestions of allopathic control of bacterial growth rates by algae-derived secondary metabolites could also explain why this lag occurs (Montfort et al. 2000; Pusceddu et al. 2009), and so bacteria that do grow are likely resistant (or become resistant) to this control. Subsequent secondary production, as indicated by [^{3}H]-leucine and [^{3}H]-glucose uptake, parallels closely photosynthetic activity. The algae are believed to generate a "phycosphere" (as first described by Bell and Mitchell 1972),

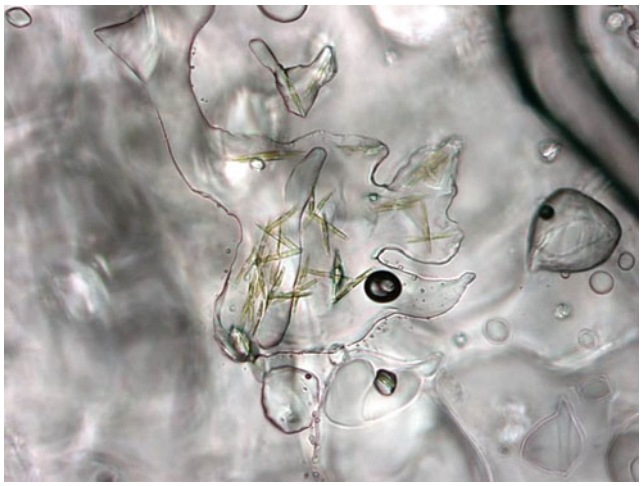


Fig. 9.5
Arctic sea-ice brine channel showing presence of diatoms (Photo from Christian Krembs and Jody Deming [from National Oceanic and Atmospheric Administration Arctic Theme Page, http://www.arctic.noaa.gov/])

a type of modified, localized niche beneficial to microbes. Bacteria, heterotrophic microeukaryotes and viruses within such zones recycle dead biomass, extracellular polymeric substances (EPS), particulate organic carbon (POC) (Herborg et al. 2001), and DOC to nutrients providing algae opportunities for further production (Lizotte 2003; Mock and Thomas 2005).

SIMCO's optimum ability to assimilate DOC is close to the prevailing ambient temperatures of the sea ice: seawater interface, about −1 °C (Hodson et al. 1981). As mentioned above, EPS and POC accumulates in sea ice due to intense primary production, and thus, relative to the underlying waters, the turnover of DOC is high (Zdanowski and Donachie 1993; Helmke and Weyland 1995). Since the DOC component is largely made up of monocarbohydrates (70–88%), EPS, mainly polysaccharide, appears to be degraded and likely represents a major food source to sea-ice bacteria (Herborg et al. 2001). Bacterial growth rates and activity increases with the advancing age of algal blooms. This is likely due to increased availability of DOC, EPS, POC, and detrital material generated from senescing algal biomass (Gunther et al. 1999; Meiners et al. 2008). Bacterial respiration rates are also highly active in SIMCO during the growing season. In a study in the Canadian High Arctic, respiration rates were on average 22-times higher than the underlying water column and that a large proportion of the primary production is converted to CO_2 (Nguyen and Maranger 2011). Aging algae tend to exude more organic matter up to some stage when they lyse (Cole 1982). This process could be also encouraged by predation by microeukaryotes, bacteria (e.g., Lovejoy et al. 1998), and via viral lysis (Gowing et al. 2004; Wells and Deming 2006a). At some point, the coupling of algal and bacterial production fades with bacterial production outstripping algal growth (Kottmeier and Sullivan 1987). This seasonal evolution of SIMCO may comprise variations in the community succession since organic matter of different types and quality could become available, allowing growth of different microbes (Zdanowski and Donachie 1993). Alternatively, sea-ice bacteria could be evolved to take advantage of the different array of nutrients available at any given time. Various bacteria in sea ice show the ability to degrade hydrocarbons derived from petroleum oils (Delille et al. 1997), including at least *Colwellia*, *Glaciecola*, *Pseudomonas*, and *Marinobacter* (Gerdes et al. 2004; Brakstad et al. 2008). Besides simple and easily consumable substrates such as sugars and amino acids, hydrocarbons derived from dying algae (perhaps algal lipid degradation products) could represent an unexpected but important food source for SIMCO.

The close association of algae and bacteria may suggest bacteria act to aid algae by provision of growth factors. Many algae require bacterial co-hosts present in order to be grown in the laboratory, and this may be due to provision of specific compounds such as vitamin B12 (Croft et al. 2005). Sea-ice algae were determined to receive sufficient supplies of vitamin B12 from bacteria in situ though availability of free cobalt in the seawater may act as a potential limitation to algal growth (Taylor and Sullivan 2008). Trace metals however could be retrieved and retained in sea ice due to EPS acting as a ligand (Hassler et al. 2011), as explained further below in the section on "Role of EPS in Sea Ice". Bacteria may also act to protect sea-ice algae from reactive oxygen species and oxidizing compounds (such as H_2O_2) arising from photosynthetic activity by virtue of catalase production (Hunken et al. 2008).

Light-Harvesting Bacteria in SIMCO

Based on research to date, SIMCO strongly favors organic carbon-dependent heterotrophs. The ability to gain energy by other means such as by light-harvesting or by inorganic autotrophic or mixotrophic process is potentially possible since resources in SIMCO may become limited at various times. Within the context of sea ice, such an ability may aid in long-term survival, especially relevant to multiyear ice. Only a few studies have explored this aspect of sea-ice microbiology, including determination of whether sea ice hosts light-harvesting bacteria. Most sea ice, at least the majority of times, is a rather aerobic environment given the low temperature and intense algal photosynthesis. As a result anoxygenic phototrophic bacteria (AAnPs) and bacteria possessing proteorhodopsin may occur in sea ice. AAnPs are strictly aerobic and use bacteriochlorophyll *a* as a light-harvesting pigment. Most known members are of the class *Alphaproteobacteria* (Yurkov and Beatty 1998). Sea-ice dwelling equivalents appear to be mainly associated with the lower parts of the ice layer (Koh et al. 2011), suggesting entrapment from the seawater rather than selective enrichment. Proteorhodopsin is a light-harvesting retinylidene cofactor-binding membrane protein able to act as a transmembrane pump and that aids in survival (DeLong and Béjà 2010). Surveys of melted sea ice have detected proteorhodopsin to be present (Koh et al. 2010). However, proteorhodopsin was not overly

common and could thus be derived from entrapped pelagic bacteria, including those of the SAR clades that only occur in low populations in SIMCO. The extremely psychrophilic, sea-ice species *Psychroflexus torquis* and *Polaribacter franzmanii* (members of the phylum *Bacteroidetes*) both possess proteorhodopsin (Bowman 2008), but the trait seems to be quite uncommon among cultured sea-ice bacteria (unpublished data from the author). Intense heterotrophic activity along with the enclosed ice channel system has been shown to lead to anaerobic zones (Rysgaard et al. 2008) In Baltic Sea ice, anaerobic phototrophs of the family *Chromatiaceae* as well as fermentative bacteria belonging to *Propionibacterium* and *Bacteroides* were observed (Petri and Imhoff 2001) that attest to this phenomenon. It is suspected the situation could be peculiar to the Baltic Sea as none of these taxa have been observed in other sea-ice zones. Overall, data suggests light-harvesting bacteria are not selected for in SIMCO under normal circumstances, but a minority of bacteria are capable of light harvesting of some sort and their occurrence could be regionally specific. The high level of shading in SIMCO may reduce any advantage light harvesting has though it may provide a survival edge outside of dense assemblages and when energy resources become limiting.

Nitrogen-Related Processes in Sea Ice

Sea-ice algae in the later stages of a bloom tend to be senescent and/or nitrogen limited and thus incorporate a high proportion of carbon that they fix into a polysaccharide-rich carbon fraction (Palmisano and Sullivan 1985). This leads to exudates high in carbon but lower in nitrogen, and other nutrients such as phosphate may also be reduced. Iron can also become less available due to slowdown in biological activity and export (Pankowski and McMinn 2008; Hassler et al. 2011). Active bacterial growth indicates efficient uptake of nitrogen and other nutrients (Bratback and Thingstad 1985). Owing to large surface area to volume ratio, bacteria are generally more adept at nutrient uptake than larger algal cells (Elser et al. 1995). This tendency perhaps leads to the transition from algal to bacterial dominance in SIMCO. Bacterial nitrogen is usually derived from inorganic sources in sea ice, especially ammonia (Tupas et al. 1994; Kuparinen et al. 2011), but given the high levels of production in sea ice, dissolved organic nitrogen, in the form of proteins and amino acids, could also be important. Addition of ammonia stimulates sea-ice bacterial growth rates and also growth efficiency (Kuparinen et al. 2011). Amino acids can be stimulatory to growth and protease activity was highest during the summer season (Christian and Karl 1995). This may suggest sea-ice bacteria produce many extracellular enzymes, allowing breakdown of peptide substrates to obtain nitrogen. These observations are also consistent with sea-ice bacteria having more copiotrophic, nutrient-dependent physiologies (Bowman et al. 1997a). It was observed that SIMCO can become oxygen depleted due to intense heterotrophic activity, encouraged within brine channels. As a result, denitrification and anaerobic ammonia oxidation can occur at appreciable rates since the DOC, ammonia, and nitrate pools can be replete (Kaartokallio 2001; Rysgaard and Glud 2004; Rysgaard et al. 2008). Sea-ice *Shewanella* spp. and *Colwellia* spp. can use nitrate as an electron acceptor for anaerobic respiration and/or to ferment sugars (Bowman et al. 1997d, 1998a); thus, it is possible other sea-ice bacteria possess a range of metabolic strategies not simply limited to aerobic chemoheterotrophy.

The Fate of SIMCO

Since the majority of sea ice is seasonal, it only provides a habitat for SIMCO for part of the year. Oceanic heat exchange causes continual slow ablation of the pack ice, even in winter. As a result, biological assemblages that occur within the ice layer eventually become exposed to the seawater (Ackley and Sullivan 1994). The brownish pigmentation of algal assemblages in sea ice (see ❯ *Fig. 9.1*) through absorption of light further introduces heat into the ice layer, and thus, the layer can rapidly disperse into the water column (Sullivan et al. 1983). The retention of the productive SIMCO layer at the base or peripheries of ice floes is a critical aspect since it is a major source of food for metazoa that graze on SIMCO (Kottmeier and Sullivan 1987). Krills are perhaps the most famous organisms that depend on SIMCO in the Antarctic pack ice, but many other zooplankton species depend upon it as well, allowing them to both reproduce during the active growing season and survive the winter (Bluhm et al. 2010; Dieckmann and Hellmer 2010).

Melting of sea ice results in a layer of relatively low salinity (~10 psu); water up to 1 m deep can form below melting ice floes. Giesenhagen et al. (1999) reported that upon seeding of this water layer with sea-ice-derived assemblage material, the pelagic bacterial population was stimulated for several days. Sea-ice microbial activity on the other hand was diminished, possibly by the combination of low salinity and higher UV-B radiation (Martin et al. 2009). The salinity stratification also as indicated above can initiate algal blooms that have obtained nutrient pulses from the dispersing sea-ice-derived material, assuming a relative absence of grazers. Sea-ice thaw also releases large amounts of exopolymeric particulates and phytodetritus (Meiners et al. 2004; Riedel et al. 2006) that is rapidly exported, influencing the productivity of benthic communities (Schewe and Soltwedel 2003; Smith et al. 2004).

Psychrophiles in Antarctic Sea Ice

There is a considerable difference in the distribution of psychrophilic and psychrotrophic bacteria between the water column and sea ice. The majority of bacteria isolated from SIMCO are psychrophilic while those from the underlying seawater are psychrotrophic (Delille 1996; Helmke and Weyland 1995; Bowman et al. 1997c). This was shown in some details by cultivation of bacteria from Antarctic fast ice and underlying seawater (Bowman et al. 1997a, c). It was also observed psychrophilic bacterial diversity was significantly enriched in sea-ice

assemblages but not in sea ice lacking significant algal accumulations. Selection of psychrophiles thus appears to occur within SIMCO. Obviously this selection is temperature independent since the sea ice and seawater temperatures are rather close (Helmke and Weyland 1995). The reasons are likely a mélange of physical, nutritional, and ecological factors. Psychrophilic bacteria were observed to be more nutritionally fastidious than psychrotolerant strains, often requiring organic nitrogen, yeast extract, and/or vitamins for growth (Bowman et al. 1997a). It can be assumed these nutrients are derived from their algal hosts upon which they are primarily epiphytic as well as the higher levels of DOC and POC within climax ice assemblages. Harder and Veldcamp (1971) observed that a psychrophilic strain regularly outgrew a psychrotrophic at low temperatures (where their growth rates were similar) when in the presence of high nutrients. This suggests that during sea-ice entrapment and subsequent blooms, SIMCO provides the combination of a stable algal-associated niche and nutrient regime to support the growth of often slow-growing, more specialized bacteria. Since sea ice is ephemeral, sea-ice psychrophiles must be able to survive in the water column at least for a time, presumably in close association with partner algae. This potentially demonstrates the close mutualism of SIMCO algae and bacteria that aids in generating the distinctiveness of SIMCO from pelagic microbial populations.

Cold-Active Enzymes of SIMCO

A fundamental aspect of psychrophily is the synthesis of cold-active enzymes, thus allowing growth to occur at temperatures well below 0 °C. In order to function effectively at low temperatures, enzymes must balance the conflicting requirements for structural stability and conformational flexibility. Psychrophilic microorganisms have thus attracted biotechnological interest (Nichols et al. 1999a, 2006; Bull et al. 2000) including applications within the chemical, food, and bioremediation industries which has been reviewed extensively over the last several years (e.g., Aguilar et al. 1998; Nichols et al. 1999a; Margesin and Schinner 2001; Bull et al. 2000; Rothschild and Mancinelli 2001; Collins et al. 2008b). This is clearly extremely ecologically significant since this adapted enzyme function allows for active secondary mineralization, and thus, sustainment of SIMCO populations is required throughout the entire polar year.

Extracellular enzymes secreted by sea-ice microbes are critical since their carbon and energy resources must be supplied from external sources. Much of this requires direct decomposition of biological macromolecules, in particular, proteins and polysaccharide, since easy-to-utilize simple compounds and nutrients are likely to be taken up rapidly and intensely competed for (Chróst 1992). Low temperature hinders the efficient mass transfer of nutrients into cells and subsequent catabolism and anabolism by metabolic pathways. Within the close confines of the sea-ice matrix, however, the environment is rather stable and the higher density of the cold brine, low temperature, and physical closeness of cells promotes a situation in which secreted enzymes can accumulate sufficiently around the cell. This level may result in higher concentration gradient of nutrients, thus overcoming the limitations of mass transfer. The production of EPS appears to aid this process also acting as an enzyme delivery vehicle and constraining diffusion away from the cell (Nevot et al. 2006; Krembs and Deming 2008; see further discussion below in relation to EPS in sea ice). Zdanowski and Donachie (1993) found that chitinases, uricases (which convert uric acid to allantoin), keritinases, proteases, lipases, and various saccharide-degrading enzymes are commonly formed by sea-ice bacteria. Typically the enzymes have optima and maxima 10–20 °C lower than typical mesophilic bacteria, such as *E. coli* and *Bacillus subtilis* (Christian and Karl 1995; Helmke and Weyland 1995; Russell 1998). Arctic sea-ice bacterial exoenzymes have been clearly shown to possess substantial activity below 0 °C (Huston et al. 2000; Groudieva et al. 2004; Yu et al. 2009). Being highly cold active, these enzymes are also generally thermolabile, displaying some inactivation at temperatures as low as 25 °C (Russell 1998), thus potentially setting the upper growth temperatures of psychrophilic life. The understanding of protein function at low temperature has considerably improved over the last 15 years and the paradigm is that the cold-active adaptations are varied, often quite unique, yet subtle (Russell 2000; Collins et al. 2008b). Enzymes do have defined physical limits as set by bacterial protein temperature-related enthalpy/entropy relationships (Ratkowsky et al. 2005); however, it can be opined that SIMCO includes the most diverse and advanced evolutionary development in this sense. Further examination of cold-active enzyme structure will enable not only insights into the nature of psychrophily but also add to the knowledge of processes of evolution of protein function. It needs to be noted that the growth of sea-ice bacteria is not only controlled by temperature adaptations of the enzymes per se, but also by the adaptations of bacteria which allow the formation of enzymes at low temperatures. The translation apparatus and other aspects of the cellular machinery of cold-adapted bacteria are, in themselves, adapted to functioning at low temperatures, and further studies of the physiology and mechanistic of psychrophilic bacteria using those from sea ice as models (e.g., *Colwellia psychrerythraea* 34 H, Methe et al. 2005) may provide insight into the *systems biology* of low temperature–associated life, as pioneered by Cavicchioli (2006).

Ice-Modifying Proteins in Sea Ice

In continually freezing (and thawing) habitats, the growth of ice crystals can be deleterious to cells due to disruption of cell membranes. To counteract this process, microorganisms up to higher life forms (such as teleost fish) form antifreeze proteins (AFPs) that are able to control the melting and growth of ice crystals (Duman and Olsen 1993). AFPs are of relevance in sea-ice ecosystems but only quite recently have received attention. Both the major Arctic and Antarctic sea-ice diatoms *F. curta* and *F. cylindrus* were found to form AFPs, belonging to a multigene family (Janech et al. 2006; Bayer-Giraldi et al. 2010), that at high

salinity could depress the freezing temperature by 2.5°C (<1°C at low salt) as well as cause striations on ice crystals themselves. This process could not only help survival against freezing but also perhaps aid in entrapment into sea ice (Bayer-Giraldi et al. 2011). The genomes of sea-ice psychrophiles (Methe et al. 2005; Bowman 2008) contain a number of ice-binding protein homologs, the activity of which has been confirmed in *Colwellia* (Raymond et al. 2007), suggesting they and other sea-ice bacteria have the capacity to modify ice crystal structures. Ice nucleation activity has been observed for various sea-ice strains and *Colwellia* phage though it was relatively weak but could potentially act to enhance survival at subzero temperatures in sea ice (Junge and Swanson 2008).

Role of EPS in Sea Ice

Bacteria and algae have been shown to have the amazing ability be able to grow in sea-ice brine channels down to −20°C (Junge et al. 2004, 2006). Some of the adaptations allowing this have been explained above; however, observations of sea-ice chemistry and biology by Prof. Jody Deming led to the consideration that additional mechanisms are also active, allowing survival in situations where bacteria interact directly with ice surfaces (Deming 2002). Based on her and colleagues research, it was believed that survival could be linked to the fact that sea-ice brine channels clearly contain high levels of exopolymeric substances (EPS), mainly polysaccharide, derived from both algae and bacteria growing in the brine (Deming 2002; Collins et al. 2008a). EPS was suspected to be present there for a reason and actually aids directly in the habitation of sea ice. EPS clearly acts as an important food source in sea ice and likely helps drive heterotrophic bacterial activity as the sea-ice algal bloom passes its peak (Riedel et al. 2006; Meiners et al. 2008). Since many EPS types often contain *N*-acetylated sugar residues (such as *N*-acetyl-D-glucosamine), the breakdown of EPS seems to help regenerate ammonia in sea ice (Riedel et al. 2007). EPS production by the sea-ice strain *Pseudoalteromonas antarctica* CAM025 was observed to be 30-fold higher at low temperature (<10°C) compared to its optimal temperature of 20°C (Nichols et al. 2005). The EPS produced during low temperature growth from this strain was also found to be richer in uronic acid sugars, potentially increasing its net negative ionic charge (Nichols et al. 2005). EPS production thus may aid in nutrient acquisition due to its anionic properties. For example, iron and cobalt availability may limit production in sea ice (Taylor and Sullivan 2008; Pankowski and McMinn 2009), and thus, effective EPS ligands binding metal ions would act to sorb vital trace metals (Hassler et al. 2011). The properties of the EPS types derived from SIMCO are only just being explored and utilized for industrial or biomedical purposes (Nichols et al. 2006). Another sea-ice-derived strain of the species *Pseudoalteromonas antarctica* was shown to form outer membrane vesicles that sloughed from cells and moved outward within the secreted EPS capsule. This research interestingly suggests EPS could act as a delivery vehicle for proteins (Nevot et al. 2006). Data suggests the EPS produced by at least some sea-ice bacteria acts as an effective cryoprotectant, protecting cells from being killed by freezing (Junge et al. 2006; Marx et al. 2009; Collins et al. 2008a). EPS from the sea-ice psychrophile *Colwellia psychrerythraea* 34 H when present in the ice resulted in the bulk ice having an improved ability to hold salt (Ewert and Deming 2011). Studies on EPS from the sea-ice algae (*Melosira arctica*) and *Colwellia* EPS also revealed they affected ice formation, creating a more complex crystalline structure, and was also able to force the ice to retain more salt (Raymond et al. 2007; Krembs et al. 2011). Overall, much evidence suggests algal and bacterial EPS protects and helps feed living cells, and simultaneously influences brine channel formation and physicochemistry. Furthermore, relatively high levels of bacteria and EPS were also found in sea-ice frost flowers (Bowman and Deming 2010), and it was suggested that wind-blown frost flower material could influence other ice areas by adding ice-nucleating particles; however, this hypothesis needs to be backed by solid evidence since Junger and Swanson (2008) suggest the nucleating activity is not strong enough to be of significance in the atmosphere.

Polyunsaturated Fatty Acid (PUFA) Synthesis by Sea-Ice Bacteria

The importance of membrane lipids by modulating the rigidity of cell membranes is a fundamental concept in cold adaptation (Morita 1975; Russell and Nichols 1999). Low temperature restricts the mass transfer of molecules across membranes, due to their increased viscosity, and would represent a major constraint for growth under the temperature cum salinity stress typical of sea ice (Nichols et al. 1999b). Certain lipids, such as PUFAs, are particularly effective at this due to their low melting point, and inherent packing chemistry has a large impact on membrane viscosity (Nichols et al. 2000). Major PUFAs produced include the omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (EHA), and stearidonic acid, all derived from alpha-linolenic acid. The other major type of PUFA includes the omega-6 fatty acids, such as arachidonic acid (ADA), which is derived from linoleic acid. Alpha-linolenic and linoleic acid are essential fatty acids in higher life forms since they cannot be synthesized de novo and are required for the optimal health of cardiovascular, nervous, and immune systems (Brett and Muller-Navarra 1997; Uauy et al. 2001). PUFA are synthesized by algae, bacteria, and other microbes such as thraustochytrids, by a combination of elongation and desaturation using specialized polyketide synthase enzyme complexes (*pfa* cluster) (Shulse and Allen 2010). Several sea-ice dwelling bacteria, all psychrophilic, produce either EPA or DHA and include members of the order *Alteromonadales* (*Colwellia*, *Shewanella*, and *Psychromonas*) while the species *Psychroflexus torquis* uniquely produces both EPA and ADA (Nichols et al. 1997) with low temperature growth enhancing EPA levels dramatically but not ADA. PUFA-producing *Photobacterium* are also common to the deep sea (Allen and Bartlett 2002). However, it must be noted that the ability to

make PUFA is not a requirement for low temperature per se nor is it limited to only cold-adapted bacteria. Deletion of *pfa* genes in the deep sea–dwelling species *Photobacterium profundum* had no effect on cold temperature growth since monounsaturated fatty acids simply substitute for the role (Allen and Bartlett 2002). Mesophilic and psychrotolerant species, including flavobacteria (e.g., Hosoya et al. 2006), gammaproteobacteria (e.g., Skerratt et al. 2002), and myxobacteria (e.g., Garcia et al. 2009), can also form PUFA. Other roles for PUFA beyond cold adaptation have yet to be discovered. *Pfa* cluster genes based on metagenome libraries are known to be widely distributed in the marine environment (Shulse and Allen 2010); however, it can be assumed PUFA does contribute to survival of various bacteria within sea ice. Many sea-ice bacteria produce high proportions of their cellular fatty acids that are either monounsaturated and/or branched, for example, in the common sea-ice dwelling bacteria of genus *Glaciecola*; virtually, all of its lipid becomes monounsaturated at low temperature (Nichols et al. 1999b) while many cold-adapted flavobacteria common in sea-ice form mainly branched chain monounsaturated fatty acids (Bowman et al. 1998c).

Virus Activity in Sea Ice

Viruses are an integral part of the microbial loop and typically outnumber bacteria by 1 or 2-orders of magnitude in seawater. Via cell lysis viruses are critical controllers of production and act to redistribute nutrients through heterotrophic processes (Thomas et al. 2001). Viruses have been detected in all forms of sea ice from frazil to multiyear ice where they can reach enormous viral particle: bacterial cell ratios (Gowing et al. 2004). Collins and Deming (2011) reported that sea-ice ratios could exceed 1,000:1 compared to the underlying seawater of usually about a 100:1 ratio. Within sea-ice brine channels, viruses concentrate substantially to values from 10^6 to 10^9 particles per ml with lower temperature brine having a higher viral concentration (Wells and Deming 2006a; Collins and Deming 2011). This enrichment in sea ice appears is due to physical, freezing-related concentration in brines since upper layers of ice had a 6-fold enrichment than lower layers. Viral to cell contact rates within sea-ice brine channels are however similar to that of the underlying seawater but rates increase as the temperature declines (Wells and Deming 2006a.). A number of phages of sea-ice bacterial hosts have been isolated, including those specific to *Colwellia* and *Flavobacterium* species (Borriss et al. 2004, 2007; Wells and Deming 2006b). All phages studied were quite host specific and were found to be as cold adapted as the bacteria themselves (Borriss et al. 2004), suggesting the phage synthesizes cold-active enzymes for its reproduction (Wells and Deming 2006b; Borriss et al. 2007). Despite the high number of viruses, viral losses did not have any influence on bacterial growth in sea ice (Gowing et al. 2004). Bacteria dwelling in sea ice thus could have extensive defenses against viral infection. Furthermore, in waters affected by ice melt, it was found grazing of bacteria considerably outstripped losses derived from viral lysis, suggesting carbon flow mainly goes to higher trophic levels (Boras et al. 2010). Nevertheless, the abundance of viruses in sea ice suggests they not only play an important role in SIMCO microbial loops and nutrient regeneration but also make sea ice a place in which lateral gene transfer is very active (Collins and Deming 2011).

Bacterial Diversity and Its Selection Within SIMCO

Elucidation of community composition is vital to understanding specific bacterial interactions in the region, especially in association with ice algae. It also allows insight into the adaptations required for survival in such extreme habitats and highlights potential biotechnological applications for novel bacteria. A morphologically diverse range of bacteria colonizing diatoms has been observed through scanning electron micrographs (Sullivan and Palmisano 1984) and by fluorescent in situ hybridization (Brinkmeyer et al. 2003; also see *Fig. 9.5*). Prosthecate, straight, and branched filamentous forms dominate the epiphytes associated with algae, while cocci, short, and long rods and fusiform bacteria are also abundant. Many epiphytic bacteria contain structural modifications of the cell wall and/or form EPS which would facilitate attachment to sea-ice algae. One chain-forming bacterium was shown to have cell surface modifications at one pole to aid attachment, while an EPS layer of another has been shown embedded in the puncta of an *Entomoneis*-like sea-ice diatom, serving to anchor the bacterium to the host (*Fig. 9.6*).

Bacterial sea-ice diversity specifically has been examined in various culture-based studies (Bowman et al. 1997a, c; Gosink et al. 1997, 1998; Junge et al. 1998; Brinkmeyer et al. 2003). Conventional 16S rRNA gene clone library analysis has also been used to identify bacterial and archaeal diversity in a variety of sea-ice types, primarily first year ice. This has included Antarctic and Arctic fast and pack ice (Brown and Bowman 2001; Junge et al. 2002; Brinkmeyer et al. 2003; Collins et al. 2010). Both community structure and diversity has been studied in Baltic Sea ice during winter (Petri and Imhoff 2001; Kaartokallio et al. 2008). More recently 454 pyrotag-based sequencing was used to assess the diversity of bacteria in Arctic multiyear ice and the underlying water (Bowman et al. 2012). Most described cultivated and molecularly detected taxa belong to classes *Gammaproteobacteria* or *Alphaproteobacteria* of phylum *Proteobacteria* and to family *Flavobacteriaceae* of the phylum *Bacteroidetes*. A minority of isolates belong to the *Actinobacteria* (mostly of genus *Arthrobacter*) and the *Firmicutes* (mostly planococci) (see both *Fig. 9.7* and *Table 9.1*). Culture-independent studies reveal lower levels of phylum *Verrucomicrobia* (mainly class *Opitutae*) (Brown and Bowman 2001; Bowman et al. 2012). Overall cultured diversity is much higher in SIMCO in first year ice (pack ice and fast ice) than the underlying water column (Bowman et al. 1997c) as can be seen in *Fig. 9.8*. In the case of high Arctic multiyear ice, in the absence of algae, diversity is also higher at least among the more

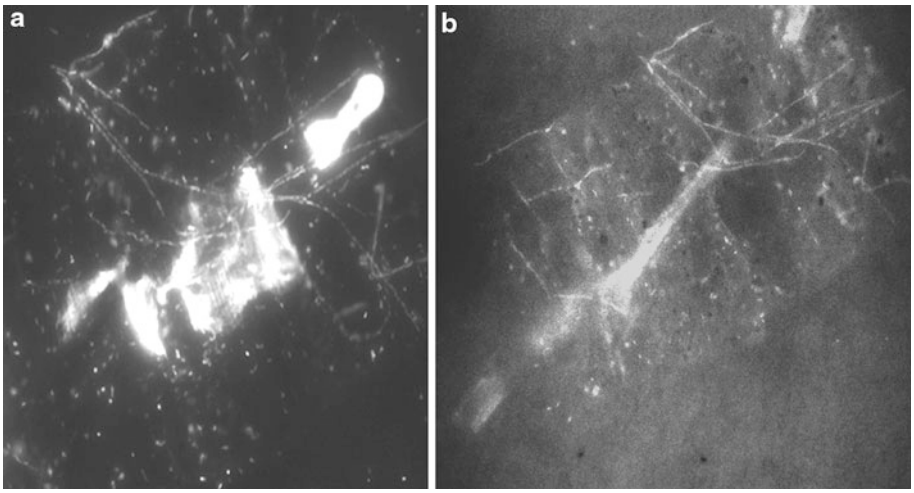


Fig. 9.6
Micrograph showing (a) distribution of bacteria, stained with DAPI on a sea-ice diatom and (b) distribution of *Flavobacteria* on the same diatom detected using a CY3-labeled *Flavobacteria*-specific oligonucleotide probe CF319 and visualized by UV epifluorescence microscopy. Sheathed filaments observed on the diatom could represent a member of the family *Saprospiraceae*

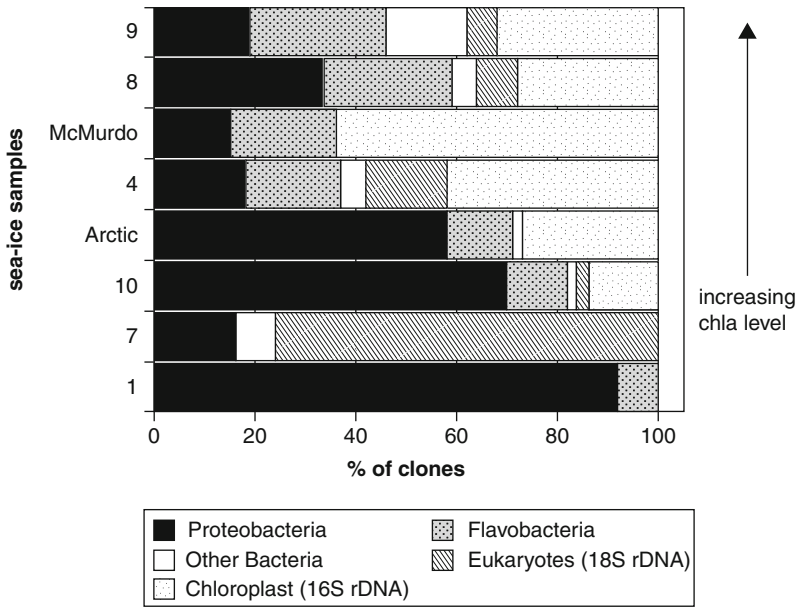


Fig. 9.7
Proportions of different bacterial and eukaryotic groups in different sea-ice samples in relation to increasing chl*a* levels. Sea-ice samples 1, 4, 7, 8, 9, and 10 are from various pack ice locations in the Southern Ocean; sea-ice sample McMurdo was collected from McMurdo Sound, Antarctica; sea-ice sample Arctic was collected from the Baffin Bay region of the Northern Open Water Polynya. Other Bacteria included are members of *Actinobacteria*, *Verrucomicrobia*, and *Chlamydiae* (presumed to be endosymbionts of protists) while *Proteobacteria* included classes *Alphaproteobacteria* and *Gammaproteobacteria*

common taxa (◉ *Fig. 9.9*). Rare taxa and unclassified taxa are not dealt with here since 454 sequencing data is subject to sequence error and chimerism and should be approached with great caution (Kunin et al. 2009). Seawater archaea as with bacteria are readily trapped from the underlying seawater during ice formation and can persist through the winter months (Collins et al. 2010). Archaea, however, do not occur in productive sea-ice habitats as shown by Brown and Bowman (2001). This has been confirmed with fluorescent in situ hybridization (FISH) counting of cells with a variety of probes in the excellent study of Brinkmeyer et al. (2003). A comparison can be made between culture clone library data and FISH data (◉ *Figs. 9.8*, ◉ *9.10*, and ◉ *9.11*) showing the strong correlations. Archaea and other seawater ultraoligotrophs that typically

Table 9.1

Sea-ice-derived species that have been given official taxonomic descriptions described to date including basic ecophysiological parameters

Species	Phylum or class	Temp. range for growth (°C)	Salinity range for growth (psu)	Authors
Octadecabacter arcticus	*Alphaproteobacteria*	<0–15		Gosink et al. 1997
Octadecabacter antarcticus	*Alphaproteobacteria*	<0–15		Gosink et al. 1997
Pseudochrobactrum glaciei	*Alphaproteobacteria*	5–40	0–60	Romanenko et al. 2008
Colwellia polaris	*Gammaproteobacteria*	4–26	10–60	Zhang et al. 2008c
Colwellia psychrerythraea	*Gammaproteobacteria*			Bowman et al. 1998a; Methe et al. 2005 (genome sequence)
Colwellia hornerae	*Gammaproteobacteria*	<0–15		Bowman et al. 1998a
Colwellia rossensis	*Gammaproteobacteria*	<0–10		Bowman et al. 1998a
Colwellia psychrotropica	*Gammaproteobacteria*	<0–25		Bowman et al. 1998a
Colwellia demingiae	*Gammaproteobacteria*	<0–15		Bowman et al. 1998a
Glaciecola punicea	*Gammaproteobacteria*	<0–25	10–90	Bowman et al. 1998b
Glaciecola pallidula	*Gammaproteobacteria*	<0–20	10–90	Bowman et al. 1998b
Marinomonas primoryensis	*Gammaproteobacteria*	4–30	10–60	Romanenko et al. 2003
Marinomonas arctica	*Gammaproteobacteria*	0–37	0–120	Zhang et al. 2008b
Marinobacter psychrophilus	*Gammaproteobacteria*	0–22	20–80	Zhang et al. 2008a
Psychromonas arctica	*Gammaproteobacteria*	0–25	10–70	Groudieva et al. 2003
Pseudoalteromonas prydzensis	*Gammaproteobacteria*	~0–30	5–150	Bowman 1998
Psychrobacter adeliensis	*Gammaproteobacteria*	2–30	0–100	Shivaji et al. 2004
Psychrobacter maritimus	*Gammaproteobacteria*	4–37	0–100	Romanenko et al. 2004
Psychrobacter salsus	*Gammaproteobacteria*	2–30	0–100	Shivaji et al. 2004
Psychromonas boydii	*Gammaproteobacteria*	<0–10	20–180	Auman et al. 2010
Psychromonas ingrahamaii	*Gammaproteobacteria*	−12 to 10	10–120	Auman et al. 2006; Riley et al. 2008 (genome sequence)
Shewanella frigidimarina	*Gammaproteobacteria*	<0–28	0–90	Bowman et al. 1997d (genome sequence available)
Shewanella gelidimarina	*Gammaproteobacteria*	<0–23	10–60	Bowman et al. 1997d
Psychrobacter glacincola	*Gammaproteobacteria*	<0–22	0–180	Bowman et al. 1997e
Aequorivita antarctica	*Bacteroidetes*	−2 to 25	5–100	Bowman and Nichols 2002
Algoriphagus ratkowskyi	*Bacteroidetes*	0–25	10–60	Bowman et al. 2003
Bizionia saleffrena	*Bacteroidetes*	−2 to 25	10–180	Bowman and Nichols 2005
Bizionia gelidisalsuginis	*Bacteroidetes*	−2 to 27	10–180	Bowman and Nichols 2005
Brumimicrobium glaciale	*Bacteroidetes*	<0–15	15–70	Bowman et al. 2003
Cellulophaga algicola	*Bacteroidetes*	−2 to 28	5–100	Bowman 2000 (genome available)

Table 9.1 (continued)

Species	Phylum or class	Temp. range for growth (°C)	Salinity range for growth (psu)	Authors
Gelidibacter algens	*Bacteroidetes*	<0–18	15–60	Bowman et al. 1997b
Gelidibacter gilvus	*Bacteroidetes*	−2 to 27	5–90	Bowman and Nichols 2005
Gelidibacter salicanalis	*Bacteroidetes*	−2 to 27	0–150	Bowman and Nichols 2005
Gillisia illustrilutea	*Bacteroidetes*	−2 to 20	0–70	Bowman and Nichols 2005
Gillisia sandarakina	*Bacteroidetes*	−2 to 20	10–80	Bowman and Nichols 2005
Gillisa hiemivivida	*Bacteroidetes*	−2 to 27	10–80	Bowman and Nichols 2005
Polaribacter franzmanii	*Bacteroidetes*	<0–10	10–70	Gosink et al. 1998
Polaribacter irgensii	*Bacteroidetes*	<−1 to 15	5–60	Gosink et al. 1998 (genome sequence available)
Polaribacter filamentus	*Bacteroidetes*	<0–10	10–70	Gosink et al. 1998 (genome sequence available)
Psychroflexus torquis	*Bacteroidetes*	−5 to 15	15–80	Bowman et al. 1998c (genome sequence available)
Psychroserpens burtonensis	*Bacteroidetes*	<0–15	15–60	Bowman et al. 1997b
Planomicrobium mcmeekinii	*Firmicutes* (*Bacilli*)	0–37	0–250	Junge et al. 1998

dominate the polar oceans are excluded to below PCR detectability (Brown and Bowman 2001; Brinkmeyer et al. 2003). Such bacteria were detected only very weakly among pyrotags in multiyear ice but were abundant in the underlying seawater (Bowman et al. 2012; see *Fig. 9.9*). This further emphasizes that SIMCO bacteria are distinct from the pelagic zone likely due to a combination of a number of factors, which can be summarized as:

1. The concentrated and intense production that occurs is a situation alien to microbes highly adapted to an oligotrophic lifestyle.
2. Freezing and high salinity conditions prevent active growth of less cold-adapted bacteria as suggested by comparative studies (Nichols et al. 1999b; Kaartokallio et al. 2005).
3. Algae-driven control (e.g., Pusceddu et al. 2009) may be effective at preventing growth of certain bacterial taxa while others are comparatively more resistant.
4. Sea-ice algal-bacterial mutualistic associations could be sufficiently integral that they provide a major competitive advantage to bacterial epiphytes during the spring bloom.

The observation of cyanobacteria in multiyear Arctic ice is likely due to infiltration from freshwater surface melt-pools where they may bloom. Such cyanobacteria-dominated communities are an extensive feature in various coastal zones where sea ice persists over many years (Vincent 1988). Some evidence suggests that SIMCO displaying high levels of primary production may increasingly be dominated by gammaproteobacteria and flavobacteria (Brown and Bowman 2001, see *Fig. 9.7*); however, the bacterial communities within SIMCO are highly heterogeneous, so this connection requires more confirmation. Further work with the now popular 454 pyrotag sequencing approach should reveal more details on community structure and beta diversity patterns. This would be useful in determining the details of community succession as shown already by Kaartokallio et al. (2008) for Baltic Sea ice.

Biogeography of Sea-Ice Bacteria

Biogeography is defined as the study of the global distribution of species, living or extinct. Such studies seek to elucidate the patterns and distributions of species within niches and is required if we are to fully understand ecosystem biodiversity, stability, functional redundancy, and extinction processes. From a microbial perspective, the study of biogeography is particularly challenging. The concept of bacterial endemism is anathema to the prevailing hypothesis that free-living bacteria are cosmopolitan in their distribution (Finlay 2002), associated with the famous catchphrase "*everything is everywhere, but the environment selects*" (Baas-Becking 1934). Detailed comparison of the community composition of analogous but geographically separate environments is required to reconcile these ideas. In the past, community comparisons were based on culture-based studies; however, molecular techniques have now opened the way for more sensitive analyses. Reviews of the concept as it pertains to the microbiota of Antarctica have been explored (Staley and Gosink 1999; Vincent 2000). Staley and Gosink (1999) suggest polar sea ice as an ideal habitat for examination of bacterial biogeography. This extreme environment provides

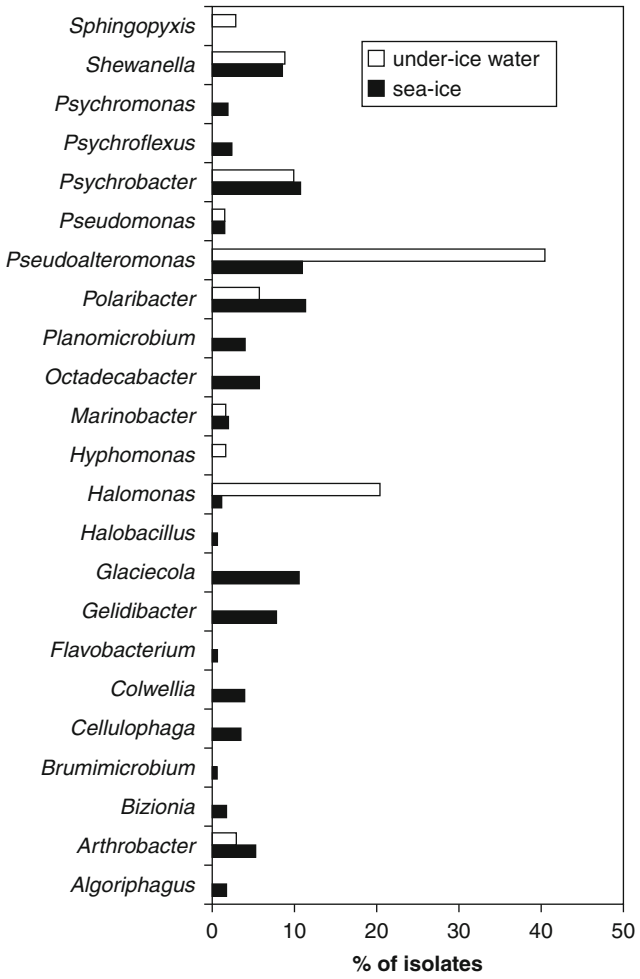


Fig. 9.8

A meta-analysis of a culture collection (210 strains) of Antarctic sea ice primarily from first year fast ice with or without visible algal assemblages (Bowman et al. 1997a, c) and under-ice water from the same location. The data shows the higher diversity of taxa from sea ice compared to the underlying seawater

selective pressure for certain types of well-adapted bacteria, such as psychrophiles as explained above. The Baas-Becking hypothesis would require that the same species of psychrophiles be found within the sea ice of both poles. For this to be possible, there would have to exist sufficient pathways for the continual exchange of psychrophilic microorganisms between the poles and if so leads to the question whether such organisms are capable of surviving transit within these pathways. Means of global traverse were proposed by Staley and Gosink (1999), including animal vectors (such as arctic terns and Antarctic skuas), cold deep sea currents (i.e., Antarctic bottom water), aerial transport, and even anthropomorphic activity. This last "method" of transfer, although only occurring recently, may prove to be a defining factor. It has been clearly observed that the ballast water of ships, people's clothing and footwear, their pets and plants have acted as vectors for the transport of many organisms around the globe since the age of human exploration.

Staley and Gosink (1999) examined three bacterial genera which were described from sea ice from both the Arctic and the Antarctic. These included the genera *Octadecabacter*, *Polaribacter*, and at the time the as yet unofficially named genus "*Iceobacter*," which is now synonymous with the genus *Psychromonas*. Their preliminary examinations led them to declare that although members of these genera occur at both poles, there was no bipolar distribution of species. Evidence for this was based primarily on DNA-DNA hybridization experiments, along with phenotypic data. Sequence analysis of 16S rRNA was inconclusive. No evidence was found that the species from each pole were more closely related than to the species from the other pole, as would be expected if speciation were occurring. They interpreted this result as an indication that 16S rRNA sequences are too highly conserved to allow for the assessment of endemic polar strains and species. Clearly opportunities still exist to explore this concept, made much easier with the capacity to generate huge DNA sequence datasets.

Dominant Bacterial Taxa in SIMCO and Their Evolutionary Origins

Various bacterial taxa are particularly dominant in sea ice and the cultivation data record captures this very effectively (◗ *Figs. 9.7–9.11*). Species of the genera *Colwellia*, *Glaciecola*, *Marinobacter*, *Octadecabacter*, *Psychrobacter*, *Polaribacter*, *Psychromonas*, and *Psychroflexus* are predominant in either or both Arctic and Antarctic sea ice (Bowman et al. 1997c; Junge et al. 2002; Brinkmeyer et al. 2003), supported by culture-independent analyses (Brown and Bowman 2001) and FISH-based analyses (Brinkmeyer et al. 2003) (◗ *Figs. 9.7–9.11*). Some of these genera are also common in the underlying water column, most notably *Polaribacter* and *Psychrobacter* (Bowman et al. 2012, ◗ *Figs. 9.6* and ◗ *9.7*). Some taxa have an observed patchiness in their distribution, including *Flavobacterium*, *Gelidibacter*, *Marinomonas*, *Pseudoalteromonas*, *Pseudomonas*, *Psychroserpens*, *Salegentibacter*, and *Shewanella*. Several species of these genera have been observed to be broadly distributed occurring in polar seawater, marine sediment, and Antarctic saline lakes. Thus, it is possible their appearance in SIMCO could be partly due to infiltration of the ice via seawater as well as general tolerances to sea-ice-specific stresses.

Most of SIMCO-associated bacterial genera are generally psychrophilic and halophilic or halotolerant (◗ *Table 9.1*); however, most if not all have more thermotolerant sister species that occur in other ecosystems. For example, the very cold-adapted *Psychroflexus torquis* has several much more thermotolerant sister species from highly saline environments, ranging from cheese brines to high altitude and tropical hypersaline lakes (e.g., Seiler et al. 2011). This suggests psychrophiles are products of comparatively (geologically speaking) "recent" evolution driven perhaps by gradually changing environments, for instance, the development of the Antarctic ice sheet and Polar Front at the beginning of the Miocene (DeConto and Pollard 2003). Evolutionary change in species could also occur due to

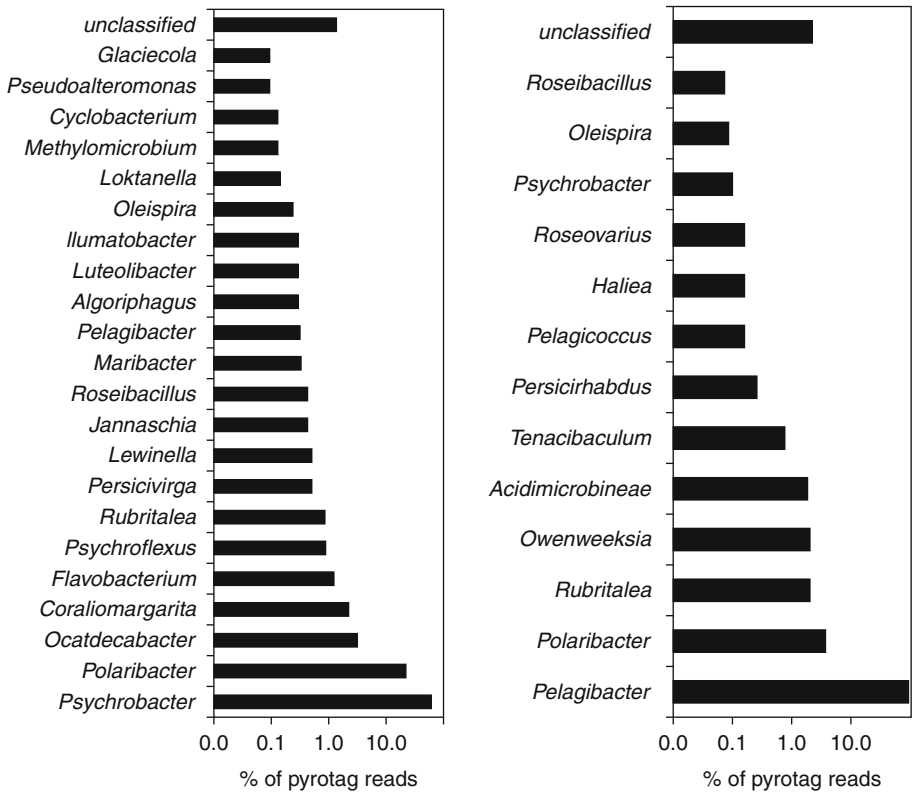


Fig. 9.9

Distribution of bacterial taxa identified via 454 pyrotag sequencing in Arctic multiyear ice (*left hand graph*) in comparison to seawater located beneath the sea ice (Bowman et al. 2012). The data does not include unclassified reads

stochastic redistribution of cells between different niches (as observed by Jaspers and Overmann 2004) where they eventually may acquire new traits for improved fitness in progressively even more extreme situations. Such stochastic processes would conceivably take place over hundreds of millions years based on the drift in 16S rRNA gene sequences (Ochman et al. 1999) of the different species. Franzmann (1996) opined that the "*use of the 16S rRNA gene as a molecular clock would suggest that the majority of Antarctic prokaryotes diverged from their nearest known non-Antarctic relatives long before a stable ice-sheet developed in Antarctica. The time of colonization (or recolonization) of Antarctic environments by individual species may have been very recent in evolutionary time.*" This conceptual foundation for the evolution of psychrophiles suggests an existence on Earth that has been much shorter than that of thermophiles (Schwartzmann 1999). Cold ecosystems are believed to have been prevalent by a series of global glaciations events during the Neoproteozoic Era 630–770 million years ago (Allen and Etienne 2008), the events of the so-called Snowball Earth. *Psychrobacter* is particularly notable for its cold adaptation as well as osmotolerance. Real-time PCR analysis demonstrated that *Psychrobacter* species were ubiquitous in polar samples but less common in temperate and tropical zone samples accompanied with temperature differentiated speciation (Rodrigues et al. 2009). This suggests this genus has successfully colonized virtually all cold biomes, including sea ice, over the last several 100 million years, perhaps coinciding with the occurrence of the Neoproteozoic glaciations events. It is thus possible that "SIMCO-specialist" bacteria have come and gone throughout Earth's history. The potential complete disappearance of all sea ice due to global climate change, perhaps within the next millennium (A. McMinn, pers. comm.), will not be a permanent deathblow for SIMCO and that the wheel will eventually turn.

Global Warming: Sea Ice as the Frontline

Current estimated increases in the concentrations of heat trapping gases in the atmosphere (e.g., CO_2, CH_4) suggest Earth's average global temperature will increase by ~1–2 °C in the next 50–100 years (Wigley and Raper 1987). Though the catastrophic implications of sea level increase for most humans living in coastal regions or low elevation islands are a moot point, sea level increase rates derived from melting icecaps will most likely be very slow and will probably have overall minimal impact, as far as can be foreseen at this stage. In other words, a 1-m increase in sea level in 1 day or a 1 week is disastrous but it is more likely a 1-m increase over centuries or millennia would have less dramatic effects. The greatest threat for increased sea level comes from the Greenland ice sheet and the western ice sheet of Antarctica with their numerous large ice shelves which encourages a greater degree of glaciological instability compared

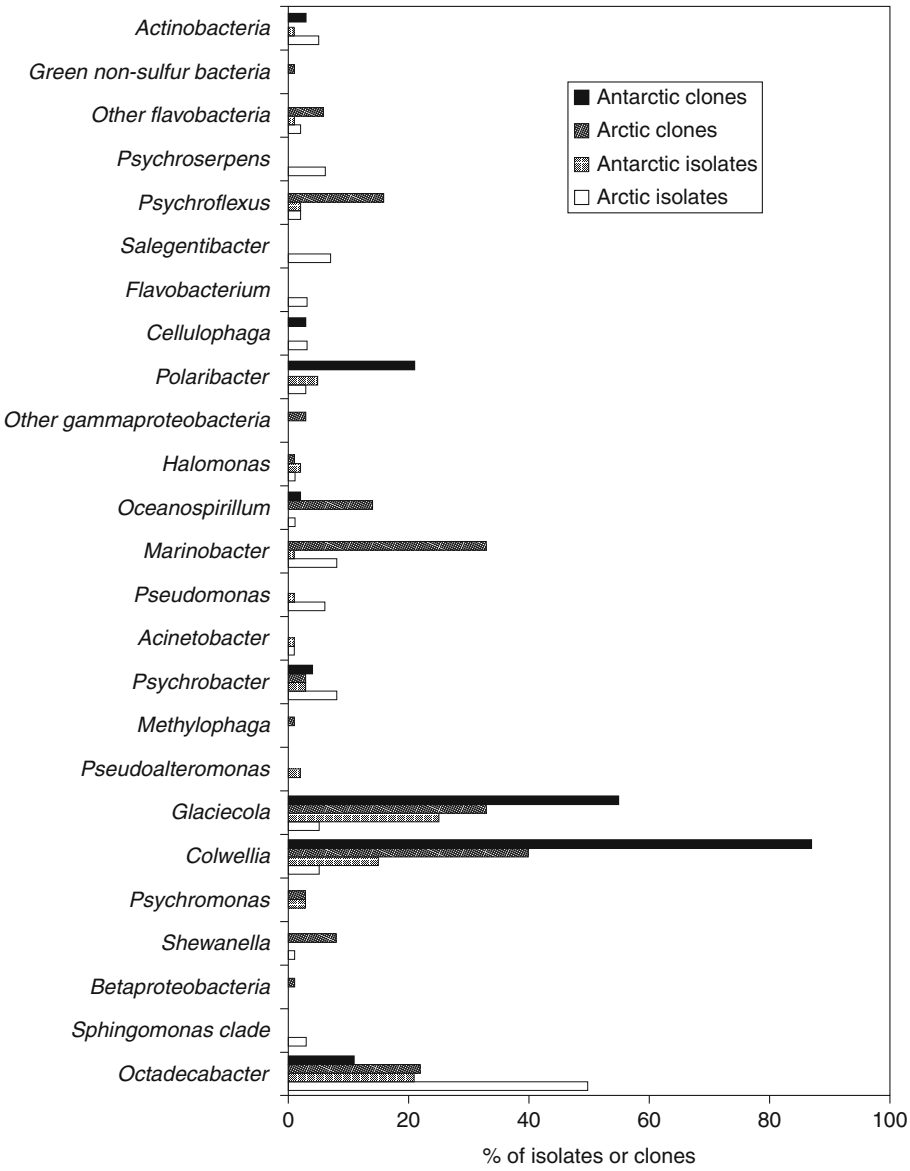


Fig. 9.10

Comparison of cultured bacteria and 16S rRNA gene sequence clone library data from first year Arctic and Antarctic pack SIMCO (Brinkmeyer et al. 2003). The data indicates predominant taxa found in both polar locations that includes *Colwellia*, *Glaciecola*, *Octadecabacter*, and *Psychrobacter*. Certain taxa show greater emphasis in sample locations and methodology (e.g., *Psychroflexus*, *Polaribacter*, and *Marinobacter*); however, this likely reflects the capacity for temporal and spatial heterogeneity of SIMCO (Brown and Bowman 2001; Kaartokallio et al. 2008)

to the much larger, thicker, and more stable eastern Antarctic ice sheet (Weertman et al. 1982). If all the sea ice in both the Arctic and around Antarctic melted totally, the sea level would only rise 4 mm (R. Grumbine, pers. comm.). Thus, the demise of sea ice will not threaten humans in terms of increased sea level. However, without sea ice, there would be obvious flow on effects which will impact on both marine and terrestrial polar fauna and flora. Studies in the Arctic Ocean suggest sea-ice melt has increased Arctic Ocean organic content while greater levels of ice melt and carbon input would increase pelagic primary production, modify food webs, affect microbial distribution, and eventually potentially reduce CO_2 sequestration via the biological pump (Sala et al. 2010; Vincent 2010). In the Antarctic, a major food source of krill would disappear and thus, higher levels of the Antarctic trophic food webs could be compromised; on the other hand, pelagic primary production would be enhanced, acting as a minor negative feedback against further change (Arrigo and Thomas 2004). Almost certainly various apex predators in the Antarctic and Arctic (polar bears, various marine bird species, seals, walruses) would become extinct through starvation. Similarly Arctic polar regions would be affected with higher trophic levels of marine-based food webs crippled. The populations and distribution of psychrophilic bacteria could be deleteriously affected, but whether this would have

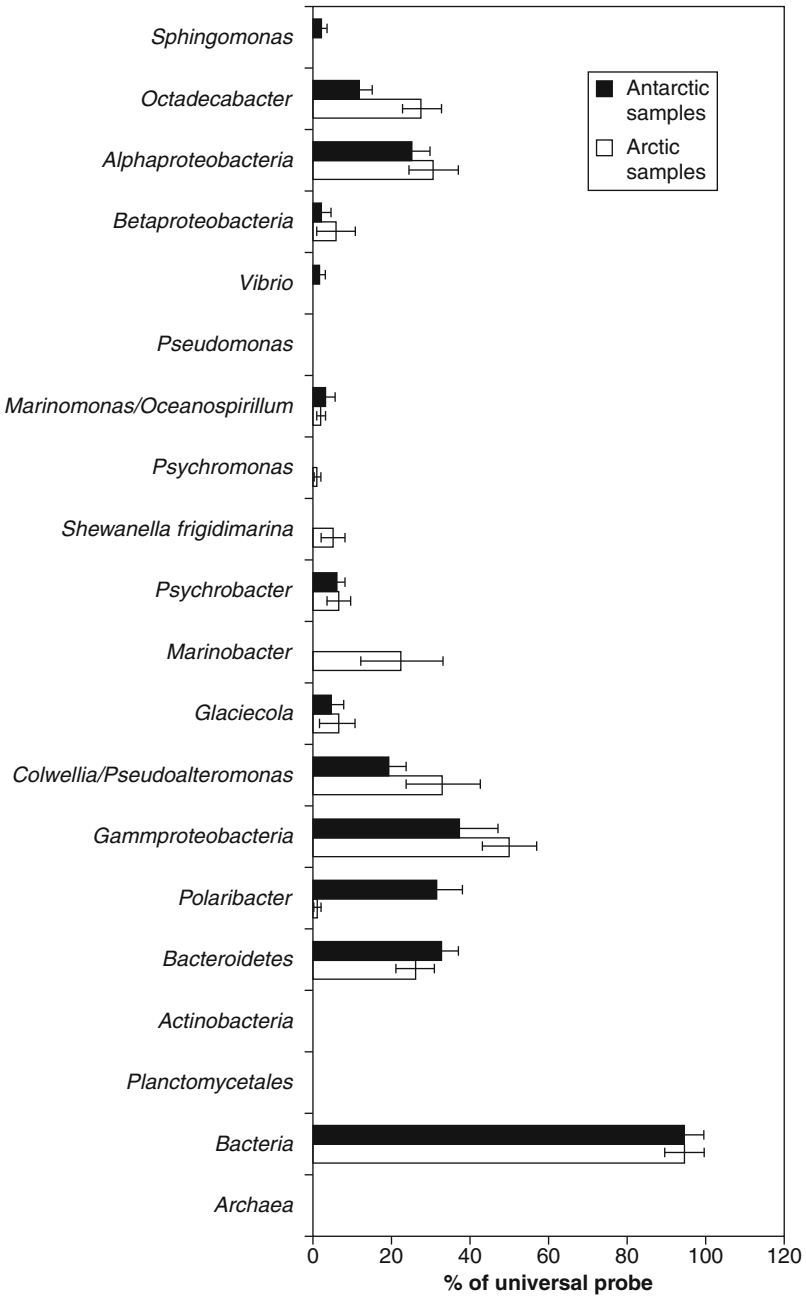


Fig. 9.11

Comparison of Arctic and Antarctic SIMCO utilizing fluorescent in situ hybridization. The values are average proportions of the total prokaryotic cell count for two separate pack ice samples (error bars equal standard deviations) (Data derived from Brinkmeyer et al. (2003))

a noticeable effect on secondary production in the polar oceans is unclear. Disruption of primary production would reduce the oceanic "biological pump" which recirculates CO_2, and global warming may also result in greater stagnation of the deep sea (increased oxygen minima zones) (Kim et al. 2001). Together these may potentially have a profound negative effect on surface productivity. Clearly the sea-ice environment in both poles are potentially threatened by global warming, as tested by research measuring changes in sea-ice surface area and the freshening of seawater (e.g., Wong et al. 1999; Tucker et al. 2001).

Conclusion

Until relatively recently, Antarctic sea ice has been considered an inhospitable environment whose inhabitants contributed little to the ecology of the polar region. However, it is now accepted that microenvironments exist in sea ice suitable for microbial growth and production and which are utilized by a variety of organisms. Bacteria, via their close association with sea-ice algae and through the dynamics of the microbial loop, are vital links within the biological cycles of Southern Ocean. A complete

understanding of these cycles will therefore require the further elucidation of the ecology of SIMCO. The biotechnological significance of sea-ice psychrophilic bacteria has not gone unnoticed, and it is expected that there will be a sustained and steady increase in the interest in EPS, cold-active enzymes, AFPs, PUFA, and other so far as yet unrevealed discoveries. The insidious threat of global warming perhaps gives us only a limited window of time in which SIMCO can be explored, and thus, more research on this crucial and interesting habitat is strongly encouraged.

References

Ackley SF, Sullivan CW (1994) Physical controls on the development and characteristics of Antarctic Sea ice biological communities- a review and synthesis. Deep Sea Research I. Oceanogr Res Paper 41:1583–1604

Ackley SF, Diekmann GS, Shen H (1987) Algal and foram incorporation into new sea ice. Eos 68:1736

Aguilar A, Ingemansson T, Magnien E (1998) Extremophile microorganisms as cell factories—support from the European Union. Extremophiles 2:367–373

Allen EE, Bartlett DH (2002) Structure and regulation of the omega-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium *Photobacterium profundum* strain SS9. Microbiology 148:1903–1913

Allen PA, Etienne JL (2008) Sedimentary challenge to Snowball Earth. Nat Geosci 1:817–825

Arrigo KR, Thomas DN (2004) Large scale of sea ice biology in the Southern Ocean. Antarctic Sci 16:471–486

Auman AJ, Breezee JL, Gosink JJ, Kämpfer P, Staley JT (2006) *Psychromonas ingrahamii* sp nov., a novel gas vacuolate, psychrophilic bacterium isolated from Arctic polar sea ice. Int J Syst Evol Microbiol 56:1001–1007

Auman AJ, Breezee JL, Gosink JJ, Schumann P, Barnes CR, Kämpfer P, Staley JT (2010) *Psychromonas boydii* sp nov., a gas-vacuolate, psychrophilic bacterium isolated from an Arctic sea-ice core. Int J Syst Evol Microbiol 60:84–92

Baas-Becking LGM (1934) Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon, The Hague

Bathmann UK, Scharek R, Klaas C, Duischar CD, Smetachek V (1997) Spring development of phytoplankton biomass and composition in major water masses of the Atlantic sector and the Southern Ocean. Deep Sea Res II 44:51–67

Bayer-Giraldi M, Uhlig C, John U, Mock T, Valentin K (2010) Antifreeze proteins in polar sea ice diatoms: diversity and gene expression in the genus *Fragilariopsis*. Environ Microbiol 12:1041–1052

Bayer-Giraldi M, Weikusat I, Besir H, Dieckmann G (2011) Characterization of an antifreeze protein from the polar diatom *Fragilariopsis cylindrus* and its relevance in sea ice. Cryobiology 63:210–219

Bell W, Mitchell R (1972) Chemotactic and growth responses of marine bacteria to algal extracellular products. Biol Bull 143:265–277

Bluhm BA, Gradinger RR, Schnack-Shiel SB (2010) Sea-ice meio- and macrofauna. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Wiley-Blackwell, Chichester, pp 357–394

Boras JA, Sala MM, Arrieta JM, Sa EL, Felipe J, Agusti S, Duarte CM, Vaque D (2010) Effect of ice melting on bacterial carbon fluxes channeled by viruses and protists in the Arctic Ocean. Polar Biol 33:1695–1707

Borriss M, Helmke E, Hanschke R, Schweder T (2004) Isolation and characterization of marine psychrophilic phage-host systems from Arctic sea-ice. Extremophiles 7:377–384

Borriss M, Lombardot T, Glockner FO, Becher D, Albrecht D, Schweder T (2007) Genome and proteome characterization of the psychrophilic *Flavobacterium* phage 11b. Extremophiles 11:95–104

Bowman JP (1998) *Pseudoalteromonas prydzensis* sp. nov., a psychrotrophic, halotolerant bacterium from Antarctic sea ice. Int J Syst Evol Microbiol 48:1037–1041

Bowman JP (2000) Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. Int J Syst Evol Microbiol 50:1861–1868

Bowman JP (2008) Genomic analysis of psychrophilic prokaryotes. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, New York, pp 265–284

Bowman JS, Deming JW (2010) Elevated bacterial abundance and exopolymers in saline frost flowers and implications for atmospheric chemistry and microbial dispersal. Geophys Res Lett 12:1041–1052

Bowman JP, Nichols DS (2002) *Aequorivita* gen. nov., a member of the family *Flavobacteriaceae* isolated from terrestrial and marine Antarctic habitats. Int J Syst Evol Microbiol 52:1533–1541

Bowman JP, Nichols DS (2005) Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp nov., *Subsaximicrobium saxinquililnus* sp nov., *Subsaxibacter broadyi* gen. nov., sp nov., *Lacinutrix copepodicola* gen. nov., sp nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. Int J Syst Evol Microbiol 55:1471–1486

Bowman JP, Brown MV, Nichols DS (1997a) Biodiversity and ecophysiology of bacteria associated with Antarctic sea ice. Antarctic Sci 9:134–142

Bowman JP, McCammon SA, Brown JL, Nichols PD, McMeekin TA (1997b) *Psychroserpens burtonensis* gen. nov, sp. nov, and *Gelidibacter algens* gen. nov, sp. nov, psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. Int J Syst Bacteriol 47:670–677

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997c) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Bowman JP, McCammon SA, Nichols DS, Skerratt JH, Rea SM, Nichols PD, McMeekin TA (1997d) *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5 omega 3) and grow anaerobically by dissimilatory Fe(III) reduction. Int J Syst Bacteriol 47:1040–1047

Bowman JP, Nichols DS, McMeekin TA (1997e) *Psychrobacter glacincola* sp. nov, a halotolerant, psychrophilic bacterium isolated from Antarctic sea ice. Syst Appl Microbiol 20:209–215

Bowman JP, Gosink JJ, McCammon SA, Lewis TE, Nichols DS, Nichols PD, Skerratt JH, Staley JT, McMeekin TA (1998a) *Colwellia demingiae* sp. nov., *Colwellia hornerae* sp. nov., *Colwellia rossensis* sp. nov. and *Colwellia psychrotropica* sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22: 6 omega 3). Int J Syst Evol Microbiol 48:1171–1180

Bowman JP, McCammon SA, Brown JL, McMeekin TA (1998b) *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. Int J Syst Evol Microbiol 48:1213–1222

Bowman JP, McCammon SA, Lewis T, Skerratt JH, Brown JL, Nichols DS, McMeekin TA (1998c) *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from Antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. Microbiology 144:1601–1609

Bowman JP, Mancuso Nichols C, Gibson JAE (2003) *Algoriphagus ratkowskyi* gen. nov., sp nov., *Brumimicrobium glaciale* gen. nov., sp nov., *Cryomorpha ignava* gen. nov., sp nov and *Crocinitomix catalasitica* gen. nov., sp nov., novel flavobacteria isolated from various polar habitats. Int J Syst Evol Microbiol 53:1343–1355

Bowman JS, Rasmussen S, Blom N, Deming JW, Rysgaard S, Sicheritz-Ponten T (2012) Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S rRNA gene. ISME J 6:11–20

Brakstad OG, Nonstad I, Faksness LG, Brandvik PJ (2008) Responses of microbial communities in Arctic sea ice after contamination by crude petroleum oil. Microb Ecol 55:540–552

Bratback G, Thingstad TF (1985) Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalisms. Mar Ecol Prog Ser 25:23–30

Brett MT, Muller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biol 38:483–499

Brinkmeyer R, Knitted K, Jargons J, Weyland H, Amman R, Helmke E (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol 69:6610–6619

Brown MV, Bowman JP (2001) A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol Ecol 35:267–275

Bull AT, Ward AC, Goodfellow M (2000) Search and discovery strategies for biotechnology: the paradigm shift. Microbiol Mol Biol Rev 64:573–606

Cavalieri DJ, Gloersen P, Parkinson CL, Comiso JC, Zwally HJ (1997) Observed hemisphere asymmetry in global sea ice changes. Science 278:1104–1106

Cavicchioli R (2006) Cold-adapted archaea. Nat Rev Microbiol 4:331–343

Christian JR, Karl DM (1995) Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in three oceanographic provinces. Limnol Oceanogr 40:1042–1049

Chróst RJ (1992) Significance of bacterial ectoenzymes in aquatic environments. Hydrobiologia 243:61–70

Clarke DB, Ackley SF (1984) Sea ice structure and biological activity in the Antarctic marginal ice zone. J Geophys Res 89:2087–2095

Cole JJ (1982) Interactions between bacteria and algae in aquatic ecosystems. Annu Rev Ecol Syst 13:291–314

Collins RE, Deming JW (2011) Abundant dissolved genetic material in Arctic sea ice Part II: viral dynamics during autumn freeze-up. Polar Biol 34:1831–1841

Collins RE, Carpenter SD, Deming JW (2008a) Spatial heterogeneity and temporal dynamics of particles, bacteria, and pEPS in Arctic winter sea ice. J Mar Syst 74:902–917

Collins T, Roulling F, Piette F, Marx J-C, Feller G, Gerday C, D'Amico S (2008b) Fundamentals of cold-adapted enzymes. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, New York, pp 211–228

Collins RE, Rocap G, Deming JW (2010) Persistence of bacterial and archaeal communities in sea-ice through the Arctic winter. Environ Microbiol 12:1828–1841

Connelly TL, Tilburg CM, Yager PL (2006) Evidence for psychrophiles outnumbering psychrotolerant marine bacteria in the springtime coastal Arctic. Limnol Oceanogr 51:1205–1210

Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438:90–93

DeConto RM, Pollard D (2003) Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO_2. Nature 421:245–249

Delille D (1996) Biodiversity and function of bacteria in the Southern Ocean. Biodivers Conserv 5:1505–1523

Delille D, Rosiers C (1996) Seasonal changes of Antarctic marine bacterioplankton and sea ice bacterial assemblages. Polar Biol 16:27–34

Delille D, Fiala M, Rosiers C (1995) Seasonal changes in phytoplankton and bacterioplankton distribution at the ice-water interface in the Antarctic neritic area. Mar Ecol Prog Ser 123:225–233

Delille D, Basseres A, Dessommes A (1997) Seasonal variation if bacteria in sea ice contaminated by diesel fuel and dispersed crude oil. Microb Ecol 33:97–105

DeLong EF, Béjà O (2010) The light-driven proton pump proteorhodopsin enhances bacterial survival during tough times. PLoS Biol 8:e1000359

Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309

Dieckmann GS, Hellmer HH (2010) The importance of sea-ice: an overview. In: Thomas DN, Dieckmann GS (eds) Sea ice, 2nd edn. Wiley-Blackwell, Chichester, pp 1–22

Duman JG, Olsen TM (1993) Thermal hysteresis protein activity in bacteria, fungi and phylogenetically diverse plants. Cryobiology 30:322–328

Eicken H, Lensu M, Leppäranta M, Tucker WB III, Gow AJ, Salmela O (1995) Thickness, structure, and properties of level summer multiyear ice in the Eurasian sector of the Arctic Ocean. J Geophys Res 100:22697–22710

El-Sayed SK (1971) Biological aspects of the pack ice ecosystem. In: Deacon G (ed) Symposium on antarctic ice and water masses. Scientific Committee on Antarctic Research, Tokyo, pp 534–554

El-Sayed SZ, Fryxell GA (1993) Phytoplankton. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 65–122

Elser JJ, Stabler LB, Hassett RP (1995) Nutrient limitation of bacterial growth and rates of bactivory in lakes and oceans: a comparative study. Aquat Microb Ecol 9:105–110

Ewert M, Deming JW (2011) Selective retention in saline ice of extracellular polysaccharides produced by the cold-adapted marine bacterium *Colwellia psychrerythraea* strain 34H. Annals Glaciol 52:111–117

Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063

Franzmann PD (1996) Examination of Antarctic prokaryotic diversity through molecular comparisons. Biodiver Conserv 5:1295–1305

Fritsen CH, Memmott JC, Ross RM, Quetin LB, Vernet M, Wirthlin ED (2011) The timing of sea ice formation and exposure to photosynthetically active radiation along the Western Antarctic Peninsula. Polar Biol 34:683–692

Garcia RO, Reichenbach H, Ring MW, Müller R (2009) *Phaselicystis flava* gen. nov., sp. nov., an arachidonic acid-containing soil myxobacterium, and the description of *Phaselicystidaceae* fam. nov. Int J Syst Evol Microbiol 59:1524–1530

Garrison DL, Buck KR (1986) Organism losses during ice melting: a serious bias in sea ice community studies. Polar Biol 10:564–572

Garrison DL, Buck KR (1989) The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsula regions. Polar Biol 10:211–219

Garrison DL, Ackley SF, Buck KR (1983) A physical mechanism for establishing algal populations in frazil ice. Nature 306:363–365

Gerdes B, Brinkmeyer R, Dieckmann G, Helmke E (2004) Influence of crude oil on changes of bacterial communities in Arctic sea-ice. FEMS Microbiol Ecol 53:129–139

Gianelli V, Thomas DN, Haas C, Kattner G, Kennedy H, Dieckmann GS, Jeffries MO, Eicken H (2001) Behavior of dissolved organic matter and inorganic nutrients during experimental sea-ice formation. Annals Glaciol 33:317–321

Giesenhager HC, Detme AE, de Wall JA, Weber A, Gradinger RR, Jochem FJ (1999) How are Antarctic planktonic microbial food webs and algal blooms affected by melting of sea ice? Microcosm simulations. Aquat Microb Ecol 20:183–201

Gleitz M, Thomas DN (1993) Variation in phytoplankton standing stock, chemical composition and physiology during sea ice formation in the southeastern Weddell Sea, Antarctica. J Exp Mar Biol Ecol 173:211–230

Gleitz M, Bathmann UV, Lochte K (1994) Build-up and decline of summer phytoplankton biomass in the eastern Weddell Sea, Antarctica. Polar Biol 14:413–422

Gosink JJ, Herwig RP, Staley JT (1997) *Octadecabacter arcticus* gen. nov., sp. nov., and *O. antarcticus*, sp.nov., non-pigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. Syst Appl Microbiol 20:356–365

Gosink JJ, Woese CR, Staley JT (1998) *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolated polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of ' *Flectobacillus glomeratus* as *Polaribacter glomeratus* comb. nov. Int J Syst Bacteriol 48:223–235

Gosselin M, Legendre L, Demers S, Ingram RG (1985) Responses of sea-ice microalgae to climatic and fortnightly tidal energy inputs (Manitounuk Sound, Hudson Bay). Can J Fish Aquat Sci 42:999–1006

Gowing MM, Garrison DL, Gibson AH, Krupp JM, Jeffries MO, Fritsen CH (2004) Bacterial and viral abundance in Ross Sea summer pack ice communities. Mar Ecol Prog Ser 279:3–12

Griffiths RP, Caldwell BA, Morita RY (1982) Seasonal changes in microbial heterotrophic activity in subarctic marine waters as related to phytoplankton primary productivity. Mar Biol 71:121–127

Grossi SM, Kottmeier ST, Sullivan CW (1984) Sea ice microbial communities III. Seasonal abundance of microalgae and associated bacteria, McMurdo Sound, Antarctica. Microb Ecol 10:231–241

Grossmann S (1994) Bacterial activity in sea ice and open water of the Weddell Sea, Antarctica: a microautoradiographic study. Microb Ecol 28:1–18

Grossmann S, Diekmann GS (1994) Bacterial standing stock, activity and carbon production of sea ice in the Weddell Sea, Antarctica. Appl Environ Microbiol 60:2746–2753

Grossmann S, Gleitz M (1993) Microbial responses to experimental sea ice formation: implications for the establishment of Antarctic sea ice communities. J Exp Mar Biol Ecol 173:273–289

Groudieva T, Grote R, Antranikian G (2003) *Psychromonas arctica* sp. nov., a novel psychrotolerant, biofilm-forming bacterium isolated from Spitzbergen. Int J Syst Evol Microbiol 53:539–545

Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, Antranikian G (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 8:475–488

Gunther S, Gleitz M, Dieckmann GS (1999) Biogeochemistry of Antarctic sea ice: a case study on platelet ice layers at Drescher Inlet, Weddell Sea. Mar Ecol Prog Ser 177:1–13

Harder W, Veldcamp H (1971) Competition of marine psychrophilic bacteria at low temperatures. Antonie Van Leeuwenhoek 37:51–63

Hassler C, Schoemann V, Mancuso-Nichols CA, Butler EC, Boyd P (2011) Saccharides enhance iron bioavailability to Southern Ocean phytoplankton. Proc Natl Acad Sci USA 108:1076–1081

Helmke E, Weyland H (1995) Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. Mar Ecol Prog Ser 11:269–287

Herborg LM, Thomas DN, Kennedy H, Haas C, Dieckmann GS (2001) Dissolved carbohydrates in Antarctic sea ice. Antarctic Sci 13:119–125

Hodson RE, Azam F, Carlucci AF, Fuhrman JA, Karl DM, Holm-Hansen O (1981) Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica. Mar Biol 61:89–94

Holm-Hansen O, Vernet M (1990) RACER: phytoplankton distribution and rates of primary production during the austral spring bloom. Antarctic J US 25:141–144

Horner RA (ed) (1985) Sea ice biota. CRC Press, Boca Raton

Horner RA, Syvertsen EE, Thomas DP, Lange C (1988) Proposed terminology and reporting units for sea ice algal assemblages. Polar Biol 8:249–253

Hosoya S, Arunpairojana V, Suwannachart C, Kanjana-Opas A, Yokota A (2006) *Aureispira marina* gen. nov., sp. nov., a gliding, arachidonic acid-containing bacterium isolated from the southern coastline of Thailand. Int J Syst Evol Microbiol 56:2931–2935

Hunken M, Harder J, Kirst GO (2008) Epiphytic bacteria on the Antarctic ice diatom *Amphiprora kufferathii* Manguin cleave hydrogen peroxide produced during algal photosynthesis. Plant Biol 10:519–526

Huston AL, Krieger-Brockett BB, Deming JW (2000) Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. Environ Microbiol 2:383–388

Janech MG, Krell A, Mock T, Kang JS, Raymond JA (2006) Ice-binding proteins from sea ice diatoms. J Phycol 42:410–416

Jaspers E, Overmann J (2004) Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Appl Environ Microbiol 70:4831–4839

Junge K, Swanson BD (2008) High-resolution ice nucleation spectra of sea-ice bacteria: implications for cloud formation and life in frozen environments. Biogeosciences 5:865–873

Junge K, Gosink JJ, Hoppe HG, Staley JT (1998) *Arthrobacter*, *Brachybacterium* and *Planococcus* isolates identified from Antarctic sea ice brine. description of *Planococcus mcmeekinii*, sp. nov. Syst Appl Microbiol 21:306–314

Junge K, Imhoff F, Staley JT, Deming JW (2002) Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol 43:315–328

Junge K, Eicken H, Deming JW (2004) Bacterial activity at-2 to-20 degrees C in Arctic wintertime sea ice. Appl Environ Microbiol 70:550–557

Junge K, Eicken H, Swanson BD, Deming JW (2006) Bacterial incorporation of leucine into protein down to -20 C with evidence for potential activity in sub-eutectic saline ice formations. Cryobiology 52:417–429

Kaartokallio H (2001) Evidence for active microbial nitrogen transformations in sea ice (Gulf of Bothnia, Baltic Sea) in midwinter. Polar Biol 24:21–28

Kaartokallio H, Laamanen M, Sivonen K (2005) Responses of Baltic Sea ice and open-water natural bacterial communities to salinity change. Appl Environ Microbiol 71:4363–4371

Kaartokallio H, Tuomainen J, Kuosa H, Kuparinen J, Martikainen PJ, Servomaa K (2008) Succession of sea-ice bacterial communities in the Baltic Sea fast ice. Polar Biol 31:783–893

Karl DM (1993) Microbial processes in the southern oceans. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 1–64

Karl DM, Holm-Hansen O, Taylor GT et al (1991) Microbial biomass and productivity in the western Bransfield Strait, Antarctica during the 1986–87 austral summer. Deep Sea Res 38:1029–1055

Kim K, Kim KR, Min DH et al (2001) Warming and structural changes in the East (Japan) Sea: a clue to future changes in global oceans? Geophys Res Lett 28:3293–3296

Kivi K, Kuosa H (1994) Late winter microbial communities in the western Weddell Sea (Antarctica). Polar Biol 14:389–399

Knox GA (ed) (1994) The biology of the southern ocean: studies in polar research. Cambridge University Press, New York

Koh EY, Atamna-Ismaeel N, Martin A, Cowie ROM, Béjà O, Davy SK, Maas EW, Ryan KG (2010) Proteorhodopsin-bearing bacteria in Antarctic sea ice. Appl Environ Microbiol 76:5918–5925

Koh EY, Phua W, Ryan KG (2011) Aerobic anoxygenic phototrophic bacteria in Antarctic sea ice and seawater. Environ Microbiol Reports 3:710–716

Kottmeier ST, Sullivan CW (1987) Late winter primary production and bacterial production in sea ice and seawater west of the Antarctic Peninsula. Mar Ecol Prog Ser 36:287–298

Kottmeier ST, Sullivan CW (1988) Sea ice microbial communities (SIMCO) 9. Effects of temperature and salinity on rates of metabolism and growth of autotrophs and heterotrophs. Polar Biol 8:293–304

Kottmeier ST, Sullivan CW (1990) Bacterial biomass and production in pack ice of Antarctic marginal ice edge zones. Deep Sea Res 37:1311–1330

Kottmeier ST, Grossi SM, Sullivan CW (1987) Sea ice microbial communities. VIII. Bacterial production in annual sea ice of McMurdo Sound, Antarctica. Mar Ecol Prog Ser 35:175–186

Krembs C, Deming JW (2008) The role of exopolymers to adaptation of microorganisms to sea-ice. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, New York, pp 247–264

Krembs C, Gradinger R, Spindler M (1999) Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. J Exp Mar Biol Ecol 243:55–80

Krembs C, Tuschling K, Juterzenka K (2002) The topography of the ice water interface—its influence on the colonization of sea ice by algae. Polar Biol 25:106–117

Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc Natl Acad Sci USA 108:3653–3658

Kunin V, Engelbrektson A, Ochman H, Hugenholtz P (2009) Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol 12:118–123

Kuparinen J, Autio R, Kaartokallio H (2011) Sea ice bacterial growth rate, growth efficiency and preference for inorganic nitrogen sources in the Baltic Sea. Polar Biol 34:1361–1373

Lange MA (1988) Basic properties of Antarctic sea ice as revealed by textural analysis of ice cores. Annals Glaciol 10:95–101

Lewis EL, Weeks WF (1971) Sea ice: some polar contrasts. In: Deacon G (ed) Antarctic ice and water masses. Scientific Committee on Antarctic Research, Cambridge, pp 23–34

Lizotte MP (2003) Microbiology. In: Thomas DN, Dieckmann GS (eds) Sea-ice: an introduction to its physics, chemistry, biology and geology. Blackwell Science, Oxford, pp 184–210

Lovejoy C, Bowman JP, Hallegraeff GM (1998) Algicidal effects of a novel marine *Pseudoalteromonas* isolate (class *Proteobacteria*, gamma subdivision) on harmful algal bloom species of the genera *Chattonella*, *Gymnodinium*, and *Heterosigma*. Appl Environ Microbiol 64:2806–2813

Margesin R, Schinner F (2001) Properties of cold-adapted microorganisms and their potential role in biotechnology. J Biotechnol 33:1–14

Martin A, Anderson MJ, Thorn C, Davy SK, Ryan KG (2011) Response of sea-ice microbial communities to environmental disturbance: an in situ transplant experiment in the Antarctic. Mar Ecol Prog Ser 424:25–37

Martin A, Hall J, Ryan K (2009) Low salinity and high level UV-B radiation reduce single-cell activity in Antarctic sea-ice bacteria. Appl Environ Microbiol 75:7570–7573

Marx JG, Carpenter SD, Deming JW (2009) Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychrerythraea* 34H. Can J Microbiol 55:63–72

Maykut GA (1985) The Ice Environment. In: Horner RA (ed) Sea ice biota. CRC Press, Boca Raton, pp 21–82

McMinn A, Skerratt J, Trull T, Ashworth C, Lizotte M (1999) Nutrient stress gradient in the bottom 5 cm of fast ice, McMurdo sound, Antarctica. Polar Biol 21:220–227

Meiners K, Brinkmeyer R, Granskog MA, Lindfors A (2004) Abundance, size distribution and bacterial colonization of exopolymer particles in Antarctic sea ice (Bellinghausen Sea). Aquat Microb Ecol 35:283–296

Meiners K, Krembs C, Gradinger R (2008) Exopolymer particles: microbial hotspots of enhanced bacterial activity in Arctic fast ice (Chukchi Sea). Aquat Microb Ecol 52:195–207

Methe BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang XJ, Moult J, Madupu R, Nelson WC, Dodson RJ, Brinkac LM, Daugherty SC, Durkin AS, DeBoy RT, Kolonay JF, Sullivan SA, Zhou LW, Davidsen TM, Wu M, Huston AL, Lewis M, Weaver B, Weidman JF, Khouri H, Utterback TR, Feldblyum TV, Fraser CM (2005) The psychrophilic lifestyle as revealed by the genome sequencing of *Colwellia psychrerythraea* 34H through genome and proteomic analyses. Proc Natl Acad Sci USA 102:10913–10918

Mitchell BG, Holm-Hansen O (1991) Observations and modeling of the Antarctic phytoplankton crop in relation to mixing depth. Deep Sea Res 38:981–1007

Mock T, Kruse M, Dieckmann GS (2003) A new microcosm to investigate oxygen dynamics during sea ice formation. Aquat Microb Ecol 30:197–205

Mock T, Thomas DN (2005) Recent advances in sea-ice microbiology. Environ Microbiol 7:605–619

Montfort P, Demers S, Levasseur M (2000) Bacterial dynamics in first year sea ice and underlying seawater of Saroma-ko Lagoon (sea of Okhotsk, Japan) and resolute Passage (High Canadian Arctic): inhibitory effects of ice algae on bacterial dynamics. Can J Microbiol 46:623–632

Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167

Nevot M, Deroncele V, Messner P, Guinea J, Mercade E (2006) Characterization of the outer membrane vesicles released by the psychrotolerant bacterium *Pseudoalteromonas antarctica* NF(3). Environ Microbiol 8:1523–1533

Nguyen D, Maranger R (2011) Respiration and bacterial carbon dynamics in Arctic sea-ice. Polar Biol 34:1843–1855

Nichols DS, Brown JL, Nichols PD, McMeekin TA (1997) Production of eicosapentaenoic and arachidonic acids by an Antarctic bacterium: response to growth temperature. FEMS Microbiol Lett 152:349–354

Nichols D, Bowman J, Sanderson K, Nichols CM, Lewis T, McMeekin T, Nichols PD (1999a) Developments with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. Curr Opin Biotechnol 10:240–246

Nichols DS, Greenhill AR, Shadbolt CT, Ross T, McMeekin TA (1999b) Physicochemical parameters for growth of the sea ice bacteria *Glaciecola punicea* 611(T) and *Gelidibacter* sp. IC158. Appl Environ Microbiol 65:3757–3760

Nichols DS, Olley J, Garda H, Brenner RR, McMeekin TA (2000) Effect of temperature and salinity stress on growth and; lipid composition of Shewanella gelidimarina. Appl Environ Microbiol 66:2422–2429

Nichols CM, Bowman JP, Guezennec J (2005) Effects of incubation temperature on growth and production of exopolysaccharides by an Antarctic sea-ice bacterium grown in batch culture. Appl Environ Microbiol 71:3519–3523

Nichols CM, Guezennec J, Bowman JP (2006) Bacterial exopolysaccharides from extreme marine environments with special consideration of the Southern Ocean, sea ice, and deep-sea hydrothermal vents: a review. Marine Biotechnol 7:253–271

Ochman H, Elwyn H, Moran NA (1999) Calibrating bacterial evolution. Proc Natl Acad Sci USA 96:12638–12643

Palmisano AC, Garrison DL (1993) Microorganisms in Antarctic sea ice. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 167–218

Palmisano AC, Sullivan CW (1985) Pathways of photosynthetic carbon assimilation in sea ice microalgae from McMurdo Sound, Antarctica. Limnol Oceanogr 30:674–678

Palmisano AC, SooHoo JB, Moe RL, Sullivan CW (1997) Sea ice microbial communities. VII. Changes in under ice spectral irradiance during the development of Antarctic sea ice microalgal communities. Mar Ecol Prog Ser 35:165–173

Pankowski A, McMinn A (2008) Ferredoxin and flavodoxin in eastern Antarctica pack ice. Polar Biol 31:1153–1165

Pankowski A, McMinn A (2009) Iron availability regulates growth, photosynthesis and production of ferredoxin and flavodoxin in Antarctic sea ice diatoms. Aquat Biol 4:273–288

Petri R, Imhoff JF (2001) Genetic analysis of sea-ice bacterial communities of the Western Baltic Sea using an improved double gradient method. Polar Biol 24:252–257

Pomeroy LR, Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat Microb Ecol 23:187–204

Priddle J, Leakey RKG, Archer SD, Murphy EJ (1996) Eukaryotic microbiota in the surface waters and sea ice of the Southern Ocean: aspects of physiology, ecology and biodiversity in a 'two-phase' ecosystem. Biodivers Conserv 5:1473–1504

Pusceddu A, Dell'Anno A, Vezzulli L, Fabiano M, Saggiomo V, Cozzi S, Catalano G, Guglielmo L (2009) Microbial loop malfunctioning in the annual sea ice at Terra Nova Bay (Antarctica). Polar Biol 32:337–346

Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE (1983) Model for bacterial culture growth rate through the entire biokinetic temperature range. J Bacteriol 154:1222–1226

Ratkowsky DA, Olley J, Ross T (2005) Unifying temperature effects on the growth rate of bacteria and the stability of globular proteins. J Theor Biol 233:351–362

Raymond JA, Fritsen C, Shen K (2007) An ice-binding protein from an Antarctic sea ice bacterium. FEMS Microbiol Ecol 61:214–221

Reimnitz E, Clayton JR, Kempema EW, Payne JR, Weber WS (1993) Interaction of rising frazil with suspended particles: tank experiments with application to nature. Cold Regions Sci Technol 21:117–135

Riedel A, Michel C, Gosselin M (2006) Seasonal study of sea-ice exopolymeric substances of the Mackenzie shelf: implication for transport of sea-ice bacteria and algae. Aquat Microb Ecol 45:195–206

Riedel A, Michel C, Gosselin M, Leblanc B (2007) Enrichment of nutrients, exopolymeric substances and microorganisms in newly formed sea ice on the Mackenzie shelf. Mar Ecol Prog Ser 342:55–67

Riley M, Staley JT, Danchin A, Wang TZ, Brettin TS, Hauser LJ, Land ML, Thompson L (2008) Genomics of an extreme psychrophile. *Psychromonas ingrahamii*. BMC Genom 9:e210

Rodrigues DF, Jesus ED, Ayala-del-Rio HL, Pellizari VH, Gilichinsky D, Sepulveda-Torres L, Tiedje JM (2009) Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*. ISME J 3:658–665

Romanenko LA, Uchino M, Mikhailov VV, Zhukova NV, Uchimura T (2003) *Marinomonas primoryensis* sp nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan. Int J Syst Evol Microbiol 53:829–832

Romanenko LA, Lysenko AM, Rohde M, Mikhailov VV, Stackebrandt E (2004) *Psychrobacter maritimus* sp.nov.and *Psychrobacter arenosus* sp.nov., isolated from coastal sea ice and sediments of the Sea of Japan. Int J Syst Evol Microbiol 54:1741–1745

Romanenko LA, Tanaka N, Frolova GM, Mikhailov VV (2008) *Pseudochrobactrum glaciei* sp nov., isolated from sea ice collected from Peter the Great Bay of the Sea of Japan. Int J Syst Evol Microbiol 58:2454–2458

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Russell NJ (1998) Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications. Adv Biochem Eng Biotechnol 61:1–21

Russell NJ (2000) Toward a molecular understanding of cold activity of enzymes from psychrophiles. Extremophiles 4:83–90

Russell NJ, Nichols DS (1999) Polyunsaturated fatty acids in marine bacteria-a dogma rewritten. Microbiology 145:767–779

Rysgaard S, Glud RN (2004) N2 production in Arctic sea ice. Limnol Oceanogr 49:86–94

Rysgaard S, Glud RN, Sejr MK, Blicher ME, Stahl HJ (2008) Denitrification activity and oxygen dynamics in Arctic sea ice. Polar Biol 31:527–537

Sala MM, Arrieta JM, Boras JA, Duarte CM, Vaque D (2010) The impact of ice melting on bacterioplankton in the Arctic Ocean. Polar Biol 33:1683–1694

Schewe I, Soltwedel T (2003) Benthic response to ice-edge-induced particle flux in the Arctic Ocean. Polar Biol 26:610–620

Schwartzmann D (1999) Life, temperature and the Earth: the self-organizing biosphere. Columbia University Press, New York

Seiler H, Bleicher A, Busse HJ, Hüfner J, Scherer S (2011) *Psychroflexus halocasei* sp. nov., isolated from an aberrant cheese surface ripening microbial consortium. Int J Syst Evol Microbiol. doi:10.1099/ijs.0.034801-0

Shivaji S, Reddy GSN, Raghavan PUM, Sarita NB, Delille D (2004) *Psychrobacter salsus* sp. nov. and *Psychrobacter adeliensis* sp. nov. isolated from fast ice from Adelie Land, Antarctica. Syst Appl Microbiol 27:628–635

Shulse CN, Allen EE (2010) Diversity and distribution of microbial long-chain fatty acid biosynthetic genes in the marine environment. Environ Microbiol 13:684–695

Skerratt JH, Bowman JP, Nichols PD (2002) *Shewanella olleyana* sp. nov., a marine species isolated from a temperate estuary which produces high levels of polyunsaturated fatty acids. Int J Syst Evol Microbiol 52:2101–2106

Skinner LC, Fallon S, Waelbroeck C, Michel E, Barker S (2010) Ventilation of the deep Southern Ocean and deglacial CO_2 rise. Science 328:1147–1151

Smith CR, Mincks S, Demaster DJ (2004) A synthesis of bentho-pelagic coupling on the Antarctic shelf: Food banks, ecosystem inertia and global climate change. deep sea research II. Top Stud Oceanogr 53:875–894

Staley JT, Gosink JJ (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. Annu Rev Microbiol 53:189–215

Staley JT, Irgens RL, Herwig RP (1989) Gas vacuolate bacteria from the sea ice of Antarctica. Appl Environ Microbiol 55:1033–1036

Style RW, Worster MG (2009) Frost flower formation on sea ice and lake ice. Geophys Res Lett 36:11501–11504

Sullivan CW, Palmisano AC (1981) Sea ice microbial communities in McMurdo Sound. Antarctic J US 16:126–127

Sullivan CW, Palmisano AC (1984) Sea ice microbial communities: distribution, abundance and diversity of sea ice bacteria in McMurdo Sound, Antarctica, in 1980. Appl Environ Microbiol 47:788–795

Sullivan CW, Palmisano AC, Kottmeier S, McGrath Grossi S, Moe R, Taylor GT (1983) The influence of light on development and growth of sea ice microbial communities in McMurdo Sound. Antarctic J US 18:177–179

Taylor GT, Sullivan CW (2008) Vitamin B-12 and cobalt cycling among diatoms and bacteria in Antarctic sea ice microbial communities. Limnol Oceanogr 53:2454–2458

Thomas DN, Dieckmann GS (2002) Ocean science—Antarctic Sea ice—a habitat for extremophiles. Science 295:641–644

Thomas DN, Kattner G, Engbrodt R, Gianelli V, Kennedy H, Haas C, Dieckmann GS, Jeffries MO, Eicken H (2001) Dissolved organic matter in Antarctic sea ice. Annals Glaciol 33:297–303

Tucker WB, Weatherly JW, Eppler DT, Farmer LD, Bentley DL (2001) Evidence for rapid thinning of sea ice in the western Arctic Ocean at the end of the 1980s. Geophys Res Lett 28:2851–2854

Tupas LM, Koike I, Karl DM, Holm-Hansen O (1994) Nitrogen metabolism by heterotrophic bacterial assemblages in Antarctic coastal waters. Polar Biol 14:195–204

Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE (2001) Essential fatty acids in visual and brain development. Lipids 36:885–895

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, London

Vincent WF (2000) Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarctic Sci 12:374–385

Vincent WF (2010) Microbial ecosystem responses to rapid climate change in the Arctic. ISME J 4:1089–1091

Wadhams P (1994) The Antarctic sea ice cover. In: Hempel G (ed) Antarctic science: global concerns. Springer, Berlin, pp 45–59

Watanabe K, Satoh H (1997) Seasonal variations of ice algae standing crop near Syowa Station, East Antarctica. Bull Plankton Soc Jpn 34:143–164

Weissenberger J, Grossmann S (1998) Experimental formation of sea ice: importance of water circulation and wave action for incorporation of phytoplankton and bacteria. Polar Biol 20:178–188

Wells LE, Deming JW (2006a) Modelled and measured dynamics of viruses in Arctic winter sea-ice brines. Environ Microbiol 8:1115–1121

Wells LE, Deming JW (2006b) Characterization of a cold-active bacteriophage on two psychrophilic marine hosts. Aquat Microb Ecol 45:15–29

Wigley TML, Raper SCB (1987) Thermal expansion of sea water associated with global warming. Nature 330:127–131

Wong APS, Bindoff NL, Church JA (1999) Large-scale freshening of intermediate waters in the Pacific and Indian oceans. Nature 400:440–443

Yu Y, Li HR, Zeng YX, Chen B (2009) Extracellular enzymes of cold adapted bacteria from Arctic sea-ice, Canada Basin. Polar Biol 32:1539–1547

Yurkov VV, Beatty JT (1998) Aerobic anoxygenic phototrophic bacteria. Microbiol Mol Biol Rev 62:695–724

Zdanowski MK, Donachie SP (1993) Bacteria in the sea ice zone between Elephant Island and the South Orkneys during the Polish sea-ice zone expedition (December 1988 to January 1989). Polar Biol 13:245–254

Zhang DC, Li HR, Xin YH, Chi ZM, Zhou PJ, Yu Y (2008a) *Marinobacter psychrophilus* sp nov., a psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1463–1466

Zhang DC, Li HR, Xin YH, Liu HC, Chen B, Chi ZM, Zhou PJ, Yu Y (2008b) *Marinomonas arctica* sp nov., a psychrotolerant bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1715–1718

Zhang DC, Yu Y, Xin YH, Liu HC, Zhou PJ, Zhou YG (2008c) *Colwellia polaris* sp nov., a psychrotolerant bacterium isolated from Arctic sea ice. Int J Syst Evol Microbiol 58:1931–1934

Zwally HJ, Parkinson CL, Comiso JC (1983) Variability of Antarctic sea ice and changes in carbon dioxide. Science 220:1005–1012

10 Coral Reef Bacterial Communities

David G. Bourne · Nicole S. Webster
Australian Institute of Marine Science, Townsville, Australia

Coral Reefs

What Comprises a Coral Reef

Tropical coral reefs are a complex ecosystem consisting of a vast array of animals, plants, microorganisms, and viruses which represent the largest structures of biological origin on the planet (Sebens 1994). The fundamental three-dimensional structure of a reef is due to Scleractinian corals which represent the largest order within the phylum Cnidaria (Daly et al. 2003). Through the deposition of calcium carbonate in their skeletons, these organisms are responsible for laying the foundations of, and building up, reef structures (see ❯ *Fig. 10.1a*). Reef-building corals which deposit aragonite and contribute to coral reef development are termed hermatypic corals, though many other coral species are ahermatypic, i.e., non-reef building. Most Scleractinian corals are colonial organisms composed of thousands of individuals, called polyps, connected by living tissue (Barnes 1987; Lalli and Parsons 1995). Each polyp has a cuplike shape with a ring of tentacles around a central opening tipped with stinging cells for defense and to capture zooplankton for heterotrophic feeding. The majority of the corals energy reserves, however, are supplied through a close symbiotic relationship with phyotosynthetic microalgae (*Symbiodinium*, commonly referred to as zooxanthellae) that reside in the corals gastrodermal cells. The coral provides the algae with a protected environment along with compounds necessary for photosynthesis. These include carbon dioxide produced by coral respiration and inorganic nutrients such as nitrates and phosphates, which are metabolic waste products of the coral. The algae in return supply organic byproducts of photosynthesis including oxygen, glucose, glycerol, and amino acids which are utilized as the building blocks of proteins, fats, and carbohydrates, as well as the synthesis of calcium carbonate ($CaCO_3$). This mutual exchange of *Symbiodinium* photosynthates and coral metabolites is the cornerstone of the high biological productivity and aragonite secreting capacity of reef-building corals (Barnes 1987; Lalli and Parsons 1995; Levinton 1995; Barnes and Hughes 1999; Sumich and Morrissey 2004).

The close symbiotic associations of corals extend past the animal and *Symbiodinium* to include an array of other microorganisms including, fungi, bacteria, and archaea. A number of studies have highlighted high Prokaryotic diversity in corals (Rohwer et al. 2001, 2002; Bourne and Munn 2005; Sunagawa et al. 2009) and reported their important role in the fitness of the whole animal (reviewed in Rosenberg et al. 2007a, b). Increasingly, studies are applying a range of classical and developing molecular methodologies to study coral /prokaryote interactions which is driving corals to become a model system for exploring invertebrate microbial interactions. However, coral reefs are not just comprised of the reef-building corals that form the complex substrata, but the accumulation of the multiple species that use these structures for habitat and foraging including fish, sponges, other cnidarians (including jellyfish), worms, crustaceans (including shrimp, cleaner shrimp, spiny lobsters, and crabs), molluscs (including cephalopods), echinoderms (including starfish, sea urchins, and sea cucumbers), ascidians, foraminifera, sea turtles, and sea snakes among countless other species (❯ *Fig. 10.1b*) (Sumich and Morrissey 2004). Therefore, defining a coral reef and the prokaryotic communities associated with a coral reef is a difficult task. Due to the complex nature of coral reef prokaryotic associations this chapter will focus primarily on keystone reef species and habitats that have been the basis for understanding coral reef bacterial processes. This will include bacteria driving water column and benthic processes of reef systems along with information on important keystone species such as corals (❯ *Fig. 10.1a*) and

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_48,

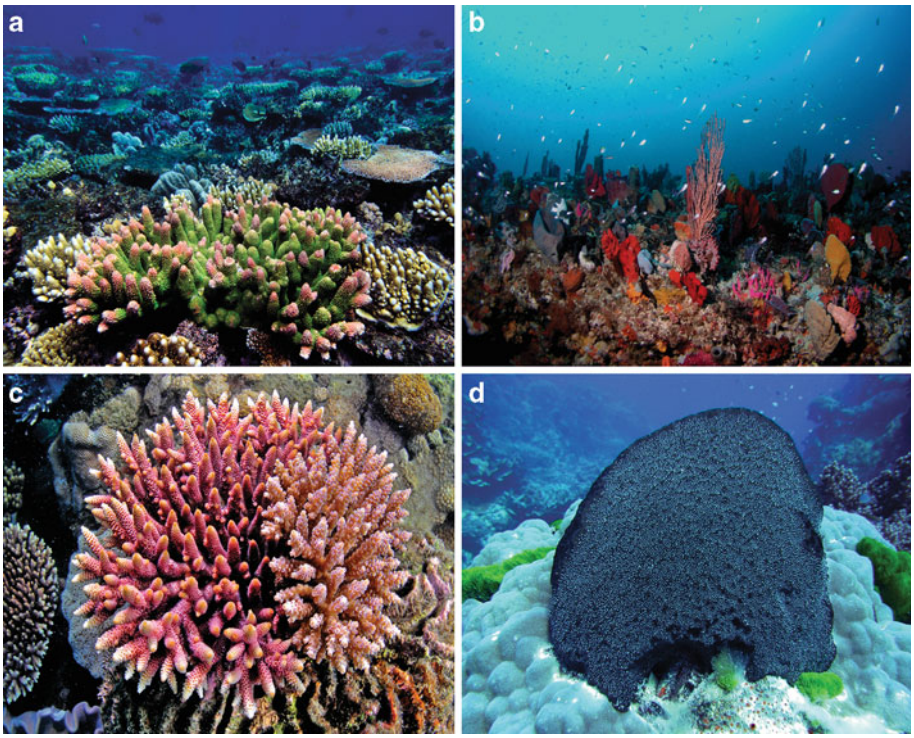


Fig. 10.1
(a) Landscape photographic image of a pristine coral reef demonstrating high coral species diversity and complex three-dimensional structure providing habitat for an array of marine organisms (Photograph: Eric Matson AIMS). (b) Image of a sponge garden reef demonstrating high species diversity and complex community structure despite few reef-building corals being present (Photograph: David Abdo AIMS). (c) Detailed image of an *Acropora millepora* coral colony next to an *Acropora tenuis* coral colony (Photograph: Eric Matson AIMS). (d) Detailed image of a sponge Rhopaloeides residing in the middle of a large coral colony (Photograph: Nicole Webster AIMS)

sponges (*Fig. 10.1d*). This chapter will focus on coral reefs that are typically found in tropical and semitropical waters, between 30° north and 30° south latitude. It should be noted that we still lack a fundamental understanding of vast areas of our oceans including coral reefs in the mesophotic zones and the deeper regions of the earth's oceans. Many species of Scleractinian coral exist in cold and deepwater environments where they are able to form relatively large reef structures and aggregations (Hovland 2008). Although these cold and deep reefs differ structurally and functionally from tropical coral reefs, they still support a diverse array of marine life, such as sponges, polychaete worms, molluscs, crustaceans, brittle stars, starfish, sea urchins, bryozoans, sea spiders, fish, and many other vertebrate and invertebrate species (Rogers 1999; Hovland 2008). These cold water and mesophotic coral reefs are an increasing research focus because of their novel biological diversity and associated prokaryotic partnerships, many of which are essential symbioses that support life in the dark reaches of the earth's oceans. These reefs have also been the focus of recent attention as their depth makes them less susceptible to natural and anthropogenic disturbance and they may therefore act as refugia for some reef species as shallow water tropical reef environments become increasingly degraded (Olson and Kellogg 2010). The study of cold water corals reefs will no doubt reveal a wealth of novel biological information from large multicellular organisms all the way down to the Bacteria, Archaea, and viruses.

Functional Importance of Coral Reefs

Tropical coral reef ecosystems are often referred to as the rainforests of the oceans since they comprise only a small fraction of the bottom surface area yet are estimated to provide habitat for over 25% of all marine species (Connell 1978). In addition to the provision of habitat and shelter, reefs are critical to nutrient cycling and central to carbon and nitrogen fixation (providing sources of essential nutrients for the marine food chain). Bacteria on coral reefs are key participants in this reef nutrient cycling which supports highly productive reef-based fisheries and contributes to coastal protection and tourism. Reefs also provide records of climatic events over paleontological time scales and more recent records of major storms and human impacts that are recorded by the changes in coral growth patterns. These records are extremely valuable for predicting the resilience of coral reefs into the future.

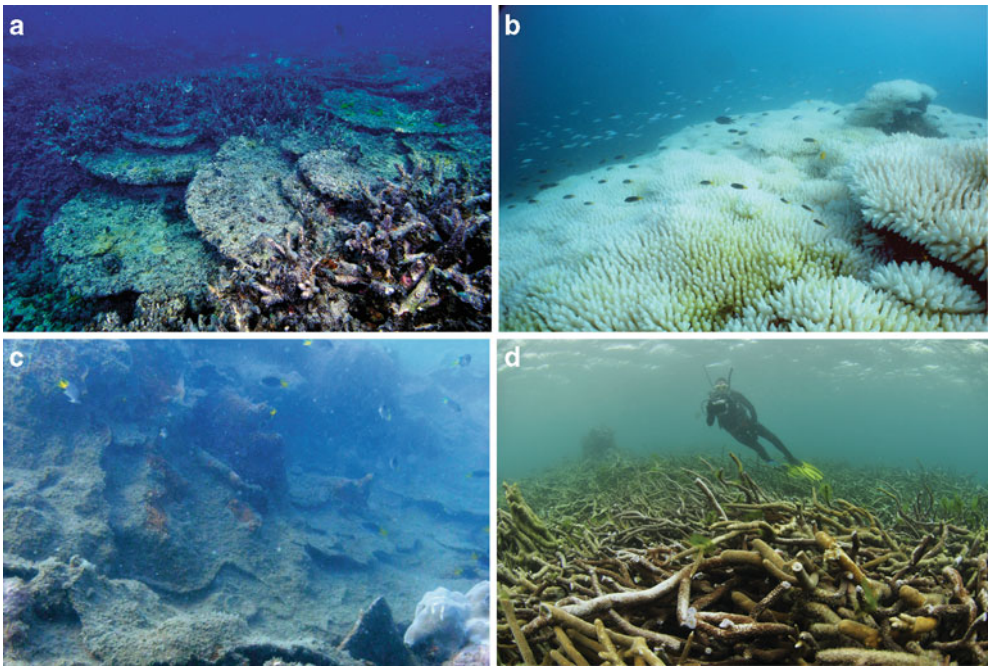


Fig. 10.2
(a) A degraded coral reef showing dead coral skeleton which over time can erode vital structure and habitat for coral reefs animals (Photograph: Eric Matson AIMS). (b) Bleached coral colonies caused by high sea surface temperatures resulting in the expulsion of the endosymbiotic *Symbiodinium* cells. Extended periods of bleaching can result in widespread coral mortality (Photograph: Ray Berkelmans AIMS). (c) Effect of excessive nutrients and sediments being carried onto coral reefs resulting in the smothering of coral and growth of algae further compromising coral health (Photograph: AIMS WQM team) (d) Structural damage of coral reefs caused by cyclone events creating waves and water surges to break coral branches and overturn large coral colonies (Photograph: Ray Berkelmans AIMS)

Reefs Under Threat

Climate change, pollution, and over-fishing are the major factors affecting the health and function of coral reefs (*Fig. 10.2a*). The earth's climate is largely driven by ocean currents and temperature. Global temperatures have increased 0.6°C over the last century and this is expected to increase sea surface temperatures by up to 1°C over the next 50 years, driving shifts in local climes and impacting directly on coral reefs. By 2100, scenarios presented by the IPCC (Intergovernmental Panel on Climate Change) predict that with current carbon dioxide (CO_2) emissions, world temperatures will increase 2–4°C with sea surface temperatures rising by 1–3°C. Such changes will lead to more frequent and severe tropical storms, a decrease in the ocean pH of 0.4–0.5 units (more acidic), a sea level rise of between 9 and 88 cm, and more extreme droughts and floods.

Sea surface temperatures (SST) that exceed the thermal threshold of coral, result in the breakdown of the symbiotic association between the coral host and its endosymbiotc *Symbiodinium* partner (*Fig. 10.2b*). Elevated SST's also cause shifts in the coral-associated bacterial populations which are known to play a role in the health of the coral holobiont (Bourne et al. 2008). Prolonged periods of higher than normal SST's can cause widespread coral mortality, with negative flow-on effects for the multitude of fish and other marine species that depend on corals for habitat and food (Jones et al. 1998). Coral-bleaching events in 1998 and 2002 were the largest yet recorded and affected large areas of reefs globally causing severe declines in coral cover (Berkelmans et al. 2004). Current modeling predicts bleaching episodes will become more common and severe (Hughes et al. 2003; Hoegh-Guldberg 2004, 2009; Hoegh-Guldberg et al. 2007). Higher SST's also result in a stressed environment with some evidence of increased disease outbreaks on coral reefs following periods of anomalously high water temperature (Bruno et al. 2007). Elevated atmospheric CO_2 associated with climate change can also directly affect coral physiology by changing the ocean carbonate chemistry. Corals rely upon a process of calcification to convert carbonates dissolved in seawater into calcium carbonate which is laid down as skeleton for growth. As atmospheric CO_2 increases so too does the acidity of the seawater, making calcification more difficult (Kleypas and Langdon 2006; Hoegh-Guldberg et al. 2007; Anthony et al. 2011). While the direct effects of ocean acidification on coral-bacterial communities are poorly understood, it is expected that they will impact upon the coral holobiont (Meron et al. 2011).

A multitude of other anthropogenic factors also impact coral reefs. Coastal development has major detrimental effects on reef ecosystems through runoff from construction, dredging,

stormwater, farming, and agricultural practices all leading to nutrient enrichment, eutrophication, and poor water quality (Fabricius 2005). Nutrient enrichment can also lead to extremes in the productivity of plankton, more variable survival of marine larvae and recruitment of coral and fish populations, and algal overgrowth of coral reef organisms (Fig. 10.2c) (Brodie and Mitchell 2005). The unsustainable removal of large and herbivorous fish species from reefs alters food webs and promotes increased growth of algae and cyanobacteria, which can cause phase shifts from coral dominated to algal-dominated reefs (Hughes 1994). Other disturbances, such as severe storms (Fig. 10.2d), population explosions of coral-feeding organisms (e.g., crown of thorns starfish), and increased disease outbreaks, have all further contributed to global declines in coral reef ecosystems. While the synergistic effects of multiple stressors are largely unknown, it is expected that reef resilience will be severely eroded.

Diversity and Functional Roles of Prokaryotic Communities on Coral Reefs

The critical roles marine microbial communities play in driving fundamental biogeochemical cycles is well recognized (Azam and Malfatti 2007; Falkowski et al. 2008). Although coral reefs represent only a small fraction of the marine ecosystem, they are extremely important for nutrient cycling in shallow, oligotrophic tropical waters. Reef productivity is largely dependent on the capture and recycling of nutrients and trace elements by reef-associated bacterial communities. Coral reef bacterial communities occupy a range of different habitats including the sediment, overlying water column, and benthic invertebrates such as corals and sponges. Breaking down the complex reef structure into habitats provides a manageable avenue to describe these highly diverse and complex microbial communities that have an important role in coral reef ecosystem functioning. The subsequent sections provide an overview of bacterial diversity and function in these individual habitats. However, it should be noted that tight benthic-pelagic coupling occurs in shallow, well-mixed tropical coral reefs with the bacterial communities in seawater, sediment, and benthic organisms being intricately linked (Garren and Azam 2012). For example, particle export which can be mediated by benthic organisms such as corals sloughing large amounts of mucus increases ecosystem productivity as it settles onto coral reef sediments or is consumed by other reef organisms thereby recycling essential nutrients back into the reef system (Wild et al. 2004a, b, 2005; Mayer and Wild 2010). Nutrients that enter warm well-lit tropical waters are also rapidly assimilated by algae and bacteria and used in the synthesis of organic matter, much of which circulates as detritus and is further colonized by bacteria and other microorganisms. The organic matter is accessible to a variety of other reef organisms in the water column and benthos as a food resource. In addition, resuspension of sediment in well-mixed reef systems can further shape microbial communities in the water column and filter-feeding organisms (see Fig. 10.3).

Prokaryotic Diversity of Reef Sediments

Coral reef sediments typical consist of sandy calcareous sands that have a large porous surface area for colonization by a diverse array of prokaryotic organisms. Estimates of microbial abundance in coral reefs sediment are in the order of 1–2 $\times$ 10^9 cells cm^{-2} (Wild et al. 2006). Bacterial fingerprinting of coral surface sediments from the Great Barrier Reef (GBR) identified between 51 and 148 bacterial operational taxonomic units (OTUs), with the highest diversity occurring in the top 3 cm of sediment (Hewson and Fuhrman 2006). Bacterial communities within calcareous reef sediments differ between nearshore and outer-shelf locations indicating that microorganisms may be useful indicators of water quality gradients (Uthicke and McGuire 2007). In GBR sediments, the communities are highly diverse with *Proteobacteria* (*Alpha*-, *Gamma*-, *Delta*-subdivisions), *Cytophaga-Flavobacterium-Bacteroides* (CFB), *Cyanobacteria*, *Planctomycetaceae*, *Verrucomicrobia*, and *Acidobacteriaceae* comprising the most abundant taxa in 16S rRNA gene clone libraries (Uthicke and McGuire 2007). In permeable reef sediments in Kaneohe Bay Hawaii, Prokaryote ribotype richness of the active microbial community was highly diverse, estimated at >1,380 bacterial ribotypes. Community structure of these sediments differed between the oxic, interfacial, and anoxic zones (Rusch et al. 2009) with *Planctomycetaceae and Proteobacteria* dominating the oxic regions and *Crenarchaeota* dominating the anoxic sediments. Application of ribosomal tag pyrosequencing of DNA and RNA extracted from sediments at this same site provided a more comprehensive analysis of prokaryotic diversity and confirmed it was dominated by the phyla *Proteobacteria*, *Firmicutes*, and *Actinobacteria* along with archaeal groups (Gaidos et al. 2011). The diversity of these sediments was comparable to terrestrial soils. The oxic surface sediments were highly heterogeneous while samples at depth were less so, further supporting microscale geochemical gradients driving much of the prokaryote diversity.

Prokaryotic communities in coral reef sediments undertake important functional roles such as nitrogen cycling (through N_2 fixation) and the metabolism of organic matter both of which are subsequently exported to the surrounding reef ecosystem. For example, Cassareto et al. (2008) observed that 28% of reef primary production is supported by endolithic algae within the coral rubble and Shashar et al. (1994) reported that 70% of nitrogen fixed within sandy reef lagoon sediments is exported to the surrounding reef (Shashar et al. 1994a; Casareto et al. 2008). Benthic bacterial communities involved in aerobic and anaerobic ammonium oxidation (such as the order *Nitrosomonadales*) dominate the permeable sediments of Checker Reef in Kaneohe Bay, Hawaii (Rusch et al. 2009). However, the archaeal populations, in particular members of the *Nitrosopumilus*, may play a more dominant role than bacteria in shunting fixed nitrogen away from primary producers toward denitrifiers (Gaidos et al. 2011). The difficulty in comparing between reefs systems is that they each have distinct biogeochemical parameters which structure and drive the microbial communities.

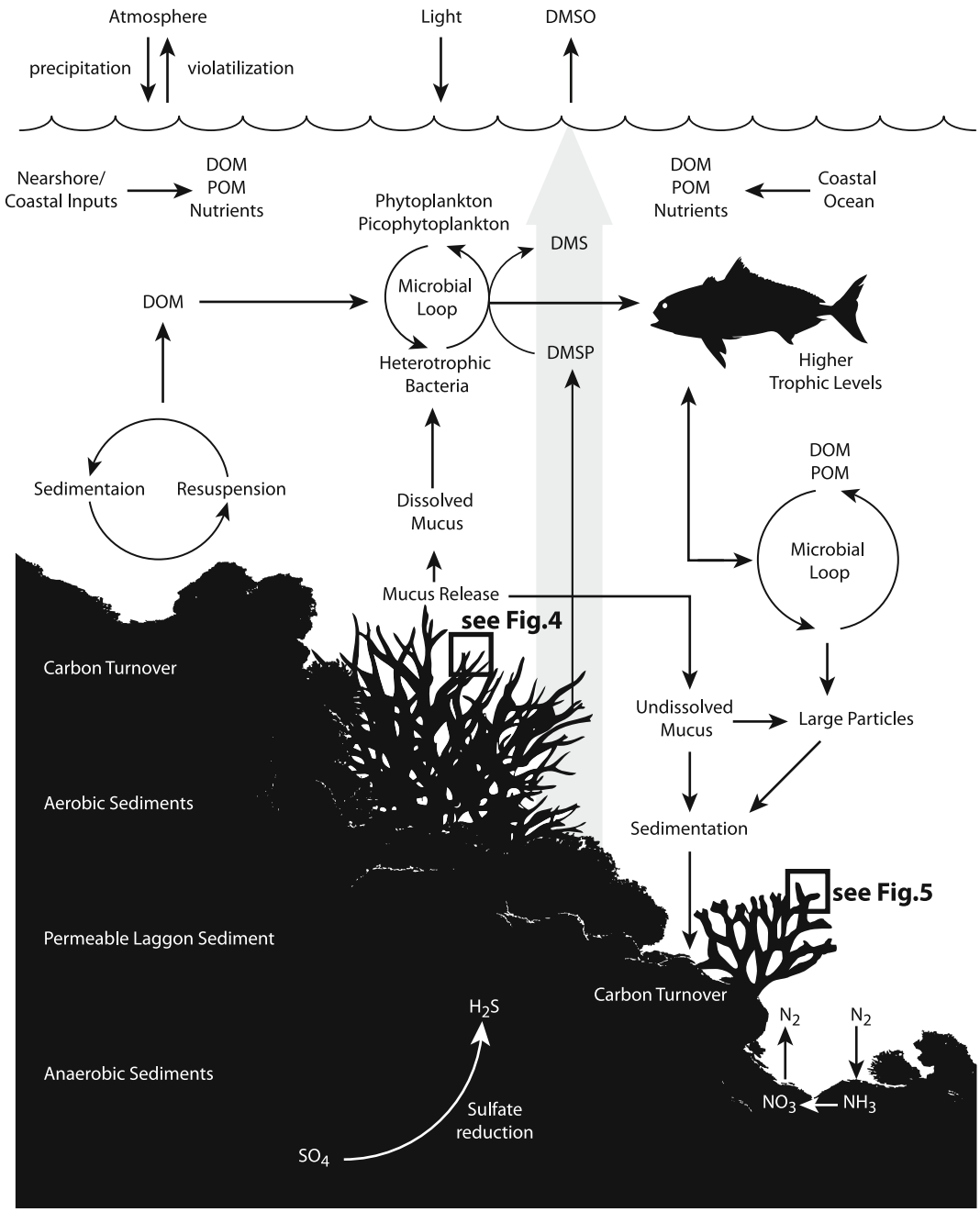


Fig. 10.3
Simplified schematic diagram of microbial processes on coral reefs. Coral reefs are able to capture and recycle nutrients allowing highly productive ecosystems to thrive in relatively nutrient poor waters. Much of this productivity is driven by microbial communities that intricately couple pelagic and benthic processes. *DOM* dissolved organic matter, *POM* particulate organic matter. *Boxes* relate to further detailed microbial processes in coral and sponge organisms, respectively

Prokaryotic Diversity of Coral Reef Waters

Planktonic Cyanobacteria

Photoautotrophic phytoplankton (the picophytoplankton) are major contributors to the biomass and primary productivity of oligotrophic reef waters (Stockner 1988). The coccoid unicellular cyanobacteria *Synechococcus* and *Prochlorococcus* are the smallest oxygenic photoautotrophs and are the most abundant picophytoplankton in tropical and subtropical waters (Partensky et al. 1999), dominating phytoplankton and primary production (Charpy and Blanchot 1999; Ferrier-Page´s and Furla 2001). Abundance of these populations vary, but often range between 1×10^4 cells ml^{-1} and 5×10^5 cells ml^{-1}

(Charpy and Blanchot 1998, 1999; Charpy 2005). Generally, *Synechococcus* spp. increase in abundance in more nutrient-enriched conditions such as coral reef lagoonal environments (Charpy 2005) while *Procholrococcus* spp. dominate the nutrient-depleted areas of the tropical oceans (Campbell et al. 1994). However, the contribution of these cyanobacteria to primary production is highly dependent on the hydrodynamics and physicochemical parameters of individual reef systems. For example, 85–90% of the picophytoplankton in the Great Astrolabe Reef Lagoon in Fiji is dominated by *Synechococcus* with *Prochlorococcus* representing <4% of the population (Charpy and Blanchot 1999) while a contrasting study from Miyako Island in Okinawa, Japan found that *Prochlorococcus* rather than *Synechococcus* dominated the picophytoplankton community (Casareto et al. 2006). On the GBR off the east coast of Australia, *Synechococcus* is generally more abundant than *Prochlorococcus* at most inshore and mid-shelf reefs, with populations correlated with salinity, shelf depth, and chlorohyll *a* concentration rather than nutrient parameters driving the community structure (Ayukai 1992; Crosbie and Furnas 2001). Mid- and outer-shelf reefs of the GBR, influenced by oceanic currents, are dominated by *Prochlorococcus* populations (Crosbie and Furnas 2001). Planktonic filamentous cyanobacterial species primarily of the genus *Trichodesmium (Oscillatoria)* are also common in coral reef waters. Periodic *Trichodesmium* blooms form dense floating algal mats that facilitate fixation of significant amounts of atmospheric nitrogen into depleted tropical waters (Bell et al. 1999). This passage of "new" fixed nitrogen into coral reef waters is thought to contribute to enhanced eutrophication, further shifting planktonic microbial communities (Bell et al. 1999; Brodie et al. 2007).

Other Bacterioplankton

The diversity and function of bacterioplankton in coral reef waters have been poorly studied compared to bacterioplankton in other marine environments (Giovannoni et al. 1996; Acinas et al. 1999; Fuhrman et al. 2006, 2008). A dynamic heterotrophic bacterial community dominates coral reef seawater, stimulated by increased organic matter from mucus and other nutrients released from corals (Torréton and Dufour 1996; van Duyl and Gast 2001; Sakka et al. 2002; Seymour et al. 2005; Torréton et al. 2007). The abundance of bacteria in reef waters varies according to local conditions, but is in the order of 1–2 x$\times$ 10^6 cells ml^{-1} (Weinbauer et al. 2010). Bacterial communities in lagoon reefs can be more active than bacteria from the open ocean (Rath et al. 1993; Weinbauer et al. 2010) and elevated levels of organic matter and bacteria correlate with phytoplankton biomass (Torréton et al. 2002). These studies are from isolated atolls that have relatively long residence times and, therefore, accumulate organic matter and bacterioplankton. In contrast, a rapidly flushing reef in French Polynesia is depleted in dissolved organic carbon and bacterioplankton relative to offshore waters (Nelson et al. 2011). Bacteria in this rapidly flushing reef system are efficient scavengers and recyclers of organic matter with the community being enriched in several bacterial classes that have unique and specific activities in these reef environments (Nelson et al. 2011). Phytoplankton and bacterial abundance on reefs can be depleted by filter-feeding members of the benthos such as sponges (see section on "Non-coral Invertebrate Prokaryotes") (Linley and Koop 1986; Yahel et al. 1998; Gast et al. 1999; van Duyl et al. 2002; Genin et al. 2009). Even within a reef, studies have shown that the number of microorganisms in the water column declines as you traverse from fore reef to back reef (Ayukai 1995). At smaller scales within the reef complex, removal and depletion of bacterioplankton and DOC is observed in coral reef cavities (Van Duyl et al. 2006; De Goeij and van Duyl 2007) due to the high coverage of suspension feeders (Coma et al. 2001). However, an increase in bacterial abundance and actively dividing cells is observed close to coral reef surfaces (4 cm away). It is likely that this stimulation of bacterial abundance is caused by the diffusion of nutrient-rich mucus into the water column. Seasonally driven ecosystem events such as coral reproduction or algal blooms can also significantly influence the abundance, diversity, and activity of bacteria in coral reef seawater (Patten et al. 2008; Wild et al. 2008; Apprill and Rappe 2011). From all these studies, we can conclude that bacterial dynamics within coral reefs systems are highly complex and dependent on many factors including (1) hydrodynamics and water residence time (rapid versus slow reef flushing by oceanic waters or nutrient-rich coastal waters), (2) benthic species abundance and diversity (high vs. low abundance of filter-feeding organisms), (3) seasonal events (coral spawning, cyclones), and (4) the scale of investigations (whole reef, within reef or microscale). Integrated comparative models of reef habitats are required to understand the specific parameters which affect the bacterial communities of coral reef water.

Few studies have directly assessed the diversity and phylogenetic composition of bacteria in coral reef seawater. Most reports use the composition of bacteria in reef seawater to compare to host-associated prokaryote associations such as those that occur in corals and sponges (see sections below). Community analysis of New Caledonian reef waters using denaturing gradient gel electrophoresis (DGGE) reported a dominance of *Roseobacter*, SAR11, *Alteromonas*, and other *Alphaproteobacteria* species (Weinbauer et al. 2010). In contrast, Polynesian reef waters studied using a combination of 16S rRNA gene T-RFLP profiling, clone libraries, and pyrosequencing were enriched in *Proteobacteria* (*Gamma-*, *Beta-* and *Delta-* subdivisions), *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* (Nelson et al. 2011). In addition, two phylotypes belonging to the bacteria SAR11 subclade of the *Alphaproteobacteria* (group 1a and group II) increased in relative abundance within the reef system compared to open water environments consistent with the findings of Weinbauer and colleagues (2010).

The Coral Microbiome

Coral Bacterial Diversity

Corals are highly complex lower invertebrates that have an abundant and diverse bacterial community that play

fundamental roles in the fitness and physiology of the animal (Rosenberg et al. 2007b; Bourne et al. 2009). The consortium of eukaryotic and prokaryotic species that make up the coral including the animal, its symbiotic dinoflagellate partner (*Symbiodinium*), plus the array of Fungi, Bacteria, Archaea, and viruses has been termed "the holobiont" (Rohwer et al. 2002; Reshef et al. 2006). Early studies into coral microbiology identified approximately 6,000 bacterial operational taxonomic units (OTUs) within three different coral species (Rohwer et al. 2002). More recent 16S rRNA tag pyrosequencing conferred similar diversity statistics for seven species of Caribbean corals ranging from 2,177 to 4,026 OTUs, with patterns of bacterial diversity potentially linked to coral morphology (Sunagawa et al. 2010). From the large number of studies that have assessed bacterial diversity of corals, most have emphasized the high bacterial diversity and reported distinct differences between the microbiology of the coral and that of the overlying seawater. Though some strong diversity patterns emerge from individual studies, currently there is no cohesive or conclusive pattern regarding species diversity of coral-associated microbes. For example, some studies report that microbial assemblages are coral host specific across geographically distant sites (Rohwer et al. 2002; Chen et al. 2011), suggesting that the coral host somehow influences the composition of prokaryotes within the holobiont. Other studies have revealed that coral-associated microbial species display site specificity, with community composition varying between location rather than coral host species (Littman et al. 2009; Barott et al. 2011) which suggests that environmental factors have some role in influencing coral-associated microbial communities. Seasonal effects have also been reported to cause shifts in coral-associated bacterial diversity with some corals sampled across different months having distinct bacterial diversity patterns (Hong et al. 2009; Ceh et al. 2011; Chen et al. 2011). The question of scale for bacterial comparisons is also important, not only across ecosystems, across reefs, or within reef systems but within individual coral colonies. For example, heterogeneity in bacterial diversity has been reported from a single coral host (Daniels et al. 2011; Sweet et al. 2011). To date, most studies investigating the diversity of coral-associated bacteria examine the whole animal, without considering that corals are complex animals with different microhabitats including a surface mucus layer, potential bacterial endosymbionts within tissues, endolithic biofilms on and within the skeletal matrix, plus microbial communities within the oral disk and gut (coelenteric gastral cavity). All of these coral microhabitats will likely have unique physiochemical environments that influence the bacterial community structure (see *Fig. 10.4*). For example, the gastral cavity of corals has previously been thought to form an open system having a robust water exchange with the surrounding seawater. However, recent studies using micromanipulation and microelectrode techniques have shown that corals have a semi-closed gastric cavity where high concentrations of essential chemical compounds can be maintained to sustain productivity (Agostini et al. 2009, 2011). These findings illustrate a need to fundamentally re-evaluate the coral symbiotic complex and highlight that essential compounds such as vitamins and nutrients are produced through internal processes driven by bacteria. The complexity of coral-bacterial interactions is enormous and is made further daunting by the fact that >800 species of corals exist in the world (Carpenter et al. 2008); though to date, we have investigated the microbial associations in only a handful of these species.

The number of formally described bacterial strains isolated from coral is very small (15 derived from SiLVA living tree database) (Yarza et al. 2008), and in stark contrast to the number of OTUs inferred from molecular studies (Rabus et al. 1996; Rohwer et al. 2002; Sunagawa et al. 2010). The number of prokaryotic cells estimated from direct *in situ* cell counts of coral mucus and coral tissue was 1×10^6 cells ml^{-1} (Garren and Azam 2010) and 10^7 per cm^2 (Koren and Rosenberg 2006; Garren and Azam 2012) respectively. Studies investigating the specific physiological roles of coral-associated microbes (such as antimicrobial activity) have isolated bacteria without formally describing the species (Kim 1994; Koh 1997; Harder et al. 2003; Kelman et al. 2006; Ritchie 2006; Gochfeld and Aeby 2008; Rypien et al. 2010). For example, a study by Ritchie (2006) isolated 776 bacterial strains from *Acropora palmata* and demonstrated that coral mucus has a role in structuring beneficial coral-associated microbial communities through antibacterial activity. Rypien et al. (2010) also observed that members of the *Gammaproteobacteria* and in particular *Vibrionales* and *Alteromonadales* had high antagonistic activity against other coral bacteria. Relative to developments in other environments, genome sequencing of single isolates has been an under-utilized approach for understanding the role of microbes in the coral holobiont. According to the Genomes Online Database, among the ~8,000 bacterial strains with genome sequences completed or under way, only a few were isolated from a coral (*Pseudoalteromonas* strains from *Montastraea annularis*, and two isolates from diseased corals—*Vibrio corallilyticus* and *Vibrio shiloi*). With the current availability and affordability of DNA sequencing, this coral-bacterial genome landscape is likely to change rapidly which will significantly enhance our understanding of the metabolic and functional potential of coral-associated bacterial strains and provide reference genomes for coral-bacterial metagenomes.

Functional Role of Coral Bacterial Communities

Though many studies have highlighted important functional roles for coral-associated bacterial communities, defining specific symbiotic functions has been incredibly difficult. Mutualistic benefits have been suggested, including fixation and passage of nitrogen and carbon to the coral host (Williams et al. 1987; Shashar et al. 1994b; Cooney et al. 2002; Rohwer et al. 2002; Lesser et al. 2004), as well as other nutrients (Knowlton and Rohwer 2003). Kimes and colleagues used a functional gene array (GeoChip 2.0) to document over 6,700 genes present in the microbiome of the coral *Montastraea faveolata*, including genes involved in carbon, nitrogen, and sulfur cycling

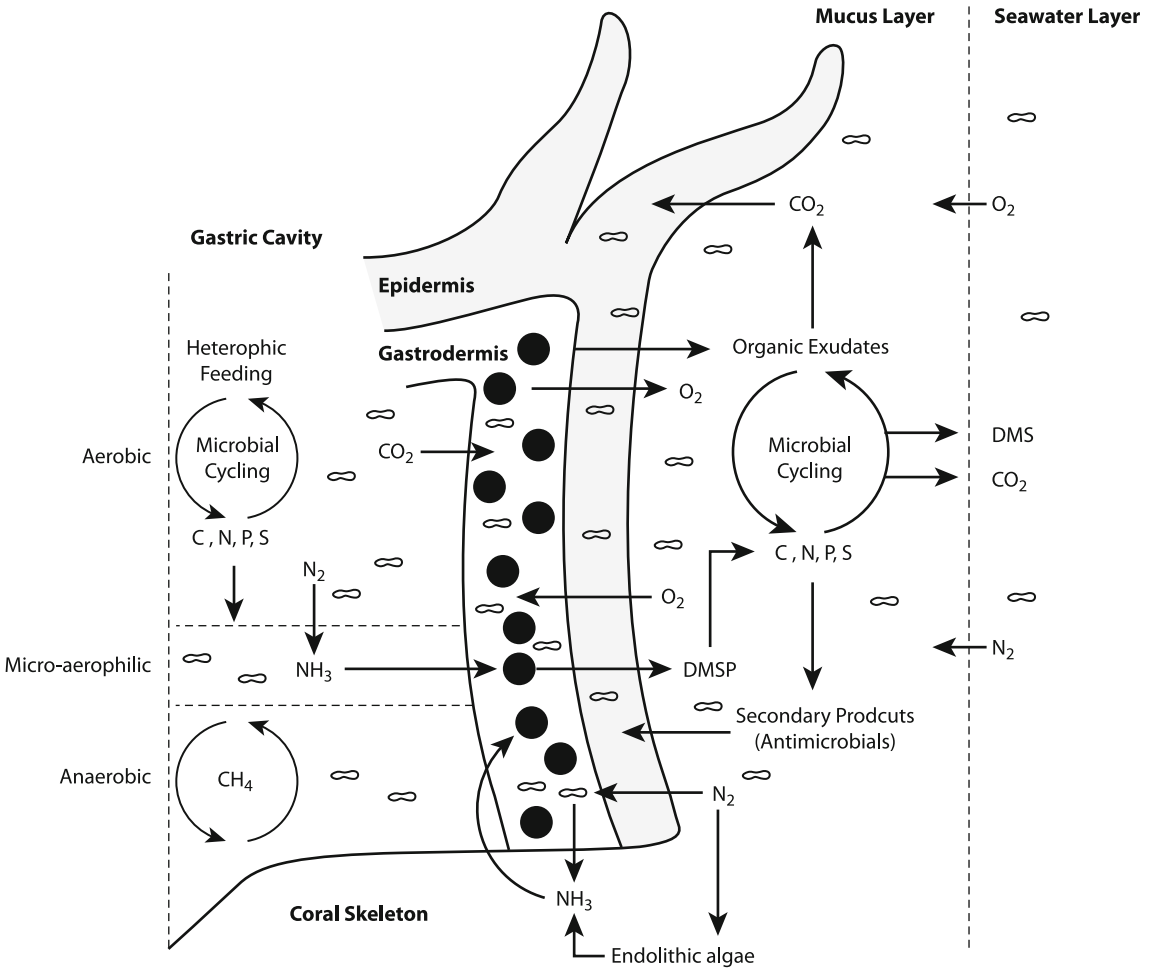


Fig. 10.4

Simplified schematic diagram of the known functional roles of prokaryotic communities in the coral holobiont. The image represents a cross section of the coral polyp with a number of microhabitats within the coral identified including: the coral mucus, epidermis, gastrodermis, basal skeleton, and gastric cavity. In each microhabitat different microbial processes are indicated. *Symbiodinium* cells in the gastrodermis are represented by *solid circles*. Bacterial communities present in the coral microhabitats are represented by *elongated characters*

(Kimes et al. 2010). A diverse array of Bacteria, Archaea, and fungal genes implicated in carbon fixation and carbon degradation were identified, including carbon fixation via the Calvin cycle (via ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*)), the reductive acetyl-CoA pathway (via acetyl-CoA carboxylase (acc)), and the reverse Kreb's cycle. A large number of genes capable of degradation of simple sugars and complex carbohydrates, such as cellulose and chitin through an array of cellulases, chitinases, mannanases, and polygalactases were also identified (Kimes et al. 2010). Through metagenomic approaches, Wegley and colleagues similarly identified genes involved in pathways dedicated for the utilization of sugars, carbohydrates, and proteins, as well as the associated transporters (Wegley et al. 2007). For example, pathways for processing glucose such as glycosyl hydrolases and transferases were observed along with genes for the utilization of monosaccharides, disaccharides, polysaccharides, and sugar alcohols. The metabolic profile of the coral microbiome was believed to reflect available compounds found in coral mucus, including high concentrations of proteins and polysaccharides, lower levels of lipids, and variable amino acids (Ducklow and Mitchell 1979; Meikle et al. 1988; Wild et al. 2004a).

Nitrogen fixation represents another contribution that can only be performed by the microbial component of the holobiont. Coral reef waters are oligotrophic with nitrogen considered to be the primary limiting nutrient for many reef ecosystems (D'Elia and Wiebe 1990; Shashar et al. 1994b; Capone 1996). Importantly, the growth and abundance of the coral endosymbiotic partner, the *Symbiodinium*, is nitrogen limited (Falkowski et al. 1993). However, gaseous nitrogen (N_2) is present in relatively high concentrations in seawater. Therefore nitrogen fixation, the reduction of N_2 into ammonia, which is performed by a group of bacteria known as diazotrophs, is an important functional role that has been suggested for

coral-associated microorganisms (Williams et al. 1987; Shashar et al. 1994b; Lesser et al. 2004). In fact, fixation of nitrogen in corals has been measured in a number of early studies using acetylene-reduction assays (Williams et al. 1987; Shashar et al. 1994b) though the actual identification of the organisms responsible has only recently been investigated.

Cyanobacteria, which are the most common and well-studied diazotrophs, have evolved unique ecological strategies for nitrogen fixation and occur in high numbers within the tissue and skeleton of some coral species (Lesser et al. 2004; Kvennefors and Roff 2009). *Symbiodinium* in the coral *Montastrea cavernosa* uses the products of nitrogen fixation by coral-associated cyanobacteria for growth and multiplication without compromising the integrity of the coral-algae symbiosis (Lesser et al. 2007). The distribution of corals with symbiotic cyanobacteria also positively correlates with depth, indicating that cyanobacteria could sustain *Symbiodinium* nutrition in low light environments (Lesser et al. 2007). Another study by Olsen and colleagues targeted a subunit of the conserved functional dinitrogenase iron protein (*nifH*) gene (responsible for nitrogen fixation in all diazotrophs), to investigate bacteria in two *Montipora* corals from Hawaii (Olson et al. 2009). Within these corals, the dominant diazotrophic bacteria were closely related to the *Vibrio* genus. Interestingly, both the studies of Olsen et al. and Lesser et al. found that the abundance of diazotrophs was positively correlated to *Symbiodinium*. Over 70% of *nifH* gene sequences retrieved from three coral species of the GBR were strongly affiliated with *Rhizobia* (Lema et al. 2012), a group of symbiotic nitrogen fixating soil bacteria that have mutualistic relationships with legume plants (Fisher 1994; Cullimore and Dénarié 2003). Further evidence for active nitrogen fixation in coral is found in recent studies showing that both the endosymbiotic algae and the coral host possess enzymes enabling ammonium assimilation (Leggat et al. 2007; Yellowlees et al. 2008; Stambler 2011) and, therefore, both could benefit from products of nitrogen fixation. All these studies provide compelling evidence that diazotrophic microbes are more than just passive members of the coral holobiont, instead representing symbiotic partners interacting in a tight physiological relationship with the coral animal and its associated microalgae and microbiota.

Active cycling of nitrogen within the holobiont was confirmed by the functional gene array of Kimes and colleagues which, in addition to genes for nitrogen fixation, detected ammonia monooxygenase (amo) genes that catalyze the oxidation of ammonia to hydroxylamine (the first step in nitrification) and genes that catalyze the reduction of hydroxylamine to nitrite (Kimes et al. 2010). Archaea identified within the holobiont are likely to be involved in ammonium assimilation and other processes of nitrogen cycling such as nitrification, ammonification, and denitrification (Wegley et al. 2007; Siboni et al. 2008; Kimes et al. 2010). By targeting known functional genes involved in these cycling pathways including nitrite reductase (*nirS* and *nirK*) and ammonia monooxygenase subunit A (*amoA*), the diversity and inferred function of many of these groups can be identified. For example, genes involved in denitrification (the reduction of nitrate to N_2 via nitrite, nitric oxide, and nitrous oxide (Jetten 2008) including nitrate reductase (*nar*), nitrite reductase (*nir*), nitric oxide reductase (*nor*), and nitrous oxide reductase (*nos*) were all detected in the study of Kimes et al. (2010). Other active nitrogen-cycling pathways in corals are dominated by the metabolism of organic nitrogen sources, such as glutamate and urea with the enzymatic genes glutamate dehydrogenase and urease, respectively, identified from Archaea (both *Crenarchaeota* and *Euryarchaeota*) and Bacteria. Metagenomic approaches to studying the coral microbiome also identified the functional subsystems common in these relevant pathways. For example, the endolithic fungal community of the coral *Porites astreoides* is likely to be important in nitrogen cycling by converting nitrate and nitrite to ammonia, enabling fixed nitrogen to cycle within the coral holobiont (Wegley et al. 2007).

Sulfur is present in large concentrations within coral tissues mainly through the production of organic molecules dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS). Recent studies have shown that coral and corals reefs are some of the largest producers of DMSP and DMS and, therefore, likely to have an important role in the biogeochemical cycling of sulfur on local scales (Broadbent and Jones 2004; Van Alstyne et al. 2006). Metabolism of these compounds typically involves bacteria, and recent studies have shown that a large proportion of bacteria closely associated with corals also have the potential to metabolize these compounds (Raina et al. 2010). This was supported by the isolation of bacteria derived from corals including members of the genera, *Roseobacter*, *Spongiobacter*, *Vibrio*, *and Alteromonas*, which are able to metabolize DMSP or DMS as the sole carbon source (Raina et al. 2009). These isolates represent a large component of the coral-associated community. For example, *Spongiobacteria* represented 59% of 16S rRNA gene sequences retrieved from clone libraries derived from *Acropora millepora* colonies around Magnetic Island (Raina et al. 2009). The DMSP metabolic pathways of bacteria are only now being elucidated (Vila-Costa et al. 2010; Curson et al. 2011; Todd et al. 2012) and it is likely that other members of the coral microbial community will have pathways for metabolizing this readily available carbon and sulfur source. For example, the coral-fungal pathogen *Aspergillus sydowii* was recently found to possess the *dddP* gene which releases DMS from DMSP (Kirkwood et al. 2010). Given the high concentration of DMSP and DMS in coral tissue it seems likely that these compounds play a role in structuring bacterial communities in corals. The metabolism of other sources of sulfur within the coral holobiont was proposed by Wegley and colleagues after identification of genes for the transport and degradation of glutathione in the metagenome of *Porites astreoides* (Wegley et al. 2007). Similar to DMSP, glutathione is likely derived from exudates of Eukaryotic partners within the coral holobiont and provides important additional carbon and sulfur metabolites for coral-associated Bacteria, Archaea, and Fungi. Inorganic sulfur is also important for the coral microbiome and a large number of sulfate reduction genes (*dsr*) from both coral-associated Bacteria and Archaea have been detected (Kimes et al. 2010).

The presence of microhabitats within the coral animal is emphasized by the retrieval of genes involved in sulfate reduction (*dsr*) and methane production (*mcr*) in coral microbiome studies (Kimes et al. 2010). Such processes occur under oxygen-depleted conditions, likely to be present in anaerobic pockets including the coral gastric cavity (Agostini et al. 2009). Anaerobic methanotrophic Archaea can be tightly coupled with sulfate reduction and complex archaeal communities can be maintained within small microhabitats of each coral polyp. The retrieval of sequences linked to methanotrophic organisms (*mmo/pmo* and *nifH* genes derived from methanotrophs) (Kimes et al. 2010; Lema et al. 2012) also indicates that tight methane transformations occur within the coral holobiont. Methane oxidation is linked to the methanogenic/sulfate coupling in stratified micro-niche habitats such as occurs in the coral gastric cavity (or other anaerobic pockets). These processes would provide additional sources of fixed carbon compounds to the holobiont.

Bacterial members of the holobiont can also produce secondary metabolites such as antibiotics (Castillo et al. 2001) with many coral isolates demonstrating high and often selective antibiotic activity against other nonspecific or opportunistic pathogenic bacteria (Ritchie 2006; Sweet et al. 2011). It is likely that there are many other functional roles for coral-associated microbial communities including the ability to scavenge limiting nutrients such as iron and other vitamins. These can then be harvested by the coral either through specific translocation mechanisms or heterotrophic feeding. The observation that corals consume their own mucus (Coles and Strathmann 1973) raises the possibility that they may farm the associated microbiota, maintaining stable bacterial communities to harvest these essential nutrients.

Non-coral Invertebrate Prokaryotes

Prokaryotic microbes are also involved in important relationships with reef invertebrates from a range of non-coral taxa including sponges, molluscs, ascidians, echinoderms, crustose coralline algae, and nematodes. Certainly, the best studied of these groups are the sponges (Porifera). Sponges are a highly diverse component of coral reefs, with an estimated 15,000 species worldwide (Hooper and Van Soest 2002) and various properties such as high filtration rates that make them an integral part of the marine ecosystem. Substantial proportions of reef habitats can be dominated by sponges with recent reports indicating that sponges cover more reef surface than hard corals on 60% of Caribbean coral reefs (Pawlik 2011). **S**ponge-microbe associations have existed for 600 million years making them one of the most ancient of all symbioses between microbes and metazoa (Wilkinson 1984). Research into sponge-microbial associations was traditionally driven by biodiscovery for novel natural products of pharmaceutical importance. In fact, many "sponge"-derived metabolites are of suspected or confirmed symbiont origin (Hochmuth and Piel 2009; Piel 2009). In the past decade the field of sponge microbiology has dramatically expanded, incorporating research on the diversity, specificity, and function of sponge-associated microbes. In the past 3 years there have been several hundred publications about various aspects of sponge microbiology and over 10,000 sponge-derived microbial sequences have been submitted to public databases (excluding next generation sequencing).

Sponges generally harbor dense and diverse microbial communities including Bacteria, Archaea, and single-celled eukaryotes (fungi and microalgae) that can comprise up to 40% of the sponge volume and have a large impact on the ecology, biology, and physiology of the host. However, not all sponges harbor a high microbial abundance (HMA); some sponge species are known as low microbial abundance (LMA) sponges, and while these species exist alongside high microbial abundance sponges, very little is known about why they host such few symbionts (Hentschel et al. 2006). The microbes are generally located within the sponge mesohyl matrix, although some sponge species contain bacteria within specialized bacteriocyte cells (reviewed in Taylor et al. 2007b) and high densities of Archaea and cyanobacteria have been detected in the pinacoderm (sponge surface tissue) (Wilkinson 1980, 1992; Webster et al. 2001a). Many of these microorganisms are vertically transmitted from adults to larvae either in the mesohyl tissue or via sperm (reviewed in Webster and Taylor 2012). For example, 28 different vertical transmission sequence clusters affiliated to 10 bacterial phyla and one archaeal phylum were detected in both adult and larval Caribbean sponges (Schmitt et al. 2008). Evidence of vertical transmission provides additional support for their role as symbionts and their importance in host health. To date, 32 bacterial phyla and candidate phyla and all major lineages of *Archaea* have been reported from sponges (reviewed in Webster and Taylor 2012; Schmitt et al. 2011). A recent phylogenetic survey of >7,500 sponge-derived sequences from the SILVA 102 database found that the major taxa inhabiting sponges were *Gammaproteobacteria* (19%), *Alphaproteobacteria* (18%) *Actinobacteria* (16%), *Firmicutes* (8%), and *Cyanobacteria* (7%) (Simister et al. 2012). A comparison of RNA- and DNA-defined communities in sponges revealed that the majority of these diverse microbes are also metabolically active within their sponge hosts (Kamke et al. 2010). In addition to this phyla-level richness, recent 454 amplicon sequencing of sponges from the Great Barrier Reef detected 3,000 bacterial OTUs (95% sequence similarity) within a single sponge species. The application of 454 amplicon sequencing to sponge microbiology has already generated an immense amount of sequence data (Webster et al. 2010; Lee et al. 2011) and has revolutionized our perception of species richness in sponges. However, the increased sequencing effort has not changed our understanding of what microbial taxa predominate. Recent 454 tag sequencing studies of Great Barrier Reef (Webster et al. 2010) and Red Sea (Lee et al. 2011) sponges revealed a dominance of bacterial taxa commonly reported in traditional 16S rRNA gene clone libraries (*Proteobacteria* (*Alpha*, *Delta*, and *Gamma*), *Chloroflexi*, *Firmicutes*, *Acidobacteria*, and *Actinobacteria*) (Taylor et al. 2007b; Simister et al. 2012).

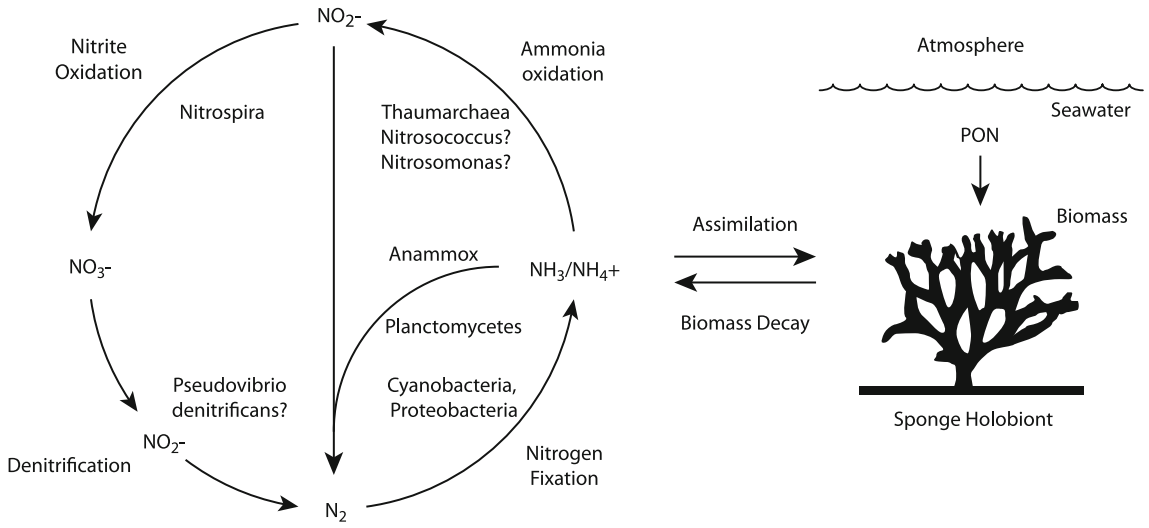


Fig. 10.5

Schematic image representing nitrogen cycling, a dominant microbial-driven process that occurs within sponge tissues. Prokaryotic communities that have been characterized to carry out the nitrogen transformation in sponge tissues are detailed. Putative microbes that are suspected to carry out some processes are represented with a *question mark*

Numerous reviews have highlighted the need to explore the specific functions of these sponge symbionts (Taylor et al. 2007a, b; Vogel 2008; Webster and Blackall 2009; Webster and Taylor 2012). However, the primary challenge with defining function lies with the development of methods that allow us to link community structure with function in these highly complex sponge microbiomes. The majority of sponge symbionts are not amenable to cultivation and phylogenetic analysis only rarely indicates the specific physiology of the symbiont. While researchers have only recently begun employing molecular tools to investigate the specific functions of sponge symbionts, they have rapidly discovered that sponge symbionts are capable of diverse metabolic processes such as nitrification (Bayer et al. 2008; Mohamed et al. 2008; Southwell et al. 2008a; Southwell et al. 2008b; Steger et al. 2008; Off et al. 2010; Schläppy et al. 2010), denitrification (Southwell et al. 2008a, b; Steger et al. 2008; Hoffmann et al. 2009; Schläppy et al. 2010), anammox (Hoffmann et al. 2009; Mohamed et al. 2010), methane oxidation (Vacelet et al. 1996), sulfate reduction (Hoffmann et al. 2005), and photosynthesis (Wilkinson 1983). Other microorganisms may contribute to host defense via the production or transport of biologically active metabolites (Unson et al. 1994; Schmidt et al. 2000).

Nitrogen metabolism has been the major research focus into the symbiotic function of sponge microbes (see *Fig. 10.5*). Nitrification, the two-step conversion of ammonia to nitrate via nitrite was first detected in sponges over 20 years ago (Corredor et al. 1988) and has since been studied intensively (Weisz et al. 2007; Bayer et al. 2008; Southwell et al. 2008a, b; Steger et al. 2008; Hoffmann et al. 2009; Mohamed et al. 2010; Off et al. 2010; Schläppy et al. 2010) with research also revealing higher nitrification rates in tropical sponges and seasonal variation in the uptake and release of ammonia in the sponge *Aplysina aerophoba* (Jimenez and Ribes 2007; Bayer et al. 2008; Schläppy et al. 2010). Nitrification is performed exclusively by ammonia oxidizers (convert ammonia into nitrite) and nitrite oxidizers (oxidize nitrite to nitrate). In sponges this symbiosis is likely a mutualistic relationship whereby the host benefits from the removal of ammonia (produced as a waste product) and the microbes benefit by obtaining energy from the inorganic nitrogen compounds. Known nitrifiers from sponges include the ammonia oxidizing Thaumarchaea and the nitrite oxidizing phylum Nitrospira (reviewed in Taylor et al. 2007b; Bayer et al. 2008). In addition, putative ammonia-oxidizing bacteria (AOB) related to the subdivisions of Gamma- (*Nitrosococcus*) and *Betaproteobacteria* (*Nitrosomonas*, *Nitrosopira*) have been detected in sponge-derived 16 S rRNA gene libraries (Hentschel et al. 2002; Diaz et al. 2004; Bayer et al. 2008; Meyer and Kuever 2008; Mohamed et al. 2010. Denitrification has also been reported in sponges from stable isotope experiments and analysis of nitrite reductase and nitric oxide reductase genes (Hoffmann et al. 2009; Schläppy et al. 2010; Siegl et al. 2011). In addition, anaerobic ammonium oxidation in sponges has been demonstrated by organisms related to anammox planctomycetes (Hoffmann et al. 2009; Mohamed et al. 2010). In reef areas with high sponge biomass, this may form a significant link in the regeneration of nitrogenous compounds (*Fig. 10.5*).

Metagenomic and whole-genome amplification approaches are proving to be valuable tools for studying sponge symbiotic functions. Sequencing of a cosmid library from the microbial community in *Aplysina aerophoba* uncovered a non-ribosomal peptide synthetase (NRPS) from a sponge-specific *Chloroflexi* (Siegl and Hentschel 2010) and a polyketide synthase (PKS) from the *Poribacteria* (Fieseler et al. 2007; Siegl and Hentschel 2010). Subsequent genome sequencing of an individual *Poribacteria* cell revealed evidence for mixotrophy whereby

autotrophic carbon fixation occurs via the Wood-Ljungdahl pathway (Siegl et al. 2011). In addition, carbon monoxide dehydrogenase has been identified in both the genome of the *Poribacteria* and also in the metagenome of the sponge *Cymbastela concentrica* (Thomas et al. 2010; Siegl et al. 2011). A mechanism which potentially enables the sponge to discriminate between symbionts and food bacteria has been proposed based on an abundance of adhesion related proteins, ankyrin repeat proteins (ARP) and tetratricopeptide repeat domain-encoding proteins (TPR) in the *Poribacteria* genome (Siegl et al. 2011), a sponge-associated *Deltaproteobacteria* genome (Liu et al. 2010), and the metagenome from *C. concentrica* (Thomas et al. 2010). These ARP and TPR proteins mediate protein-protein interactions in eukaryotes and over 20% of the ARP and TPR detected in the metagenome of *C. concentrica* had signal peptides for extracellular secretion in Gram-negative bacteria suggesting an interaction with surrounding cells (Thomas et al. 2010).

A range of other functional properties have been described for sponge symbionts based on the genome content. The *Poribacteria* genome and the metagenome from *C. concentrica* both contained genes associated with the metabolism of vitamin B_{12} which would provide a source of this vitamin for the host and may also serve as a cofactor for the enzymes involved in autotrophic carbon fixation (Thomas et al. 2010; Siegl et al. 2011). The evolution of bacterial genomes for symbiotic relationships is thought to involve transposable insertion elements which facilitate the removal of non-required genes and/or the rearrangement of genes to form new structures or pathways. A large number of transposable insertion elements was recently detected in the metagenome of the sponge *C. concentrica* (Thomas et al. 2010). The deltaproteobacterium from *C. concentrica* also contained proteins associated with cell attachment and infection, was capable of aerobic growth, contained the capacity to remove toxins from the sponge and has an apparent role in nutrient transport.

While genomic and metagenomic sequencing is advancing our understanding of sponge symbiotic function, sequence homology approaches are dependent on the quality and content of sequence databases which can be problematic in organisms that are distantly related to their nearest relatives and for which many of the genes have not yet been defined. For instance, 40% of the protein-coding genes in the *Poribacteria* genome had unknown functions. Furthermore, the presence of functional genes in the genome does not necessarily correlate with metabolic activity.

Microbial symbionts have also been described in many other reef invertebrates. For example, bivalves are known to contain endosymbiotic bacteria in their gills which can fix CO_2 with energy obtained through the oxidation of reduced sulfur compounds (Krueger and Cavanaugh 1997), gutless oligochaetes contain chemoautotrophic bacterial endosymbionts below their cuticles (Dubilier et al. 1999), bacterial symbionts present in nudibranch's are thought to play a role in reproduction as they breakdown the mucus layer and egg capsule during intercapsular development (Klussmann-Kolb and Brodie 1999) and the extracellular bacteria that many echinoderms possess between their ectoderm and complex surface cuticle are thought to play a role in nutrition and nitrogen metabolism (Walker and Lesser 1989; Lesser and Blakemore 1990; Lesser and Walker 1992; Burnett and McKenzie 1997). There are also many other reef invertebrate microbial associations where the symbiotic functions are as yet undescribed.

Specificity of Microbial-Host Communities

Partitioning of microbial communities within a reef system is a common conclusion from many diversity studies with coral-associated microbial communities clearly distinct from those in the overlying seawater. For example, Bourne and Munn (2005) reported that the water column 1 m above coral colonies was dominated by *Alphaproteobacteria* while coral samples were dominated by *Gammaproteobacteria*. Other studies also highlight that *Proteobacteria* and cyanobacteria are very common in reef waters (Frias-Lopez et al. 2002).

Many sponge-associated microorganisms appear to be specific to their sponge hosts. In fact, phylogentically unrelated sponges from geographically separated regions are known to share many microbial phylotypes that have not been recorded from the surrounding seawater, sediment, or other hosts (Hentschel et al. 2002; Taylor et al. 2007b; Lafi et al. 2009). Sponge-specific sequence clusters were first described by Hentschel and colleagues in 2002 (Hentschel et al. 2002) and were defined as groups of at least three 16S rRNA gene sequences that (1) are more similar to each other than to sequences from other (non-sponge) sources; (2) are derived from two or more sponge species, or the same species from different locations; (3) are supported by three independent phylogenetic tree-building approaches (neighbor-joining, maximum parsimony, maximum likelihood). A detailed phylogenetic comparison of all publicly available 16S rRNA sequences in 2007 placed 32% of sponge-derived sequences into these sponge-specific sequence clusters (Taylor et al. 2007b). This concept was revisited in 2010 using 16S and 18S rRNA sequences available in the SILVA database. Overall, 27% of the 7,546 sponge-derived microbial sequences fell into monophyletic, sponge-specific sequence clusters that spanned 14 bacterial phyla, the Archaea, and the Fungi. Such clusters were particularly common in the *Chloroflexi*, *Cyanobacteria*, "*Poribacteria*," *Betaproteobacteria*, and *Acidobacteria* with the largest cluster occurring for the cyanobacterium "*Synechococcus spongiarum*," which contained 245 sequences from 40 sponge species (Simister et al. 2012).

The question of how geographically separated sponges acquire and maintain these highly specific bacterial symbioses when the microbes are apparently absent from the surrounding seawater has intrigued evolutionary biologists and microbial ecologists for many years. The application of 454 tag sequencing to study vertical versus horizontal symbiont acquisition in the GBR sponge *Rhopaloeides odorabile* determined that half of the previously described sponge-specific microbes were found exclusively in adults and larvae—implying vertical transmission

of these groups. The remaining taxa including the candidate phyla "*Poribacteria*" (which until this point had only ever been found in sponges) were also detected at very low abundance among the seawater sequence tags. Thus, members of the rare seawater biosphere may serve as seed organisms for widely occurring symbiont populations in sponges and their host association might therefore have evolved much more recently than was previously assumed.

The existence of geographic or host-specific subpopulations of sponge microbes was recently investigated using 454 amplicon sequencing with 32 sponge species from 8 global locations. The taxonomic richness detected in this study comprised 25 bacterial phyla with *Proteobacteria*, *Chloroflexi*, and *Poribacteria* being the most diverse. Similarity analysis of the sponge-derived microbial communities revealed that tropical sponges shared more similar microbial communities than they did with subtropical sponges, although no correlation with host phylogeny was detected. Overall, sponges had a small "core" bacterial community which was comprised of very few OTUs with global distribution. In contrast, a large species-specific bacterial community was present which was verified by many OTUs occurring in only a single sponge species. It is likely that the core microbial community is environmentally acquired whereas the species-specific community may be maintained through vertical transmission. While different sponges host distinct bacterial species, the sponge-derived bacteria are still often more closely related to each other than they are to any non-sponge-derived microbes.

Environmental Stress Shifting Diversity and Function of Coral Reef Prokaryotic Communities

The genetic plasticity of microorganisms allows them to rapidly shift their metabolic capabilities, host range, function, and community dynamics in response to changing environmental conditions. To predict the response and resilience of reef ecosystems and host populations subjected to environmental stress, we therefore need to understand how their associated microbiota is affected. The impact of anthropogenic disturbance on coral reef prokaryotic communities was investigated by metagenomic analysis along a water quality gradient in the Northern Line Islands (from Kingman to Kiritimati) with increasing human population. Human impacts associated with land use and fishing were reflected by shifts in the microbial diversity and metabolic function with a distinct move toward heterotrophy, including potential pathogens at the highest populated and impacted island of Kiritimati (Dinsdale et al. 2008).

Environmental disturbance destabilizes coral-bacterial associations (Knowlton and Rohwer 2003) and early identification of these microbial community shifts may provide a bioindicator for stress and declining coral fitness. For example, Bourne and colleagues (Bourne et al. 2008) demonstrated that as corals bleach in response to elevated seawater temperatures the microbial community shifts, but then returns to normal once the environmental stress is removed. The coral-associated bacterial communities underwent changes prior to any visible signs of stress (e.g., bleaching) in the coral host. Vega-Thurber and colleagues also identified functional shifts in the associated microbiome of the coral *Porites compressa* subjected to abiotic stressors (temperature, nutrients, dissolved organic carbon, and pH) (Vega Thurber et al. 2008). An increase in the abundance of microbial genes involved in stress resistance and virulence were observed along with changes in community metabolism. These included shifts in secondary metabolism profiles, sulfur and nitrogen metabolism, motility and chemotaxis, fatty acid and lipid utilization pathways. A study by Littman and colleagues (2011) directly compared the microbiomes of healthy and bleached *Acropora millepora* during a natural bleaching event and similarly identified an increase in virulence genes, a shift from autotrophy to heterotrophy along with increases in genes associated with the metabolism of fatty acids, proteins, simple carbohydrates, phosphorus, and sulfur (Littman et al. 2011).

Reduced ocean pH driven by rising atmospheric carbon dioxide concentrations is predicted to have a major impact on calcifying organisms such as corals (Hoegh-Guldberg et al. 2007). Reduced seawater pH also causes shifts in the coral-associated bacterial community closely reflecting the changes that occur in stressed and diseased corals, in particular an increased abundance of *Vibrionaceae* and *Altermonadaceae* under acidified conditions (Meron et al. 2011). Reduced water quality associated with excessive nutrient input can also alter coral-associated microbial community diversity and activity. For example, Kline and colleagues (Kline et al. 2006) demonstrated that increased levels of dissolved organic carbon disrupted the coral-associated microbial community in the surface mucus layer by stimulating growth rates of specific microorganisms with impacts for host health. Similarly, the release of dissolved nutrients by algae stimulates microbial activity with patterns of increasing coral stress correlating to proximity to the algae (Smith et al. 2006). The impact of poor water quality and elevated nutrients associated with fish farms on the bacterial communities associated with *Porites cylindrica* was recently assessed (Garren et al. 2009). In the high effluent treatment characterized by elevated levels of DOC and chlorophyll *a*, the coral-associated bacterial community underwent a rapid and dramatic change which included an increase in previously identified human and coral pathogens such as *Arcobacter*, *Fusobacterium*, and *Desulfovibrio* spp. However, bacterial communities were able to recover their original composition after 22 days, revealing the resilience of corals and their associated bacteria to eutrophication.

With the exception of corals, very little research has addressed the resilience of reef invertebrate-microbe partnerships to a changing climate or anthropogenic stress. Recent studies with sponges have examined the effects of elevated seawater temperatures (Lemoine et al. 2007; López-Legentil et al. 2008, 2010; Webster et al. 2008a, 2011a) and heavy metals (Webster et al. 2001b; Selvin et al. 2007) on the symbiotic microbial associations. These studies report shifts in the stable symbiotic microbial communities that closely correlate with

a decline in sponge health. For instance, the giant barrel sponge *Xestospongia muta* is dominated by a *Synechococcus*-type cyanobacteria and a stable *Crenarchaeota* community that reflects the archaeal community found in other sponge species (López-Legentil et al. 2008, 2010). López-Legentil and colleagues demonstrated that this archaeal symbiosis is maintained when the sponge undergoes cyclical bleaching from which it is able to recover, but shifts to reflect the archaeal community in the surrounding sediment when the sponges become fatally bleached (López-Legentil et al. 2010). This shift precedes an increased expression of *amoA*, most likely a result of elevated ammonia associated with tissue death (López-Legentil et al. 2010). In the sponge *Halichondria bowerbanki* changes in the microbial community have been detected by DGGE in individuals exposed to seawater temperatures only 1–2°C above ambient. These changes included the disappearance of specific microbes from thermally stressed sponges and the appearance of new microbes, potentially indicating that a rare species had increased in relative frequency within the microbial community (Lemoine et al. 2007). Major changes in the symbiotic microbial community were also detected in the GBR sponge *Rhopaloeides odorabile* when adult sponges were exposed to temperatures of 33°C. These included the loss of the primary culturable symbiont within 24 h, a rapid increase in *Bacteroidetes* and *Firmicutes* and a community shift away from known sponge symbionts toward a microbial community reflecting that of diseased corals (Webster et al. 2008a). In contrast to the adult sponges, larval *R. odorabile* exhibit a markedly higher thermal tolerance, with adverse health effects and a concomittant microbial shift not occurring until 36°C (Webster et al. 2011a). This shift involved the loss of previously described symbionts (in particular the *Nitrospira*, *Chloroflexi*, and a *Roseobacter* lineage) and the appearance of novel *Gammaproteobacteria* that were not detected at lower temperatures. These studies with *R. odorabile* reveal distinct thermal tolerances in each of the life history stages and confirm that sponge larvae can maintain highly stable symbioses at seawater temperatures exceeding those predicted under climate change. By revealing that shifts in sponge-associated microbial communities occur in conjunction with necrosis and mortality of the host, evidence is provided for a strong link between host health and the stability of symbiont communities.

Crustose coralline algae (CCA) are the key reef-building primary producers and are known to induce larval metamorphosis and recruitment for many coral species (Heyward and Negri 1999). Reef biofilms (particularly microorganisms associated with CCA) are also important as settlement cues for a variety of marine invertebrates, including corals (Negri et al. 2001; Webster et al. 2004). The microbial community associated with the CCA *Neogoniolithon fosliei* was found to be sensitive to 32°C which is only 2–4°C above the mean maximum annual SST (Webster et al. 2011b). After 7 days at 32°C, a large shift in microbial community structure occurs including an increase in *Bacteroidetes* and a reduction in *Alphaproteobacteria*, in particular the loss of the primary strain (with high sequence similarity to a described coral symbiont). Concomitant with the microbial shift were clear indications of stress in the host CCA including bleaching and a reduction in maximum quantum yield. A 50% reduction in the ability of *N. fosliei* to induce coral larval metamorphosis at 32°C accompanied the changes in microbiology, pigmentation, and photophysiology of the CCA. This research demonstrates how thermal stress influences microbial associations on CCA with subsequent downstream impacts on coral recruitment which is critical for reef regeneration and recovery from climate-related mortality events.

To better predict the consequences of these microbial shifts in response to environmental perturbations, we need to understand the functional mechanisms that link symbiotic community structure and host health. Furthermore, additional research is required to assess the adaptive capacity of invertebrate-prokaryote symbioses and how the resilience of the partnerships may be affected by possible functional redundancy provided by a rare microbial biosphere.

Disease in Coral Reef Communities

Coral Diseases

Recent decades have seen a global increase in reports of diseases that affect many marine species including fish, seals, dolphins, shellfish (oysters, scallops, abalone, and clams), starfish, urchins, sponges, sea grass, kelp, coralline algae, and corals (Harvell et al. 1999, 2002; Lafferty et al. 2004; Bally and Garrabou 2007; Haapkyla et al. 2007). Determining if these reported outbreaks are due to improved monitoring, changed environmental conditions, opportunistic or emerging pathogens, or reduced host resistance and resilience is a challenging question. Nevertheless, environmental drivers including rising seawater temperatures and eutrophication compromise the physiological fitness of coral reef organisms, contributing to increased prevalence of diseases globally.

Diseases and disruptions to symbioses as a result of environmental stressors have the potential to cause major impacts on population levels, biodiversity, and community structure of coral reef ecosystems by causing shifts in the abundance of various groups. For example, disease outbreaks in the Caribbean have caused unprecedented widespread changes in reef ecosystems through the loss of key reef organisms and coral cover (Aronson and Precht 2001; Porter et al. 2001; Weil 2004; Weil et al. 2006). In other regions of the world such as the GBR there is evidence that temperature stress is also causing a decline in reef health and coral cover (Willis et al. 2004; Bruno et al. 2007; Sweatman et al. 2011) with an established link between coral disease outbreaks and warm temperature anomalies at sites with high coral cover (Bruno et al. 2007). Increases in SSTs may affect the frequency and severity of disease outbreaks by increasing the prevalence and virulence of pathogens, facilitating invasions of new pathogens, or reducing host resistance and resilience (Sutherland et al. 2004). Harvell and colleagues highlighted that *Vibrio*-associated diseases are increasing worldwide with

increasing temperatures being one of the driving factors (Harvell et al. 2002). For example, a recent study correlated the long-term effects of ocean warming on marine prokaryotic communities and observed an increase in the dominance of members of the *Vibrio* genus, in particular *Vibrio cholera*, within the plankton-associated community of the North Sea (Vezzulli et al. 2012). Climate-linked mass mortality events of benthic invertebrates in the temperate Northwestern (NW) Mediterranean Sea have also been reported (Cerrano et al. 2000; Linares et al. 2008; Garrabou et al. 2009) and *Vibrio* infections have been identified as triggering some of these disease outbreaks (Vezzuli et al. 2010, 2012). In the coral *Paramuricea clavata*, an elevated abundance of *Vibrio* was observed on diseased colonies and inoculation of *Vibrio coralliilyticus* onto healthy colonies reproduced disease symptoms in laboratory infection experiments.

Considerable research effort has focused on the identification of the microbial agent(s) responsible for widespread coral disease outbreaks. Although there have been some successes, the complexity of the coral holobiont, a limited understanding of host physiology and the lack of systematic deductive approaches to disease investigations means that many uncertainties remain (Bourne et al. 2009). Poor definitive disease descriptions contribute to confusion in coral disease studies and difficulties in identifying causative agents (Work et al. 2008). Current disease descriptions are based on field observations and documenting disease signs *in situ*, which represent macroscopic characteristics of lesions that overlook the host response to disease at the cellular level. Unfortunately, corals display few macroscopic signs indicative of stress and therefore many diseases, including environmental stress, predation, and infectious disease, often manifest with similar visible signs (Bruckner and Bruckner 2002; Patterson et al. 2002; Work et al. 2008). Comprehensive case definitions systematically characterizing diseases at the gross, microscopic, immunological, and microbial level are therefore urgently required (Work and Aeby 2006). In addition, development of rapid and robust coral disease diagnostic methods that underscore disease comparison across regional scales is required (Pollock et al. 2011). Definitive identification of microbial agents linked to the underlying disease etiologies will be extremely useful for potential management and mitigation strategies of disease outbreaks.

The exact number of different coral diseases is unknown but studies estimate 18 to 29 different syndromes (Sutherland et al. 2004; Weil 2004) (see *Fig. 10.6a–e*, e.g., of coral diseases). Of these classified diseases, approximately eight pathogens have been identified as the causative agents for the onset of lesions and half of these microorganisms belong to the *Vibrionaceae* family (reviewed in Rosenberg et al. 2007b). Identification of vibrios as disease agents is consistent with their recognized role as marine halophiles commonly associated with diseases of humans and marine animals including bivalves (Sugumar et al. 1998; Garnier et al. 2007) and crustacea (Lavilla-Pitogo et al. 1998; Bourne et al. 2007; Gómez et al. 2009; Shields 2011).

Vibrio shiloi was identified as the causative agent of bleaching in the invasive Mediterranean coral *Oculina patagonica* (Ben-Haim et al. 1999; Banin et al. 2000). Detailed experimental work identified many aspects of the infection process (reviewed in Rosenberg et al. 2007b) and provided the first detailed analysis of the cellular processes leading to disease lesions in corals. However, the complexity of the coral holobiont and the shifting baseline of pathogen-host interactions is highlighted by the fact that this bacterium is no longer found in association with bleached coral (Reshef et al. 2006). Similarly, the aspergillosis epizootic affecting Caribbean gorgonians has waned since a peak between 1994 and 1997 (Kim and Harvell 2004) and the causative fungus *Aspergillus sydowii* is now found associated with healthy corals (Toledo-Hernandez et al. 2008). These results further highlight the potential for host adaptive capacity (Reshef et al. 2006; Rosenberg et al. 2007b). Though *Vibrio shiloi* represented the first model system for coral-pathogen interactions, recent investigations have focused on *Vibrio coralliilyticus* infections of corals. *Vibrio coralliilyticus* was first isolated from bleaching and diseased *Pocillopora damicornis* in the eastern Indo Pacific (Ben-Haim et al. 2003b) and has since been isolated from numerous diseased corals from geographically separated areas of the Indian, Pacific, and Atlantic oceans, Red Sea, Caribbean Sea, and the Mediterranean (Sussman et al. 2008; Pollock et al. 2010; Vezzuli et al. 2010). The genome sequencing of two strains (Santos et al. 2011; Kimes et al. 2012) combined with NMR-based metabolomic (Borouierdi et al. 2009) and antimicrobial investigations (Vizcaino et al. 2010) has improved our fundamental understanding of the physiology and pathogenicity of *Vibrio coralliilyticus*. The virulence of the organism is linked to expression of toxic proteases (VcpA) that cause the breakdown of coral tissue (Ben-Haim et al. 2003a; Sussman et al. 2009; Santos et al. 2011). Though a number of other classic bacterial virulence factors including those involved in motility, host degradation, secretion, antimicrobial resistance, and transcriptional regulation have been identified in *V. coralliilyticus* and shown to be upregulated at higher temperatures (Kimes et al. 2012). The response of the coral host *Pocillopora damicornis* to infection by *Vibrio coralliilyticus* has been investigated at both the physiological and transcriptomic gene levels (Vidal-Dupiol et al. 2011). Interestingly, during these studies, invasion of coral tissue by the pathogen only occurred at higher seawater temperatures, consistent with expression of bacterial virulence factors at these higher temperatures. Infection also elicited an immune response in the host coral with several targeted candidate immune genes showing a distinct response at a transcriptomic expression level (Vidal-Dupiol et al. 2011).

The casual link between human activities and an identified coral pathogen has recently been highlighted by investigation of white pox (acroporid serratiosis) infection of *Acropora palmata* in the Caribbean (Patterson et al. 2002; Sutherland et al. 2010, 2011). These studies highlight that a human commensal pathogen (*Serratia marcescens*) enters the marine environment through sewage contamination and causes coral disease. The pathogenic strain was isolated from human wastewater, diseased corals, and corallivorous snails which could potentially be acting as the reservoir and transmission vector of the disease. The *Serratia marcescens* strain was taxonomically characterized and confirmed to originate from human waste. The pathogen was

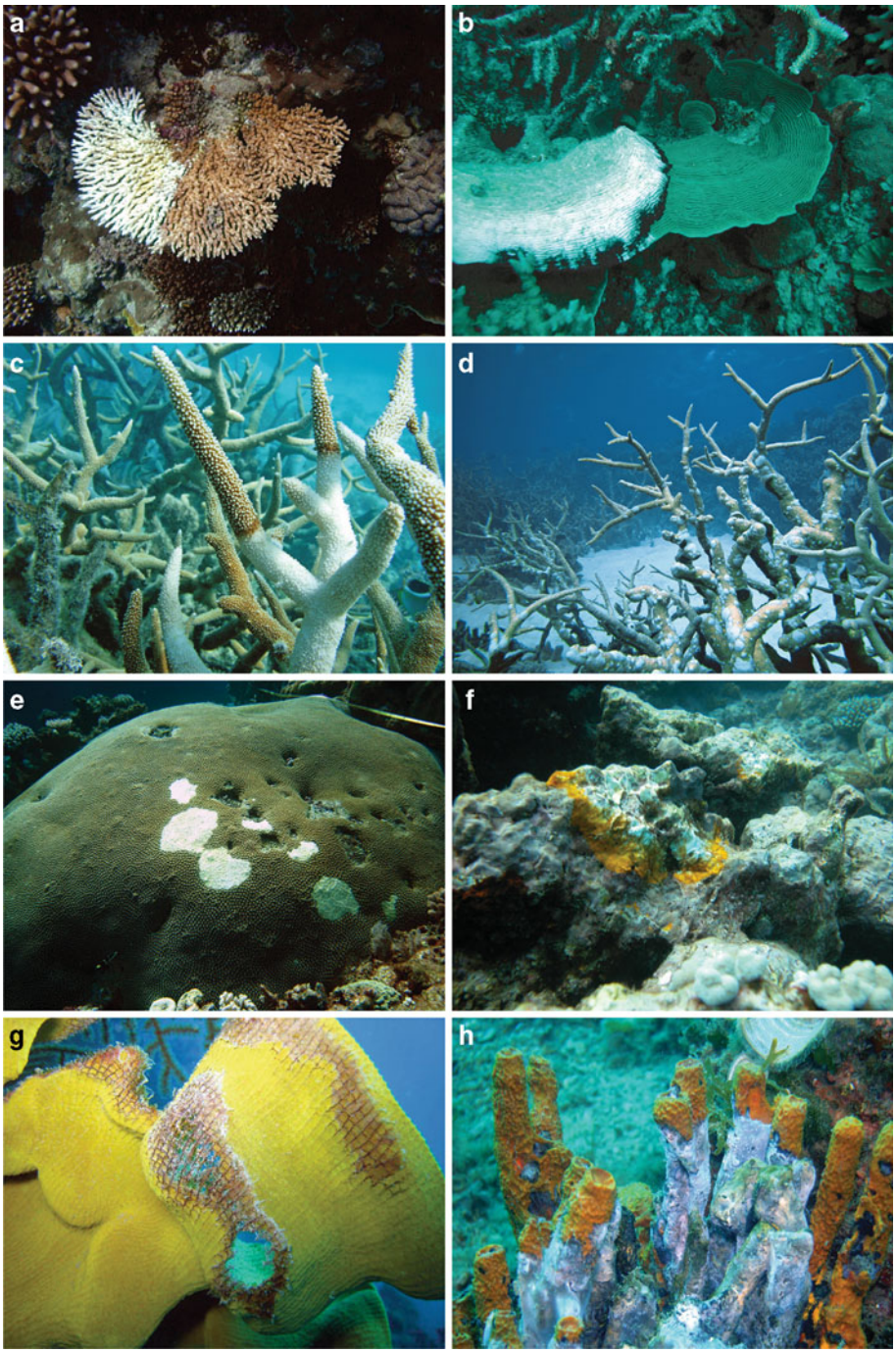


Fig. 10.6

Field appearance of syndromes and disease affecting coral reef organisms. (a) White syndrome on *Acropora* sp. from the GBR (Photograph; AIMS LTMP). (b) Black Band disease illustrating the complex polymicrobial black lesion that migrates across the coral killing the underlying tissue (Photograph; AIMS LTMP) (c) Brown band disease on branching *Acropora* sp. from the GBR (Photograph; AIMS LTMP) (d) Coral Tumors on branching *Acropora* sp. from the GBR (Photograph; AIMS LTMP). The cause of this disease is unknown though possibly involves a viral infection. (e) Ulcerative white spots on *Porites sp*. coral colony. (f) Coralline Lethal Orange Disease (CLOD) on crustose coralline algae (Photograph; AIMS LTMP). (g) Diseased GBR sponge *Ianthella basta* (Photograph; Heidi Luter AIMS). (h) Diseased Slovenian sponge *Aplysina aerophoba* (Photograph; Joana Xavier)

able to cause the symptoms of white pox disease in infection assays, satisfying Koch's postulates for disease causation (Sutherland et al. 2010, 2011).

Other studies have examined microbial community differences between healthy and diseased corals to understand potential factors which may trigger disease outbreaks (Cooney et al. 2002; Frias-Lopez et al. 2002; Sunagawa et al. 2009). Investigations on intra-colonial variation in the associated bacterial community of diseased and healthy tissue on a single coral colony have demonstrated that in some cases colony lesions cause a "whole coral" shift in the microbiota (Pantos et al. 2003; Barneah et al. 2007). Significant changes in microbial functional genes were detected when healthy *Montastraea faveolata* individuals and yellow band disease infected colonies were compared (Kimes et al. 2010). The changes in microbial metabolism profiles were assessed using a functional gene array (GeoChip 2.0) and indicated distinct responses for the surface mucus and tissue communities in the diseased state, in particular nutrient cycling genes changed in the microbial community inhabiting the tissue. High-density 16S rRNA gene microarrays and clone libraries detected an increase in bacterial diversity and distinct community shifts for healthy compared to White-Plague type II disease affected *Montastraea faveolata* colonies (Sunagawa et al. 2009). Previously identified marine pathogens from the *Alteromonadaceae*, *Rhodobacteraceae*, *Campylobacteraceae*, and *Vibrionaceae* families increased in the diseased coral samples, though the putative primary pathogen for this disease, *Aurantimonas corallicida*, was not detected in any sample. A meta-analysis comparing coral-associated microbial communities using 16S rRNA gene sequence data retrieved from 32 studies, identified patterns of unique microbial taxa associated with healthy, bleached, and diseased coral samples (Mouchka et al. 2010). *Proteobacteria* groups dominated all sample types, though bleached corals displayed higher proportions of *Vibrio* and *Acidobacteria* sequences. The dominant taxa in diseased corals included *Rhodobacter*, *Clostridia*, *Cyanobacteria*, and fewer *Oceanospiirillum* sequences compared to healthy coral samples. Though direct comparisons of bacterial communities associated with healthy and diseased coral colonies can provide insight into community dynamics, shifts and changes in the diseased bacterial community are often due to secondary colonization as the disease progresses. This is further promoted by the elevated organic nutrients associated with decaying tissue which can stimulate the rapid growth of opportunistic species. There is an obvious requirement for definitive case descriptions of each coral disease outbreak to elucidate biotic and abiotic causative agents (Work et al. 2008; Bourne et al. 2009).

The difficulty in studying coral diseases is exemplified by the long history of work on black band disease (BBD) which was first identified in 1973 (Antonius 1973) but despite 40 years of subsequent research still represents an enigma. This global disease infects a multitude of coral species and has large impacts on coral populations especially since the band can move up to 2 cm per day often killing entire coral colonies in a matter of days to weeks (Kuta and Richardson 1997). The black band lesion comprises a dark pigmented polymicrobial mat dominated by phototrophic cyanobacteria, along with sulfate-reducing and oxidizing bacteria, marine fungi, and heterotrophic bacteria (*Fig. 10.6b*). The lesion migrates over live coral colonies causing necrosis of the underlying coral tissue (reviewed in Richardson 2004). Numerous studies have characterized this complex microbial community and proposed various microbial groups that may be important in disease virulence (Richardson 1997; Cooney et al. 2002; Frias-Lopez et al. 2004; Viehman et al. 2006; Sato et al. 2010). However, due to the anoxic and sulfide-rich environment in the lower parts of the microbial mat (Carlton and Richardson 1995; Richardson 1997) it appears that the biogeochemical conditions within the lesion (rather than a specific pathogen) are the major cause for the derived coral mortality associated with this disease (Glas et al. 2012).

Diseases of Other Reef Organisms

In recent years reports of sponge disease have increased dramatically with sponge populations throughout the Mediterranean and Caribbean particularly affected and an increased prevalence of disease also recorded for sponges in Papua New Guinea, the Great Barrier Reef, and Cozumel, Mexico (reviewed in Webster 2007). As with corals, these disease events often occur in unrelated species, span broad geographic ranges, and sponges present with a wide array of physiological symptoms (Gochfeld et al. 2007; Wulff 2007; Webster et al. 2008b; Luter et al. 2010a, b; Maldonado et al. 2010). These epidemics can have severe impacts on the survival of sponge populations, the ecology of the reef, and the fate of any associated biota. For example, a severe epidemic in the Caribbean caused the loss of 70–95% of sponge specimens (Galstoff 1942), an epidemic in the Mediterranean commercial sponge fishery reduced the annual output by over 90% (Vacelet 1994) and 60% of the commercial sponges in the Ligurian Sea succumbed to disease (Gaino et al. 1992). While numerous reviews have highlighted the increasing incidence of sponge disease and emphasized the importance of identifying sponge pathogens (Webster 2007; Webster and Blackall 2009), very few studies have comparatively examined the microbial communities of diseased and healthy sponges.

To date, only a single sponge pathogen has been confirmed and this was a novel *Alphaproteobacteria* strain which affected the GBR sponge *R. odorabile* causing extensive necrosis (Webster et al. 2002). This sponge pathogen produces a collagenase enzyme which degrades the sponge skeletal fibers (Mukherjee et al. 2009). Throughout 2008–2009 a disease epidemic severely affected populations of *Ircinia fasciculata* in the Western Mediterranean and African coasts (Maldonado et al. 2010). The symptoms included small pustules on the sponge surface which converged to form large necrotic lesions. TEM indicated that a bacterium with twisted rod morphology was responsible for the infection. The infection proceeded from the outside to the inner body and sponges reacted by laying down concentric barriers of collagen, reminiscent of a primitive immune response. This protective mechanism was often sufficient to

stop the spread of the disease. Aplysina red band syndrome (ARBS) is another disease that affects *Aplysina* sponges throughout the Caribbean (Olson et al. 2006; Gochfeld et al. 2007). Symptoms include a rust-coloured leading edge often followed by a trail area of necrotic tissue (Olson et al. 2006). Microscopic examination of the red band indicated that a cyanobacterium was consistently responsible for the coloration, although its role in disease causation is still not known. A second "black patch" disease was reported to affect over 40% of the *Aplysina aerophoba* population in Slovenia (Webster et al. 2008b) (*Fig. 10.6h*). Microbial characterization in diseased and healthy sponges using DGGE detected multiple sequences that were exclusively present in diseased *A. aerophoba*. Most notably, a *Deltaproteobacteria* with similarity to a strain implicated in coral black band disease was detected in all sponge lesions but absent from healthy tissue (Webster et al. 2008b).

Other unverified disease agents which have been reported for sponges include fungi, viruses, cyanobacteria, and bacterial strains within the *Bacillus* and *Pseudomonas* genera (reviewed in Webster 2007). Identifying the causative agents of disease is problematic in sponges due to the presence of diverse assemblages of Bacteria, Archaea, and other Eukaryotes such as algae and Fungi. This is further complicated by the fact that, in contrast to corals, sponge skeletons rapidly degrade after mortality, leaving behind no telltale sign that a disease event has even occurred. As with corals, microbiological investigations in sponges often detect a shift in the symbiotic bacterial community in diseased individuals but are unable to ascribe this shift to a specific pathogen (Bourne 2005; Cervino et al. 2006; Olson et al. 2006; Sussman et al. 2008; Webster et al. 2008b; Angermeier et al. 2011). In general, diseased sponges and corals tend to host a higher abundance of *Bacteroidetes*, *Epsilon-*, and *Deltaproteobacteria* than do their healthy counterparts (Frias-Lopez et al. 2002; Pantos and Bythell 2006; Webster et al. 2008b). Disease outbreaks in the sponge *I. basta* have recently occurred on reefs throughout the GBR, Torres Strait, and Papua New Guinea (Cervino et al. 2006; Luter et al. 2010a, b) (*Fig. 10.6g*). Comprehensive comparisons of diseased and healthy individuals by bacterial cultivation, histology, TEM, and molecular analysis of 16S rRNA genes failed to reveal any potential pathogens. In addition, the diseased state could not be transmitted to healthy individuals even during direct-contact infection/damage assays (Luter et al. 2010b). Similarly, the common Caribbean barrel sponge *Xestospongia muta*, whose population is actually increasing with declining coral cover (McMurray et al. 2010), is affected by a disease known as sponge orange band syndrome (SOB) which occurs in conjunction with fatal bleaching in this species (Cowart et al. 2006). Scanning and TEM revealed that SOB is accompanied by massive destruction of the pinacoderm and DGGE with cyanobacteria-specific primers indicated a shift in the cyanobacterial community away from the *Synechococcus*/*Prochlorococcus* clade of sponge-specific symbionts toward nonspecific cyanobacteria reflecting the community of the ambient environment and including lineages such as *Leptolyngbya* which have been associated with diseased corals (Angermeier et al. 2011). To date, no putative pathogens have been described for SOB and, as with *I. basta*, infection assays with healthy *X. muta* were unable to transmit the disease. These studies suggest that microorganisms may not be responsible for the disease-like symptoms in *I. basta* and *X. muta* and this may also be the case for many of these other disease-like mortality events in sponges.

There is some evidence for correlations between sponge disease and environmental factors such as elevated temperatures and urban / agricultural runoff. Mass sponge mortalities have occurred during abnormally high seawater temperatures (Vicente 1989; Cerrano et al. 2000), including a recent die off that affected 80–100% of the *I. fasciculata* populations in the western Mediterranean (Cebrian et al. 2011). Whether these mortality events involve pathogenic agents, cause a breakdown in important microbial symbioses, or relate solely to exceeding the sponge's physiological thresholds is a critical question for future research. Recently, coral disease-associated microbes were reported from healthy specimens of *Agelas tubulata* and *Amphimedon compressa* on Florida reefs (Negandhi et al. 2010) and the sea fan pathogen *Aspergillus sydowii* was isolated from healthy *Spongia obscura* in the Bahamas (Ein-Gil et al. 2009). These findings indicate that sponges may act as a reservoir for potential coral pathogens.

Calcareous coralline algae (CCA) are important reef builders as they deposit layers of calcium carbonate and are the primary source of chemical morphogens reported for coral larvae (Morse et al. 1996; Heyward and Negri 1999). Coralline lethal orange disease (CLOD) is the most common infection of CCA throughout the South Pacific (Littler and Littler 1995; Aeby et al. 2008) with symptoms including a bright orange band with slimy, stringy material spreading across the algal surface, leaving behind bare algal skeleton which is rapidly colonized by turf and filamentous algae (*Fig. 10.6f*). While a specific etiological agent has not been determined for CLOD, a consortium of *Planococcus*, *Bacillus*, and *Pseudomonas* species is consistently associated with the condition (Cervino et al. 2005). CCA are also affected by a range of other syndromes including coralline fungal disease, coralline white band syndrome, coralline target syndrome, and coralline cyanophyte disease (Littler and Littler 1998; Vargas-Angel 2010). Because of the importance of CCA to reef building, consolidation and recruitment, the loss of CCA to disease has the potential to significantly impact the health and ecology of coral reefs and their ability to recover from disturbance.

Periodic epizootics of other coral reef organisms have also been recorded in recent decades. For example, a severe disease epidemic resulted in the mass mortality of *Diadema antillarum* (the long-spined black sea urchin) populations in the Caribbean between 1983 and 1984 (Lessios et al. 1984; Lessios 1998). This widespread mortality event represented the most extensive disease outbreak of a marine animal recorded to date with populations reduced by 97% and no significant recovery of the urchin observed (Hughes 1994; Lessios 1998). No pathogen was identified as the causative agent in this disease outbreak, though the collapse of the urchin population was credited with the accelerated degradation of coral reefs throughout the Caribbean,

contributing to algal-dominated reef ecosystems due to the reduction of this important herbivore (Hughes 1994; Lessios et al. 2001).

Future Directions and Conclusions

Studying the function and diversity of prokaryotic communities in coral reefs is a challenging endeavor; however, the combination of existing approaches with developing techniques such as high-throughput sequencing and imaging analyses will provide a wealth of knowledge for coral reef microbiology in the next decade. One challenge will be integrating the emerging and overwhelming quantity of "omics" data (metagenomics, metatranscriptomics, and metaproteomics) to better understand functional microbial processes within reef holobionts and linking this data with patterns of microbial diversity.

While metagenome sequence data provides valuable information about genes with the potential for being expressed, it cannot determine which genes are functional. In addition, since total DNA is sequenced, it is not possible to distinguish between actively growing cells and dormant or dead cells. Restricting analysis to the active community members is now possible by extracting only DNA that has incorporated stable isotopes or bromodeoxyuridine during replication. Using combined transcriptomic and metabolomic approaches, expressed genes can be identified and their activities interpreted. However, one unique challenge for coral studies is the complex symbioses which require the separation of coral, *Symbiodinium*, and prokaryote DNA/RNA/protein fractions. This is not easily achieved with approaches such as metagenomics since mitochondrial DNA often dominates the DNA dataset (Vega Thurber et al. 2008; Littman et al. 2011), highlighting the critical need for development and optimization of coral-specific "omics" protocols.

Direct visualization of individual microorganisms and measurement of their microbial activity within niche habitats is becoming a reality and can be applied to coral reef organisms. Fluorescence *in situ* hybridization (FISH) protocols for corals have been developed and optimized by Ainsworth and coworkers (Ainsworth et al. 2006, 2008; Ainsworth and Hoegh-Guldberg 2009) and can now be combined with techniques such as nano resolution secondary ion mass spectrometry (NanoSIMS) to visualize the microorganisms and map their metabolic activity through uptake and processing of labeled isotope substrates (Lechene et al. 2007). In addition, techniques such as laser microscope dissection (LMD) can remove single cells from complex samples and can be combined with single-cell genome sequencing to provide a comprehensive view of which species are present, their metabolic potential, cell quantification, and metabolic activity. Such advanced techniques must also link to established scientific investigations to combine ecological, histological, and microbiological studies. The application of "omic" and advanced imaging approaches to coral microhabitats will enable us to map diversity and function, thereby elucidating the role these prokaryotic communities have in the fitness of the coral holobiont. These approaches will also be applied to studies of reef diseases and environmental stress. Ultimately, combining these approaches will enable a better understanding of which microbes are alive and active, and which enzymes and pathways function in coral reef microbial communities under different conditions. Then, we can begin to truly comprehend coral reef microbial communities from the microscopic to the global scale.

References

Acinas SG, Anton J, Rodriguez-Valera F (1999) Diversity of free-living and attached bacteria in offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl Environ Microbiol 65:514–522

Aeby G, Work T, Fenner D, Didonato E (2008) Coral and crustose coralline algae disease on the reefs of American Samoa. In: Proceedings of the 11th international coral reef symposium, Ft. Lauderdale, FL

Agostini S, Suzuki Y, Casareto BE, Nakano Y, Hidaka M, Badrun N (2009) Coral symbiotic complex: hypothesis through vitamin B12 for a new evaluation Galaxea. J Coral Reef Stud 11:1–11

Agostini S, Suzuki Y, Higuchi T, Casareto B, Yoshinaga K, Nakano Y, Fujimura H (2011) Biological and chemical characteristics of the coral gastric cavity. Coral Reefs 31:1–10

Ainsworth TD, Hoegh-Guldberg O (2009) Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. Aquat Biol 4:289–296

Ainsworth TD, Fine M, Blackall LL, Hoegh-Guldberg O (2006) Fluorescence *in situ* hybridization and spectral imaging of coral-associated bacterial communities. Appl Environ Microbiol 72:3016–3020

Ainsworth TD, Hoegh-Guldberg O, Leggat W (2008) Imaging the fluorescence of marine invertebrates and their associated flora. J Micros-Oxf 232:197–199

Angermeier H, Kamke J, Abdelmohsen UR, Krohne G, Pawlik JR, Lindquist NL, Hentschel U (2011) The pathology of sponge orange band disease affecting the Caribbean barrel sponge *Xestospongia muta*. FEMS Microbiol Ecol 75:218–230

Anthony KRN, Maynard JA, Diaz-Pulido D, Mumby PJ, Marshall PA, Cao L, Hoegh-Guldberg O (2011) Ocean acidification and warming will lower coral reef resilience. Glob Chang Biol 17:1798–1808

Antonius A (1973) New observations on coral destruction in reefs. In: 10th Meeting of the Association of Island Marine Laboratories of the Caribbean. University of Puerto Rico: Association of Island Marine Laboratories of the Caribbean, p. 3

Apprill A, Rappe MS (2011) Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii USA. Aquat Microb Ecol 62:251–266

Aronson R, Precht WF (2001) White-band diseases and the changing face of Caribbean coral reefs. Hydrobiologia 460:25–38

Ayukai T (1992) Picoplankton dynamics in Davies reef lagoon, the great barrier reef, Australia. J Plankton Res 14:1593–1606

Ayukai T (1995) Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. Coral Reefs 14:141–147

Azam F, Malfatti F (2007) Microbial structuring of marine ecosystems. Nat Rev Microbiol 5:782–791

Bally M, Garrabou J (2007) Thermodependent bacterial pathogens and mass mortalities in temperature benthic communities: a new case of emerging disease linked to climate change. Glob Chang Biol 13:2078–2088

Banin E, Israely T, Kushmaro A, Loya Y, Orr E, Rosenberg E (2000) Penetration of the coral-bleaching bacterium *Vibrio shiloi* into *Oculina patagonica*. Appl Environ Microbiol 66:3031–3036

Barneah O, Ben-Dov E, Kramarsky-Winter E, Kushmaro A (2007) Characterization of black band disease in Red Sea stony corals. Environ Microbiol 9:1995–2006

Barnes RD (1987) Invertebrate zoology. Harcourt Brace Jovanovich College Publishers, Fort Worth

Barnes RSK, Hughes RN (1999) An introduction to marine ecology. Blackwell Science, Oxford, UK

Barott KL, Rodriguez-Brito B, Janouškovec J, Marhaver KL, Smith JE, Keeling P, Rohwer FL (2011) Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. Environ Microbiol Rep 13:1192–1204

Bayer K, Schmitt S, Hentschel U (2008) Physiology, phylogeny and in situ evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. Environ Microbiol 10:2942–2955

Bell PRF, Elmetri I, Uwins P (1999) Nitrogen fixation by *Trichodesmium* spp. In the Central and Northern Great barrier reef lagoon: relative importance of the fixed-nitrogen load. Mar Ecol Prog Ser 186:119–126

Ben-Haim Y, Banim E, Kushmaro A, Loya Y, Rosenberg E (1999) Inhibition of photosynthesis and bleaching of zooxanthellae by the coral pathogen *Vibrio shiloi*. Environ Microbiol 1:223–229

Ben-Haim Y, Zicherman-Keren M, Rosenberg E (2003a) Temperature-regulated bleaching and lysis of the coral *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. Appl Environ Microbiol 69:4236–4242

Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, Rosenberg E (2003b) *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. Int J Syst Evol Microbiol 53:309–315

Berkelmans R, De'ath G, Kininmonth S, Skirving WJ (2004) A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns, and predictions. Coral Reefs 23:74–83

Borouierdi AFB, Vizcaino MI, Meyers A, Pollock EC, Huynh SL, Schock TB et al (2009) NMR-based microbial metabolomics and the temperature-dependent coral pathogen *Vibrio coralliilyticus*. Environ Sci Technol 43:7658–7664

Bourne DG (2005) Microbiological assessment of a disease outbreak on corals from Magnetic Island (Great Barrier Reef, Australia). Coral Reefs 24:304–312

Bourne DG, Munn CB (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. Environ Microbiol 7:1162–1174

Bourne DG, Høj L, Webster NS, Payne M, Skindersøe M, Givskov M, Hall MA (2007) Aspects of the microbiology of phyllosoma rearing of the ornate rock lobster *Panulirus ornatus*. Aquaculture 268:274–287

Bourne DG, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral associated microbial communities during a bleaching event. ISME J 2:350–363

Bourne DG, Garren M, Work TM, Rosenberg E, Smith G, Harvell CD (2009) Microbial disease and the coral holobiont. Trends Microbiol 17:554–562

Broadbent AD, Jones GB (2004) DMS and DMSP in mucus ropes, coral mucus, surface films and sediment pore waters from coral reefs in the Great Barrier Reef. Mar Freshw Res 55:849–855

Brodie J, Mitchell A (2005) Nutrients in Australian tropical rivers changes with agricultural development and implications for receiving environments. Mar Freshw Res 56:279–302

Brodie J, De'ath G, Devlin M, Furnas M, Wright M (2007) Spatial and temporal patterns of near-surface chlorophyll a in the Great Barrier Reef lagoon. Mar Freshw Res 58:342–353

Bruckner AW, Bruckner RJ (2002) Coral predation by *Sparisima viride* and lack of relationship with coral disease. Bali 2002:1245–1249

Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, Harvell CD et al (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5: 1220–1227

Burnett WJ, McKenzie JD (1997) Subcuticular bacteria from the brittle star *Ophiactis balli* (Echinodermata: Ophiuroidea) represent a new lineage of extracellular marine symbionts in the alpha subdivision of the class Proteobacteria. Appl Environ Microbiol 63:1721–1724

Campbell L, Nolla HA, Vaulot D (1994) The importance of Prochlorococcus to community structure in the central North Pacific Ocean. Limnol Oceanogr 39:954–961

Capone DG (1996) Coral reef ecosystems in the context of the marine nitrogen cycle. In: Bjork M, Semesi AK, Pedersen M, Bergman B (eds) Current trends in marine botanical research in the East African region. Ord & Vetande, Uppsala, pp 61–76

Carlton RG, Richardson LL (1995) Oxygen and sulfide dynamics in a horizontally migrating cyanobacterial mat: Black band disease of corals. FEMS Microbiol Ecol 18:155–162

Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A et al (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560–563

Casareto BE, Charpy L, Blanchot J, Suzuki Y, Kurasawa K, Ishikawa Y (2006) Phototrophic prokaryotes in Bora Bay, Miyako Island, Okinawa, Japan. In: Proceedings of 10th international coral reef symposium, Okinawa, Japan, vol 1, pp 844–853

Casareto BE, Charpy L, Langlade MJ, Suzuki T, Ohba H, Niraula M, Suzuki Y. (2008) Nitrogen fixation in coral reef environments. In: Proceedings of the 11th international coral reef symposium, Fort Lauderdale, FL, vol 2, pp 896–900

Castillo I, Lodeiros C, Nunez M, Campos I (2001) In vitro evaluation of antibacterial substances produced by bacteria isolated from different marine organisms. Rev Biol Trop 49:1213–1222

Cebrian E, Uriz MJ, Garrabou J, Ballesteros E (2011) Sponge mass mortalities in a warming Mediterranean Sea: are cyanobacteria-harboring species worse off? PLoS One 6:e20211

Ceh J, van Keulen M, Bourne DG (2011) Coral-associated bacterial communities on Ningaloo Reef, Western Australia. FEMS Microbiol Ecol 75:134–144

Cerrano C, Bavestrello G, Bianchi CN, Cattaneovietti R, Bava S, Morganti C et al (2000) A catstrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-Western Mediterranean), summer 1999. Ecol Lett 3:284–293

Cervino JM, Littler MM, Littler DS, Polson S, Goreau TJ, Brooks B, Smith GW (2005) Identification of microbes associated with coralline lethal algal disease and its relationship to glacial ice melt (global warming). Phytopathology 95:120–121

Cervino JM, Winiarski-Cervino K, Polson SW, Goreau T, Smith GW (2006) Identification of bacteria associated with a disease affecting the marine sponge *Ianthella basta* in New Britain, Papua New Guinea. Mar Ecol Prog Ser 324:139–150

Charpy L (2005) Importance of photosynthetic picoplankton in coral reef ecosystems. Life Environ 55:217–223

Charpy L, Blanchot J (1998) Photosythetic picoplankton in French Polynesian atol lagoon: estimation of taxacontribution to biomass and production by flow cytometry. Mar Ecol Prog Ser 162:57–70

Charpy L, Blanchot J (1999) Picophytoplankton biomass, community structure and productivity in the Great Astrolabe Lagoon, Fiji. Coral Reefs 18:255–262

Chen C-P, Tseng C-H, Chen CA, Tang S-L (2011) The dynamics of microbial partnerships in the coral *Isopora palifera*. ISME J 5:728–740

Coles SL, Strathmann R (1973) Observations on coral mucus "flocs" and their potential trophic significance. Limnol Oceanogr 18:673–678

Coma R, Ribes M, Gili J-M, Hughes RN (2001) The ultimate opportunists: consumers of seston. Mar Ecol Prog Ser 219:305–308

Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302–1310

Cooney RP, Pantos O, Le Tissier MD, Barer MR, O'Donnell AG, Bythell JC (2002) Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. Environ Microbiol 4:401–413

Corredor JE, Wilkinson CR, Vicente VP, Morell JM, Otero E (1988) Nitrate release by Caribbean reef sponges. Limnol Oceanogr 33:114–120

Cowart JD, Henkel TP, McMurray SE, Pawlik JR (2006) Sponge orange band (SOB): a pathogenic-like condition of the giant barrel sponge *Xestospongia muta*. Coral Reefs 25:513

Crosbie ND, Furnas MJ (2001) Abundance distribution and flow-cytometric characaterization of picophytoprokaryote populations in central (17 degrees S) and southern (20 degrees S) shelf waters of the Great Barrier Reef. J Plankton Res 23:809–828

Cullimore J, Dénarié J (2003) How legumes select their sweet talking symbionts. Science 302:575

Curson ARJ, Sullivan MJ, Todd JD, Johnston AWB (2011) DddY, a periplasmic dimethysulfoniopropionate lyase found in taxonomically diverse species of Proteobacteria. ISME J 5:1191–1200

Daly M, Fautin DG, Cappola VA (2003) Systematics of the Hexacorallia (Cnidaria: Anthozoa). Zoo J Linn Soc 139:419–437

Daniels CA, Zeifman A, Heym K, Ritchie KB, Watson CA, Berzins I, Breitbart M (2011) Spatial heterogeneity of bacterial communities in the mucus of *Montastraea annularis*. Mar Ecol Prog Ser 426:29–40

De Goeij JM, van Duyl FC (2007) Coral cavities are sinks of dissolved organic matter. Limnol Oceanogr 52:2608–2617

D'Elia CF, Wiebe WJ (1990) Biogeochemical nutrient cycles in coral reef ecosystems. In: Dubinsky Z (ed) Coral reefs. Elsevier, Amsterdam, pp 49–74

Diaz MC, Akob D, Cary CS (2004) Denaturing gradient gel electrophoresis of nitrifying microbes associated with tropical sponges. Boll Mus Ist Biol Univ Genova 68:279–289

Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM et al (2008) Functional metagenomic profiling of nine biomes. Nature 452: U628–U629

Dubilier N, Amann R, Erséus C, Muyzer G, Park S, Giere O, Cavanaugh CM (1999) Phylogenetic diversity of bacterial endosymbionts in the gutless marine oligochete *Olavius loisae* (Annelida). Mar Ecol Prog Ser 178:271–280

Ducklow HW, Mitchell R (1979) Bacterial populations and adaptations in the mucus layers on living corals. Limnol Oceanogr 24:715–725

Ein-Gil N, Ilan M, Carmeli S, Smith GW, Pawlik JR, Yarden O (2009) Presence of *Aspergillus sydowii*, a pathogen of gorgonian sea fans in the marine sponge *Spongia obscura*. ISME J 3:752–755

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar Pollut Bull 50:125–146

Falkowski PG, Dubinsky Z, Muscatine L, McCloskey LR (1993) Population control in symbiotic corals. Bioscience 43:453–464

Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. Science 320:1034–1039

Ferrier-Pages C, Furla P (2001) Pico- and nanoplankton biomass and production in the two largest atoll lagoons of French Polynesia. Mar Ecol Prog Ser 211:63–76

Fieseler L, Hentschel U, Grozdanov L, Schirmer A, Wen G, Platzer M et al (2007) Widespread occurrence and genomic context of unusually small polyketide synthase genes in microbial consortia associated with marine sponges. Appl Environ Microbiol 73:2144–2155

Fisher HM (1994) Genetic regulation of nitrogen fixation in rhizobia. Microbiol Rev 58:352–386

Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. Appl Environ Microbiol 68:2214–2228

Frias-Lopez J, Klaus JS, Bonheyo GT, Fouke BW (2004) Bacterial community associated with black band disease in corals. Appl Environ Microbiol 70:5955–5962

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S (2006) Annually reoccuring bacterial communities are predicatable from ocean conditions. Proc Natl Acad Sci USA 103:13104–13109

Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown BE, Green JL, Brown JH (2008) A latitudinal diversity gradient in planktonic marine bacteria. Proc Natl Acad Sci USA 105:7774–7778

Gaidos E, Rusch A, Ilardo M (2011) Ribosomal tag pyrosequencing of DNA and RNA from benthic coral reef microbiota: community spatial structure, rare members and nitrogen-cycling guilds. Environ Microbiol 13:1138–1152

Gaino E, Pronzato R, Corriero G, Buffa P (1992) Mortality of commercial sponges: incidence in two Mediterranean areas. Bolletino di Zoologia 59:79–85

Galstoff PS (1942) Wasting disease causing mortality of sponges in the West Indies and Gulf of Mexico. In: Proceedings of the VIII American Science Congress, Washington, DC, pp 411–421

Garnier M, Labreuche Y, Garcia C, Robert M, Nicolas JL (2007) Evidence for the involvement of pathogenic bacteria in summer mortalities of the pacific oyster *Crassostrea gigas*. Microbial Ecology 53:187–196

Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonne P, Cigliano M et al (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Glob Chang Biol 15:1090–1103

Garren M, Azam F (2010) New method for counting bacteria associated with coral mucus. Appl Environ Microbiol 76:6128–6133

Garren M, Azam F (2012) New directions in coral reef microbial ecology. Environ Microbiol 14:833–844

Garren M, Raymundo L, Guest J, Harvell CD, Azam F (2009) Resilience of coral-associated bacterial communities exposed to fish farm effluent. PLoS One 4: e7319

Gast GJ, Jonkers PJ, van Duyl FC, Bak RPM (1999) Bacteria, flagellates and nutrients in island fringing coral reef waters: influence of the ocean, the reef and eutrophication. Bull Mar Sci 65:523–538

Genin A, Monismith SG, Reidenbach MA, Yahel G, Koseffb JR (2009) Intense benthic grazing of phytoplankton in a coral reef. Limnol Oceanogr 54:938–951

Giovannoni SJ, Rappe MS, Vergin KL, Adair NL (1996) 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria. Proc Natl Acad Sci USA 93:7979–7984

Glas MS, Sato Y, Ulstrup KE, Bourne DG (2012) Biogeochemical conditions determine virulence of Black Band Disease in corals. ISME J 6:1526–1534

Gochfeld DG, Aeby GS (2008) Antibacterial chemical defenses in Hawaiian corals provide possible protection from disease. Mar Ecol Prog Ser 362:119–128

Gochfeld DJ, Schlöder C, Thacker RW (2007) Sponge community structure and disease prevalence on coral reefs in Bocas del Toro, Panama. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro, pp 335–342

Gómez AC, Bourne DG, Hall M, Owens L, Høj L (2009) Molecular diagnosis of *Vibrio harveyi* in aquaculture: potential gene targets for real-time PCR detection. Aquaculture 287:1–10

Haapkyla J, Seymour AS, Trebilco J, Smith D (2007) Coral disease prevalence and coral health in the Wakatobi Marine Park, south-east Sulawesi, Indonesia. J Mar Biol Assoc UK 87:403–414

Harder T, Lau SCK, Dobretsov S, Fang TK, Qian P-Y (2003) A distinctive epibiotic bacterial community on the soft coral *Dendronephthya* sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis. FEMS Microbiol Ecol 43:337–347

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ et al (1999) Review: marine ecology - emerging marine diseases - climate links and anthropogenic factors. Science 285:1505–1510

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Ecology - Climate warming and disease risks for terrestrial and marine biota. Science 296:2158–2162

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters FEMS Microbiol Ecol 55:167–177

Hewson I, Fuhrman JA (2006) Spatial and vertical biogeography of coral reef sediment bacterial and diazotroph communities. Mar Ecol Prog Ser 306:79–86

Heyward AJ, Negri AP (1999) Natural inducers for coral larval metamorphosis. Coral Reefs 18:273–279

Hochmuth T, Piel J (2009) Polyketide synthases of bacterial symbionts in sponges – Evolution-based applications in natural products research. Phytochemistry 70:1841–1849

Hoegh-Guldberg O (2004) Coral reefs and projections of future change. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer, Berlin, pp 463–484

Hoegh-Guldberg O (2009) Climate change and coral reefs: Trojan horse or false prophecy? Coral Reefs 28:569–575

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E et al (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Hoffmann F, Larsen O, Theil V, Rapp H-T, Pape T, Michaelis W, Reitner J (2005) An anaerobic world in sponges. Geomicrobiology 22:1–10

Hoffmann F, Radax R, Woebken D, Holtappels M, Lavik G, Rapp HT et al (2009) Complex nitrogen cycling in the sponge *Geodia barretti*. Environ Microbiol 11:2228–2243

Hong M-J, Yu Y-T, Chen CA, Chiang P-W, Tang S-L (2009) Influence of species specificity and other factors on bacteria associated with the coral *Stylophora pistillata* in Taiwan. Appl Environ Microbiol 75:7797–7806

Hooper JNA, Van Soest RWM (2002) Systema Porifera: a guide to the classification of sponges. Kluwer Academic / Plenum Publishers, New York

Hovland M (2008) Deep-water coral reefs: unique Biodiversity hotspots. Praxis Publishing (Springer), Chichester

Hughes TP (1994) Catastrophies, phase shifts and large-scale degradation of a Caribbean coral reef. Science 263:1547–1551

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C et al (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929–933

Jetten MSM (2008) The microbial nitrogen cycle. Environ Microbiol 10:2903–2909

Jimenez E, Ribes M (2007) Sponges as a source of dissolved inorganic nitrogen: nitrification mediated by temperate sponges. Limnol Oceanogr 52:948–958

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. Plant Cell Physiol 21:1219–1230

Kamke J, Taylor MW, Schmitt S (2010) Activity profiles for marine sponge-associated bacteria obtained by 16S rRNA vs 16S rRNA gene comparisons. ISME J 4:498–508

Kelman D, Kashman Y, Rosenberg E, Kushmaro A, Loya Y (2006) Antimicrobial activity of Red Sea corals. Mar Biol 149:357–363

Kim K (1994) Antimicrobial activity in Gorgonian corals (Coelenterata, Octocorallia). Coral Reefs 13:75–80

Kim K, Harvell CD (2004) The rise and fall of a six-year coral-fungal epizootic. Am Nat 164:S52–S63

Kimes NE, Van Nostrand JD, Weil E, Zhou J, Morris PJ (2010) Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. Environ Microbiol 12:541–556

Kimes NE, Grim CJ, Johnson WR, Hasan NA, Tall BD, Kothary MH, et al (2012) Temperature regulation of virulence factors in the pathogen *Vibrio coralliilyticus*. ISME J 6:835–846

Kirkwood M, Todd JD, Rypien KL, Johnston AWB (2010) The opportunistic coral pathogen *Aspergillus sydowii* contains dddP and makes dimethyl sulfide from dimethylsulfoniopropionate. ISME J 4:147–150

Kleypas JA, Langdon CI (2006) Coral reefs and changing seawater chemistry. In: Phinney JT, Hoegh-Guldberg O, Kleypas J, Skirving W, Strong A (eds) Coral reefs and climate change: science and management. American Geophysical Union, Washington, DC, pp 73–110

Kline DI, Kuntz NM, Breitbart M, Knowlton N, Rohwer F (2006) Role of elevated organic carbon levels and microbial activity in coral mortality. Mar Ecol Prog Ser 314:119–125

Klussmann-Kolb A, Brodie GD (1999) Internal storage and production of symbiotic bacteria in the reproductive system of a tropical marine gastropod. Mar Biol 133:443–447

Knowlton N, Rohwer F (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. Am Nat 162:S51–S62

Koh EGL (1997) Do Scleractinian corals engage in chemical warfare against microbes? J Chem Ecol 23:379–398

Koren O, Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral *Oculina patagonica* in summer and winter. Appl Environ Microbiol 72:5254–5259

Krueger DM, Cavanaugh CM (1997) Phylogenetic diversity of bacterial symbionts of *Solemya* hosts based on comparative sequence analysis of 16S rRNA genes. Appl Environ Microbiol 63:91–98

Kuta KG, Richardson LL (1997) Black band disease and the fate of diseased coral colonies in the Florida Keys. In: Proceedings of the 8th international coral reef symposium, Panama, vol 1, pp 575–578

Kvennefors E, Roff G (2009) Evidence of cyanobacteria-like endosymbionts in Acroporid corals from the Great Barrier Reef. Coral Reefs 28:547

Lafferty KD, Porter JW, Ford SE (2004) Are diseases increasing in the ocean? Annu Rev Ecol Evol Syst 35:31–54

Lafi FF, Fuerst JA, Fieseler L, Hentschel U (2009) Widespread distribution of *Poribacteria* in Demospongiae. Appl Environ Microbiol 75:5695–5699

Lalli CM, Parsons TR (1995) Biological oceanography: an introduction. Butterworth-Heinemann Ltd., Oxford, UK

Lavilla-Pitogo CR, Leaño EM, Paner MG (1998) Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent vibrios in the rearing environment. Aquaculture 164:337–349

Lechene CP, Luyten Y, McMahon G, Distel DL (2007) Quantitative imaging of nitrogen fixation by individual bacteria within animal cells. Science 317:1563–1566

Lee OO, Wang Y, Yang J, Lafi FF, Al-Suwailem A, Qian P-Y (2011) Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. ISME J. doi:10.1038/ismej.2010.165

Leggat W, Hoegh-Guldberg O, Dove S, Yellowlees D (2007) Analysis of an EST library from the dinoflagellate (*Symbiodinium* sp.) symbiont of reef-building corals. J Phycol 43:1010–1021

Lema KA, Willis BL, Bourne DG (2012) Coral form specific associations with diazotrophic bacteria. FEMS Microb Ecol (in press)

Lemoine N, Buell N, Hill A, Hill M (2007) Assessing the utility of sponge microbial symbiont communities as models to study global climate change: a case study with *Halichondria bowerbanki*. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Série Livros, Museu Nacional, Rio de Janeiro, pp 419–425

Lesser MP, Blakemore RP (1990) Description of a novel symbiotic bacterium from the brittle star, *Amphipholis squamata*. Appl Environ Microbiol 56:2436–2440

Lesser MP, Walker CW (1992) Comparative study of the uptake of dissolved amino acids in sympatric brittle stars with and without endosymbiotic bacteria. Comp Biochem Physiol 101B:217–223

Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997–1000

Lesser MP, Falcon LI, Rodriguez-Roman A, Enriquez S, Hoegh-Guldberg O, Iglesias- Prieto R (2007) Nitrogen fixation by symbiotic cyanobactieria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. Mar Ecol Prog Ser 347:143–152

Lessios HA (1998) Mass mortality of *Diadema antillarum* in Teh Caribbean: what have we learned? Ann Rev Ecol Syst 19:371–393

Lessios HA, Garrido MJ, Kessing BD (2001) Demographic history of *Diadema antillarum*, a keystone herbivore on Caribbean reefs. Proc Roy Soc B: Biol Sci 268:2347–2353

Lessios HA, Cubit JD, Robertson DR, Shulman MJ, Parker MR, Garrity SD, Levings SC (1984) Mass mortality of *Diadema antillarum* on the Caibbean coast of Panama. Coral Reefs 3:173–182

Levinton JS (1995) Marine biology: function, biodiversity, ecology. Oxford University Press, New York

Linares C, Coma R, Garrabou J, Díaz D, Cabala M (2008) Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. J Appl Ecol 45:688–699

Linley EAS, Koop K (1986) Significance of pelagic bacteria as a trophic resource in a coral reef lagoon, One Tree Island, Great Barrier Reef. Mar Biol 92: 457–464

Littler M, Littler D (1995) Impact of CLOD pathogen on Pacific coral reefs. Science 267:1356–1360

Littler M, Littler D (1998) An undescribed fungal pathogen of reef-forming crustose coralline algae discovered in American Samoa. Coral Reefs 17:144

Littman R, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Environ Microbiol Rep 3:651–660

Littman RA, Willis BL, Pfeffer C, Bourne DG (2009) Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. FEMS Microbiol Ecol 68:152–163

Liu MY, Kjelleberg S, Thomas T (2010) Functional genomic analysis of an uncultured delta-proteobacterium in the sponge *Cymbastela concentrica*. ISME J 5:427–435

López-Legentil S, Song B, McMurray SE, Pawlik JR (2008) Bleaching and stress in coral reef ecosystems: *hsp70* expression by the giant barrel sponge *Xestospongia muta*. Mol Ecol 17:1840–1849

López-Legentil S, Erwin PE, Pawlik JR, Song B (2010) Effects of sponge bleaching on ammonia-oxidizing *Archaea*: distribution and relative expression of

ammonia monoxygenase genes associated with the giant barrel sponge *Xestospongia muta*. Microb Ecol 60:561–571

Luter HM, Whalan S, Webster NS (2010a) Prevalence of tissue necrosis and brown spot lesions in a common marine sponge. Mar Freshw Res 61: 481–484

Luter HM, Whalan S, Webster NS (2010b) Exploring the role of microorganisms in the disease-like syndrome affecting the sponge *Ianthella basta*. Appl Environ Microbiol 76:5736–5744

Maldonado M, Sánchez-Tocino L, Navarro C (2010) Recurrent disease outbreaks in corneous demosponges of the genus *Ircinia*: epidemic incidence and defense mechanisms. Mar Biol 157:1577–1590

Mayer FW, Wild C (2010) Corl mucus release and following particle trapping contribute to rapid nutrient recycling in a Northern Red Sea fringing reef. Mar Freshw Res 61:1006–1014

McMurray SE, Henkel TP, Pawlik JR (2010) Demographics of increasing populations of the giant barrel sponge *Xestospongia muta* in the Florida Keys. Ecology 91:560–570

Meikle P, Richards GN, Yellowlees D (1988) Structural investigations on the mucus from 6 species of coral. Mar Biol 99:187–193

Meron D, Atias E, Iasur Kruh L, Elifantz H, Minz D, Fine M, Banin E (2011) The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. ISME J 5:51–60

Meyer B, Kuever J (2008) Phylogenetic diversity and spatial distribution of the microbial community associated with the Caribbean deep-water sponge Polymastia cf. corticata by 16S rRNA, aprA, and amoA gene analysis. Microbial Ecol 56:306–321

Mohamed NM, Saito K, Tal Y, Hill RT (2010) Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. ISME J 4:38–48

Mohamed NM, Enticknap JJ, Lohr JE, McIntosh SM, Hill RT (2008) Changes in bacterial communities of the marine sponge *Mycale laxissima* on transfer into aquaculture. Appl Environ Microbiol 74:1209–1222

Morse ANC, Iwao K, Baba M, Shimoike K, Hayashibara T, Omori M (1996) An ancient chemosensory mechanism brings new life to coral reefs. Biol Bull 191:149–154

Mouchka ME, Hewson I, Harvell DC (2010) Coral-associated bacterial assemblages: current knowledge and the potential for climate-driven impacts. Intergr Comp Biol 50:662–674

Mukherjee J, Webster NS, Llewellyn LE (2009) Purification and characterization of a collagenolytic enzyme from a pathogen of the Great Barrier Reef sponge *Rhopaloeides odorabile*. PLoS One 4:e7177

Negandhi K, Blackwelder PL, Ereskovsky AV, Lopez JV (2010) Florida reef sponges harbor coral disease-associated microbes. Symbiosis 51: 117–129

Negri AP, Webster NS, Hill RT, Heyward AJ (2001) Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser 223:121–131

Nelson CE, Alldredge AL, McCliment EA, Amaral-Zettler LA, Carlson CA (2011) Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. ISME J 5:1374–1387

Off S, Alawi M, Spieck E (2010) Enrichment and physiological characterization of a novel *Nitrospira*-like bacterium obtained from a marine sponge. Appl Environ Microbiol 76:4640–4646

Olson JB, Kellogg CA (2010) Microbial ecology of corals, sponges and algae in mesophotic coral environments. FEMS Microbiol Ecol 73:17–30

Olson JB, Gochfeld DJ, Slattery M (2006) Aplysina red band syndrome: a new threat to Caribbean sponges. Dis Aquat Organ 71:163–168

Olson ND, Ainsworth TD, Gates RD, Takabayashi M (2009) Diazotrophic bacteria associated with Hawaiian montipora corals: diversity and abundance in correlation with symbiotic dinoflagellates. J Exper Mar Biol Ecol 371:140–146

Pantos O, Bythell JC (2006) Bacterial community structure associated with white band disease in the elkhorn coral *Acropora palmata* determined using culture independent 16S rRNA techniques. Dis Aquat Organ 69:79–88

Pantos O, Cooney RP, Le Tissier MDA, Barer MR, O'Donnell AG, Bythell JC (2003) The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. Environ Microbiol 5:370–382

Partensky F, Blanchot J, Vaulot D (1999) Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review. In Charpy L, Larkum AWD (eds) Marine cyanobacteria. Bulletin de l'Institut Oce´ anographique, Monaco, pp 457–475

Patten NL, Mitchell JG, Middelboe M, Eyre BD, Seuront L, Harrison PL, Glud RN (2008) Bacterial and viral dynamics during a mass coral spawning period on the Great Barrier Reef. Aquat Microb Ecol 50:209–220

Patterson KL, Porter JW, Ritchie KE, Polson SW, Mueller E, Peters EC et al (2002) The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. Proc Natl Acad Sci U S A 99: 8725–8730

Pawlik JR (2011) The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems. Bioscience 61:888–898

Piel J (2009) Metabolites from symbiotic bacteria. Nat Prod Rep 26:338–362

Pollock FJ, Morris PJ, Willis BL, Bourne DG (2011) The urgent need for robust coral disease diagnostics. PLoS Pathog 7:e1002183

Pollock FJ, Wilson B, Johnson WR, Morris PJ, Willis BL, Bourne DG (2010) Phylogeny of the coral pathogen *Vibrio coralliilyticus*. Environ Microbiol Rep 2:172–178

Porter JW, Dustan P, Jaap WC, Patterson KL, Kosmynin V, Meier OW et al (2001) Patterns of spread of coral disease in the Florida Keys. Hydrobiologia 460:1–24

Rabus R, Fukui M, Wilkes H, Widdle F (1996) Degradative capacities and 16S rRNA-targeted whole-cell hybridization of sulfate-reducing bacteria in an anaerobic enrichment culture utilizing alkylbenzenes from crude oil. Appl Environ Microbiol 62:3605–3613

Raina J-B, Tapiolas D, Willis BL, Bourne DG (2009) Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. Appl Environ Microbiol 75:3492–3501

Raina J-B, Dinsdale E, Willis BL, Bourne DG (2010) Do organic sulphur compounds DMSP and DMS drive coral microbial associations? Trends Microbiol 18:101–108

Rath H, Schiller C, Herndl GJ (1993) Ectoenzymatic activity and bacterial dynamics along a trophic gradient in the Caribbean Sea. Mar Ecol Prog Ser 102:89–96

Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. Environ Microbiol 8:2068–2073

Richardson LL (1997) Occurrence of the black band disease cyanobacterium on healthy corals of the Florida Keys. Bull Mar Sci 61:485–490

Richardson LL (2004) Black band disease. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer, Berlin, pp 325–336

Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1–14

Rogers AD (1999) The biology of *Lophelia pertusa* (Linnaeus 1758) and other deep-water reef-forming corals and impacts from human activities. Int Rev Hydrobiol 84:315–406

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10

Rohwer F, Breitbart M, Jara J, Azam F, Knowlton N (2001) Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. Coral Reefs 20: 85–91

Rosenberg E, Kellogg CA, Rohwer F (2007a) Coral microbiology. Oceanography 20:146–154

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007b) The role of microorganisms in coral health, disease and evolution. Nat Rev 5:355–362

Rusch A, Hannides AK, Gaidos E (2009) Diverse communities of active Bacteria and Archaea along oxygen gradients in coral reef sediments. Coral Reefs 28:15–26

Rypien KL, Ward JR, Azam F (2010) Antagonistic interactions among coral-associated bacteria. Environ Microbiol 12:28–39

Sakka A, Legendre L, Gosselin M, Niquil N, Delesalle B (2002) Carbon budget of the planktonic food web in an atoll lagoon (Takapoto, French Polynesia). J Plankton Res 24:301–320

Santos E, Alves N, Dias G, Mazotto AM, Vermelho A, Vora G et al (2011) Genomics and proteomics of the coral pathogen *Vibrio coralliilyticus* reveal a vast virulence repertoire. ISME J 5:1471–1483

Sato Y, Willis BL, Bourne DG (2010) Successional changes in bacterial communities during the development of black band disease on the reef coral, *Montipora hispida*. ISME J 4:203–214

Schläppy M-L, Schöttner S, Lavik G, Kuypers M, de Beer D, Hoffmann F (2010) Evidence of nitrification and denitrification in high and low microbial abundance sponges. Mar Biol 157:593–602

Schmidt EW, Obraztsova AY, Davidson SK, Faulkner DJ, Haygood MG (2000) Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel delta-proteobacterium, "Candidatus Entotheonella palauensis". Mar Biol 136:969–977

Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Appl Environ Microbiol 74:7694–7708

Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N et al (2011) Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. ISME J 6:564–576

Sebens K (1994) Biodiversity of coral reefs: what are we losing and why? Amer Zool 34:115–133

Selvin J, Shanmugha Priya S, Seghal Kiran G, Thangavelu T, Sapna Bai N (2007) Sponge-associated marine bacteria as indicators of heavy metal pollution. Microbiol Res 164:352–363

Seymour JR, Patten N, Bourne DG, Mitchell JG (2005) Spatial dynamics of virus-like particles and heterotrophic bacteria within a shallow coral reef system. Mar Ecol Prog Ser 288:1–8

Shashar N, Feldstein T, Cohen Y, Loya Y (1994a) Nitrogen fixation (acetylene reduction) on a coral reef. Coral Reefs 13:171–174

Shashar N, Cohen Y, Loya Y, Sar N (1994b) Nitrogen fixation (acetylene reduction) in stony corals: evidence for coral-bacteria interactions. Mar Ecol Prog Ser 111:259–264

Shields JD (2011) Diseases of spiny lobsters: a review. J Invertebr Pathol 106: 79–91

Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. Environ Microbiol 10:2979–2990

Siegl A, Hentschel U (2010) PKS and NRPS gene clusters from microbial symbiont cells of marine sponges by whole genome amplification. Environ Microbiol Rep 2:507–513

Siegl A, Kamke J, Hochmuth T, Piel J, Richter M, Liang C et al (2011) Single-cell genomics reveals the lifestyle of *Poribacteria*, a candidate phylum symbiotically associated with marine sponges. ISME J 5:61–70

Simister RL, Deines P, Botté ES, Webster NS, Taylor MW (2012) Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. Environ Microbiol 14:517–524

Smith JE, Shaw M, Edwards RA, Obura D, Pantos O, Sala E et al (2006) Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. Ecol Lett 9:835–845

Southwell MW, Popp BN, Martens CS (2008a) Nitrification controls on fluxes and isotopic composition of nitrate from Florida Keys sponges. Mar Chem 108:96–108

Southwell MW, Weisz J, Martens CS, Lindquist N (2008b) In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnol Oceanogr 53:986–996

Stambler N (2011) Zooxanthellae: the yellow symbionts inside animals. In: Dubinsky A, Stambler N (eds) Coral reefs: an ecosystem in transition Part 3. Springer, Netherlands, pp 87–106

Steger D, Ettinger-Epstein P, Whalan S, Hentschel U, de Nys R, Wagner M, Taylor MW (2008) Diversity and mode of transmission of ammonia-oxidizing archaea in marine sponges. Environ Microbiol 10:1087–1094

Stockner JG (1988) Phototrophic picoplankton: an overview from marine and freshwater ecosystems. Limnol Oceanogr 33:765–775

Sugumar G, Nakai T, Hirata Y, Matsubara D, Muroga K (1998) *Vibrio splendidus* biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* larvae. Dis Aquat Organ 33:111–118

Sumich JL, Morrissey JF (2004) An introduction to the biology of marine life. Jones and Bartlett, Sudbury, MA

Sunagawa S, Woodley CM, Medina M (2010) Threatened corals provide underexplored microbial habitats. PLoS One 5:e9554

Sunagawa S, DeSantis TZ, Piceno YM, Brodie EL, DeSalvo MK, Voolstra CR et al (2009) Bacterial diversity and white plague disease-associated community changes in the Caribbean coral *Montastraea faveolata*. ISME J 3:512–521

Sussman M, Willis BL, Victor S, Bourne DG (2008) Coral pathogens indentified for White Syndrome (WS) epizootics in the Indo-Pacific. PLoS One 3:e2393

Sussman M, Mieog JC, Doyle J, Victor S, Willis BL, Bourne DG (2009) Vibrio zinc-metalloprotease causes photoinactivation of coral endosymbionts and coral tissue lesions. PLoS One 4:e4511

Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar Ecol Prog Ser 266:273–302

Sutherland KP, Shaban S, Joyner JL, Porter JW, Lipp EK (2011) Human pathogen shown to cause disease in the threatened Eklhorn coral *Acropora palmata*. PLoS One 6:e23468

Sutherland KP, Porter JW, Turner JW, Thomas BJ, Looney EE, Luna TP et al (2010) Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, *Acropora palmata*. Environ Microbiol 12:1122–1131

Sweatman H, Delean S, Syms C (2011) Assessing loss of coral cover on Australia's Great Barrier Reef over two decades, with implications for longer-term trends. Coral Reefs 30:521–531

Sweet MJ, Croquer A, Bythell JC (2011) Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs 30:39–52

Taylor MW, Thacker RW, Hentschel U (2007a) Genetics. Evolutionary insights from sponges. Science 316:1854–1855

Taylor MW, Radax R, Steger D, Wagner M (2007b) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347

Thomas T, Rusch D, DeMaere MZ, Yung PY, Lewis M, Halpern A et al (2010) Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. ISME J 4:1557–1567

Todd JD, Kirkwood M, Newton-Payne S, Johnston AWB (2012) DddW, a third DMSP lyase in a model Roseobacter marine bacterium, *Ruegeria pomeroyi* DSS-3. ISME J 6:223–226

Toledo-Hernandez C, Zuluaga-Montero A, Bones-Gonzalez A, Rodriguez JA, Sabat AM, Bayman P (2008) Fungi in healthy and diseased sea fans (*Gorgonia ventalina*): is *Aspergillus sydowii* always the pathogen? Coral Reefs 27:707–714

Torréton J-P, Dufour P (1996) Temporal and spatial stability of bacterioplankton biomass and productivity in an atoll lagoon. Aquat Microb Ecol 11:251–261

Torréton J-P, Pagès J, Talbot V (2002) Relationship between bacterioplankton and phytoplankton biomass, production and turnover rate in Tuamotu atoll lagoons. Aquat Microb Ecol 28:267–277

Torréton J-P, Rochelle-Newall E, Jouon A, Faure V, Jacquet S, Douillet P (2007) Correspondence between the distribution of hydrodynamic time parameters and the distribution of biological and chemical variables in a semi-enclosed coral reef lagoon. Estuar Coast Shelf Sci 74:766–776

Unson MD, Holland ND, Faulkner DJ (1994) A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Mar Biol 119:1–11

Uthicke S, McGuire K (2007) Bacterial communities in great barrier reef calcareous sediments: contrasting 16S rDNA libraries from nearshore and outer shelf reefs. Estuar Coast Shelf Sci 72:188–200

Vacelet J, Fiala-Médioni A, Fisher CR, Boury-Esnault N (1996) Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous cladorhizid sponge. Mar Ecol Prog Ser 145:77–85

Van Alstyne K, Schupp P, Slattery M (2006) The distribution of dimethylsulfoniopropionate in tropical Pacific coral reef invertebrates. Coral Reefs 25:321–327

van Duyl F, Gast G, Steinhoff W, Kloff S, Veldhuis M, Bak R (2002) Factors influencing the short-term variation in phytoplankton composition and biomass in coral reef waters. Coral Reefs 21:293–306

van Duyl FC, Gast GJ (2001) Linkage of small-scale spatial variations in DOC, inorganic nutrients and bacterioplankton growth with different coral reef water types. Aquat Microb Ecol 24:17–26

Van Duyl FC, Scheffers SR, Thomas FIM, Driscoll M (2006) The effect of water exchange on bacterioplankton depletion and inorganic nutrient fluxes in coral cavities. Coral Reefs 25:23–36

Vargas-Angel B (2010) Crustose coralline algal diseases in the U.S.-Affiliated Pacific Islands. Coral Reefs 29:943–956

Vega Thurber RLV, Barott KL, Hall D, Liu H, Rodriguez-Mueller B, Desnues C et al (2008) Metagenomic analysis indicates that stressors induce production of herpeslike viruses in the coral *Porites compressa*. Proc Nat Acad Sci USA 105:18413–18418

Vezzuli L, Previati M, Pruzzo C, Marchese A, Bourne DG, Cerrano C (2010) Vibrio infection triggering mass mortality events in a warming Mediterranean Sea. Environ Microbiol 12:2007–2019

Vezzulli L, Brettar I, Pezzati E, Reid PC, Colwell RR, Höfle MG, Pruzzo C (2012) Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios. ISME J 6:21–30

Vicente VP (1989) Regional commercial sponge extinction in the West Indies: are recent climatic changes responsible? Mar Ecol Prog Ser 10:179–191

Vidal-Dupiol J, Ladrière O, Meistertzheim A-L, Fouré L, Adjeroud M, Mitta G (2011) Physiological responses of the scleractinian coral *Pocillopora damicornis* to bacterial stress from *Vibrio coralliilyticus*. Journal of Experimental Biology and Ecology 214:1533–1545

Viehman S, Mills DK, Meichel GW, Richardson LL (2006) Culture and identification of *Desulfovibrio* spp. from corals infected by black band disease on Dominican and Florida Keys reefs. Dis Aquat Organ 69:119–127

Vila-Costa M, Rinta-Kanto JM, Sun S, Sharma S, Porestsky R, Moran MA (2010) Transcriptomic analysis of a marine bacterial community enriched with dimethylsulfoniopropionate. ISME J 4:1410–1420

Vizcaino MI, Johnson WR, Kimes NE, Williams K, Torralba M, Nelson KE et al (2010) Antimicrobial resistance of the coral pathogen *Vibrio coralliilyticus* and Caribbean sister phylotypes isolated from a diseased octocoral. Microb Ecol 59:646–657

Vogel G (2008) The inner lives of sponges. Science 320:1028–1030

Walker CW, Lesser MP (1989) Nutrition and development of brooded embryos in the brittlestar *Amphipholis squamata*: do endosymbiotic bacteria play a role? Mar Biol 103:519–530

Webster NS (2007) Sponge disease: a global threat? Environ Microbiol 9:1363–1375

Webster NS, Blackall LL (2009) What do we really know about sponge- microbial symbioses? ISME J 3:1–3

Webster NS, Taylor MW (2012) Marine sponges and their microbial symbionts: love and other relationships. Environ Microbiol 14:335–346

Webster NS, Watts JE, Hill RT (2001a) Detection and phylogenetic analysis of novel crenarchaeote and euryarchaeote 16S ribosomal RNA gene sequences from a Great Barrier Reef sponge. Marine Biotechnol 3:600–608

Webster NS, Cobb RE, Negri AP (2008a) Temperature thresholds for bacterial symbiosis with a sponge. ISME J 2:830–842

Webster NS, Negri AP, Webb RI, Hill RT (2002) A spongin-boring alpha proteobacterium is the etiological agent of disease in the Great Barrier Reef sponge, *Rhopaloeides odorabile*. Mar Ecol Prog Ser 232:305–309

Webster NS, Botté ES, Soo RM, Whalan S (2011a) The larval sponge holobiont exhibits high thermal tolerance. Environ Microbiol Rep 3:756–762

Webster NS, Soo R, Cobb R, Negri AP (2011b) Elevated seawater temperature causes a microbial shift on crustose coralline algae with implications for the recruitment of coral larvae. ISME J 5:759–770

Webster NS, Webb RI, Ridd MJ, Hill RT, Negri AP (2001b) The effects of copper on the microbial community of a coral reef sponge. Environ Microbiol 3: 19–31

Webster NS, Xavier JR, Freckelton M, Motti CA, Cobb R (2008b) Shifts in microbial and chemical patterns within the marine sponge *Aplysina aerophoba* during a disease outbreak. Environ Microbiol 10:3366–3376

Webster NS, Smith LD, Heyward AJ, Watts JE, Webb RI, Blackall LL, Negri AP (2004) Metamorphosis of a scleractinian coral in response to microbial biofilms. Appl Environ Microbiol 70:1213–1221

Webster NS, Taylor MW, Behnam F, Lücker S, Rattei T, Whalan S et al (2010) Deep sequencing reveals exceptional diversity and modes of -transmission for bacterial sponge symbionts. Environ Microbiol 12:2070–2082

Wegley L, Edwards RA, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ Microbiol 9:2707–2719

Weil E (2004) Coral diseases in the wider Caribbean. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer-Verlag, Berlin, pp 35–68

Weil E, Smith G, Gil-Agudelo DL (2006) Status and progress in coral reef disease research. Dis Aquat Organ 69:1–7

Weinbauer MG, Kerros ME, Motegi C, Wilhartitz IC, Rassoulzadegan F, Torréton J-P et al (2010) Bacterial community composition and potential controlling mechanisms along a trophic gradient in a barrier reef system. Aquat Microb Ecol 60:15–28

Weisz J, Hentschel U, Lindquist N, Martens C (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Mar Biol 152:475–483

Wild C, Woyt H, Huettel M (2005) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87–98

Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells within highly porous calcareous reef sands. Mar Freshw Res 57:415–420

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed MYM, Jorgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70

Wild C, Rasheed MYM, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. Mar Ecol Prog Ser 267:159–171

Wild C, Mayr C, Wehrmann L, Schöttner S, Naumann M, Hoffmann F, Rapp HT (2008) Organic matter release by cold water corals and its implication for fauna–microbe interaction. Mar Ecol Prog Ser 372:67–75

Wilkinson CR (1980) Cyanobacteria symbiotic in marine sponges. In: Schwemmler W, Schneck HEA (eds) Endocytobiology, endosymbiosis and cell biology. De Gruyter, Berlin, pp 553–563

Wilkinson CR (1983) Net primary productivity in coral reef sponges. Science 219:410–412

Wilkinson CR (1984) Immunological evidence for the Precambrian origin of bacterial symbioses in marine sponges. Proc Roy Soc Lond (B) 220: 509–517

Wilkinson CR (1992) Symbiotic interactions between marine sponges and algae. In: Reisser W (ed) Algae and symbioses: plants, animals, fungi, viruses, interactions explored. Biopress, Bristol, pp 112–128

Williams WM, Viner AB, Broughton WJ (1987) Nitrogen fixation (acetylene-reduction) associated with the living coral *Acropora variabilis*. Mar Biol 94:531–535

Willis BL, Page CA, Dinsdale EA (2004) Corals disease on the Great Barrier Reef. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer, Heildelberg, pp 69–104

Work TM, Aeby GS (2006) Systematically describing gross lesions in corals. Dis Aquat Organ 70:155–160

Work TM, Richardson LL, Reynolds TL, Willis BL (2008) Biomedical and veterinary science can increase our understanding of coral disease. J Exp Mar Biol Ecol 362:63–70

Wulff JL (2007) Disease prevalence and population density over time in three common Caribbean coral reef sponge species. J Mar Biol Assoc UK 87:1715–1720

Yahel G, Post AF, Fabricius KE, Marie D, Vaulot D, Genin A (1998) Phytoplankton distribution and grazing near coral reefs. Limnol Oceanogr 43:551–563

Yarza P, Richter M, Peplies J, Euzeby J, Amann R, Schleifer K-H et al (2008) The All-Species Living Tree project: A 16S rRNA-based phylogenetic tree of all sequenced type strains. Syst Appl Microbiol 31:241–250

Yellowlees D, Rees TAV, Leggat W (2008) Metabolic interactions between algal symbionts and invertebrate hosts. Plant Cell Environ 31:679–694

Ecophysiology

11 Planktonic Versus Sessile Life of Prokaryotes

*Kevin C. Marshall**

Because of the extremely small size of most prokaryotic organisms, the limits on what is meant by the terms planktonic and sessile require definition. According to the *Oxford English Dictionary*, planktonic refers to "drifting or floating organic life found at various depths in the ocean or fresh water." At the micrometer level, a planktonic habitat for prokaryotes can also encompass water films around soil particles, saliva in the mouth, fluids in the intestinal lumen, serum in blood vessels, and urine in the bladder and urinary tract. Sessile, on the other hand, means "immediately attached, without a footstalk." Again, one can extend this definition to include those prokaryotes directly adhering to surfaces, those attaching by means of a holdfast at the end of a prostheca (e.g., *Caulobacter*), those embedded in biofilms developing as a result of extracellular polymer production by bacteria colonizing surfaces, and those colonizing mucus excreted by higher organisms (as in the gastrointestinal tract and the mucigel of plant roots).

Most microbiologists, oriented by their training to the study of pure cultures, regard suspension culture as the normal state for growth of these organisms. This is particularly true for research into the physiology and biochemistry of bacteria, whereby homogeneous suspensions of bacteria are readily harvested and manipulated for experimental purposes. The reality of prokaryotic life in natural habitats is that many organisms spend part or all of their life spans attached to surfaces (Marshall 1976). However, recently there has been a veritable explosion in research devoted to understanding the behavior of bacteria at surfaces (Beachey 1980; Bitton and Marshall 1980; Marshall 1984; Savage and Fletcher 1985).

Many questions arise regarding the association of bacteria with surfaces. It is my aim in this chapter to consider the current state of knowledge concerning the following questions: How do prokaryotes adhere to surfaces? Is there a single, all-embracing mechanism or a range of mechanisms of adhesion in different organisms? Are some prokaryotes especially adapted to a sessile existence? Are particular organisms homogeneous in their adhesive characteristics, or are they variable in their response to surfaces? Once attached to a surface, do prokaryotes always remain in a sessile state or do they return to the planktonic state at some stage? Do prokaryotes gain any real advantage from being associated with surfaces? Are certain prokaryotes specifically adapted to the colonization of excreted mucous layers? Are sessile bacteria in a different physiological state from planktonic organisms; that is, do prokaryotes exhibit a physiological response to contact with a surface? If they show such responses, what physicochemical factors are responsible for inducing the responses?

Mechanisms of Adhesion to Surfaces

Full details of proposed mechanisms of adhesion of prokaryotes to solid surfaces have been presented elsewhere (Marshall 1985, 1986a), so only a brief outline will be presented in this paper.

Transport Processes

Water currents induced by temperature and gravity (fluid dynamic forces) provide the major mechanism for the transport

*Deceased

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_49,

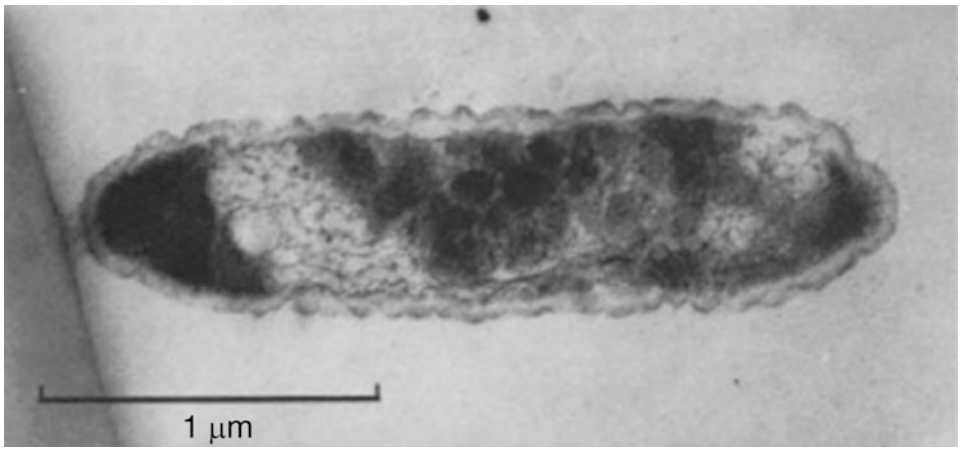


Fig. 11.1
Perpendicular adhesion of a marine bacterium to a solid plastic surface. The extracellular polymeric substances bridging between the cell and the surface are present only at the adhesive pole of the cell (Courtesy of R. H. Cruickshank)

of planktonic bacteria over large distances. When bacteria and other particles in flowing water are transported to the region of the boundary layer near a solid surface, a lift force directs the bacteria toward the surface where fluid frictional forces slow them down (Characklis 1981a) and deposit them in the vicinity of the surface.

Sedimentation is of significance only when bacteria are aggregated together or are attached to particles. Individual bacteria behave essentially as colloidal particles (Marshall 1976) and tend to remain in suspension. Nutrient gradients may become established across the boundary layer near some surfaces, and these may provide opportunities for chemotactic responses toward the surfaces by motile bacteria. Brownian motion can account for random movement of very small bacteria within the quiescent water of a boundary layer near a surface (Marshall 1976).

Long-Range Forces

Bacteria in the vicinity of a solid–liquid interface frequently show an instantaneous but reversible attraction to the interface (Marshall et al. 1971a), and an attempt has been made to explain this reversible attraction by means of the colloid stability (DLVO named for the originators—Derjaguin, Landau, Verwey, and Overbeek) theory. That this attraction is reversible is shown by the fact that the bacteria can be removed from the solid surface by gentle shearing forces. The DLVO theory accounts, at least in part, for the attraction of a negatively charged bacterium to a negatively charged substratum surface at the "secondary attraction minimum" resulting from the interaction between London-van der Waals attraction forces and electrical repulsion forces in the overlapping double layers of cations surrounding the negatively charged surfaces. In terms of the DLVO theory, a bacterial cell would be held at a distance of some 10 nm from the surface by repulsion forces.

Problems in applying DLVO theory to biological systems have been raised by Pethica (1980) and Rutter and Vincent (1980), especially when the complexity of the bacterial cell envelope and the extracellular components are taken into account. However, Busscher and Weerkamp (1987) have argued strongly in favor of such long-range forces in the initial attraction of bacteria to surfaces.

Short-Range Forces

Certain bacteria irreversibly attach to surfaces very rapidly (Fletcher 1980), whereas other bacteria require a significant time of exposure to the surface before becoming firmly attached (Marshall et al. 1971a). Irreversible attraction is shown by the fact that the bacteria cannot be removed by moderate shear forces. What is the mechanism of this firm adhesion of bacteria to surfaces? Early observations indicated that polymer bridging by extracellular components of cells to the substratum surface (*Fig. 11.1*) resulted in firm adhesion (Marshall and Cruickshank 1973; Fletcher and Floodgate 1973), and these observations have been confirmed for many systems (Corpe 1980; Costerton et al. 1981). These extracellular polymers have a small radius of curvature and can overcome any repulsion barrier near a surface and, thus, can bind the cell to the surface using a variety of short-range forces. These forces include (1) chemical bonds (electrostatic, covalent, and hydrogen), (2) dipole interactions (dipole-dipole, dipole-induced dipole, and ion-dipole), and (3) hydrophobic interactions (Rutter and Vincent 1980).

Adhesion to surfaces in nature is generally considered to be *nonspecific*. That is, the bacteria adhere to a wide variety of different inanimate, and possibly animate, surfaces with varying degrees of adhesive strength. Bridging polymers involved in most cases of nonspecific adhesion are either extracellular polysaccharides, proteins, or glycoproteins. The precise mechanism whereby such polymers interact with

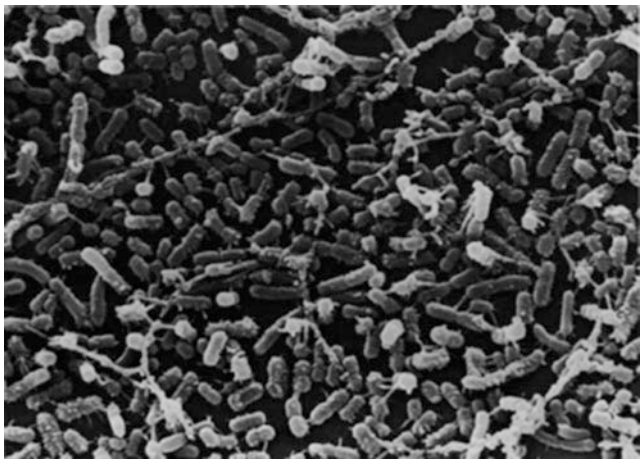


Fig. 11.2
Colonization of a glass surface, rendered hydrophobic by treatment with silane, by a marine bacterium after 16-h exposure. The condensed extracellular polymeric substances are clearly visible, as a result of drying on a cold stage (Courtesy of T. Neu)

a range of substratum surfaces is not known, but it almost certainly involves various combinations of the short-range forces listed above.

Specific adhesion involves lectin-receptor-type mechanisms, in which a proteinaceous substance (lectin) on the bacterial surface reacts with a complementary carbohydrate receptor on another cell type (Switalski et al. 1989). The best-described examples of specific adhesion involve the attachment of pathogenic bacteria to the host cell surfaces they infect. However, specific attachment of bacteria to the heterocysts of the cyanobacterium *Anabaena* has been described (Lupton and Marshall 1981).

Thermodynamic Approach to Bacterial Adhesion

Various workers have attempted to relate the extent of bacterial adhesion to the variation in surface free energy of the substratum, with very variable results (Dexter et al. 1975; Fletcher and Loeb 1979). More detailed studies revealed that, in addition to the substratum-surface free energy, it was necessary to consider the bacterium-surface free energy and the surface tension of the liquid (Absolom et al. 1983; Pringle and Fletcher 1983). The change in free energy associated with bacterial adhesion (ΔF_{adh}) is given by

$$\Delta F_{adh} = \gamma_{BS} - \gamma_{BL} - \gamma_{SL}$$

where γ_{BS}, γ_{BL}, and γ_{SL} are the bacterium-substratum, bacterium-liquid, and substratum-liquid interfacial tensions, respectively. Bacterial adhesion is favored if the process results in a free energy decrease. In general, Absolom et al. (1983) found good agreement between bacterial adhesion to a variety of substrata and the adhesion behavior predicted by the thermodynamic model.

Detachment of Bacteria from Surfaces

Not all cells remain adherent at the surface. Mechanisms of detachment include fluid shear forces (Marshall et al. 1971a), changes in surface free energy of the substratum (Busscher et al. 1986) or the organism (Rosenberg et al. 1983; Fattom and Shilo 1984), reproductive mechanisms (Power and Marshall 1988), and enzymatic degradation of adhesive structures. In most cases, however, the majority of adhering bacteria remain at the surface, where they are capable of growth, reproduction (*Fig. 11.2*) (Lawrence and Caldwell 1987; Power and Marshall 1988; Szewzyk and Schink 1988), and even biofilm formation. A *biofilm* consists of cells immobilized at a substratum surface and frequently embedded in an organic polymer matrix of microbial origin (Characklis and Marshall 1990). Other practical aspects of bacterial detachment from surfaces will be considered in later sections.

Occurrence of Sessile Prokaryotes

Microbial Succession at Surfaces

Early reports indicated that very small bacteria were the primary colonizers of surfaces immersed in seawater and were succeeded by conventional rod-shaped and, somewhat later, by prosthecate bacteria (Marshall et al. 1971b). It was realized that the initial colonizing organisms were starvation-survival forms (Morita 1982) that eventually produced cellular growth at surfaces and thus gave rise to rod-shaped forms (Dawson et al. 1981; Power and Marshall 1988). Early colonizing organisms tend to be Gram-negative bacteria, particularly species of *Pseudomonas*, *Flavobacterium*, and *Achromobacter*, followed later by prosthecate bacteria (Corpe 1973). Gram-positive bacteria have rarely been recorded on surfaces in aquatic habitats, although there have been recent reports of significant numbers of Gram-positive bacteria on surfaces associated with groundwater (Kölbel-Boelke and Hirsch 1989) and on the seagrass *Zostera capricorni* (Angles 1988). The numbers, overall biomass, and diversity of attached microorganisms increased with increasing time of immersion of a surface (Jordan and Staley 1976). Scanning electron microscopic studies also have revealed a progression from rod-shaped primary colonizers to prosthecate forms and then to a complex biofilm whose composition varies with the nature of the exposed surface and with time (Gerchakov et al. 1977; Marszalek et al. 1979; Dempsey 1981). Even in illuminated waters, microalgae are not primary colonizers of surfaces (Marshall et al. 1971b; Corpe 1973; Jordan and Staley 1976), but extensive development of diatoms, fungi, and protozoa has been observed following bacterial biofilm formation (Gerchakov et al. 1977; Marszalek et al. 1979).

Biologically inert substrata, such as stainless steel or glass, were colonized rapidly following immersion in seawater and produced a complex, two-tiered, microfouling layer (Gerchakov et al. 1977; Marszalek et al. 1979; Dempsey 1981). The first stage of colonization consisted mainly of bacteria followed by

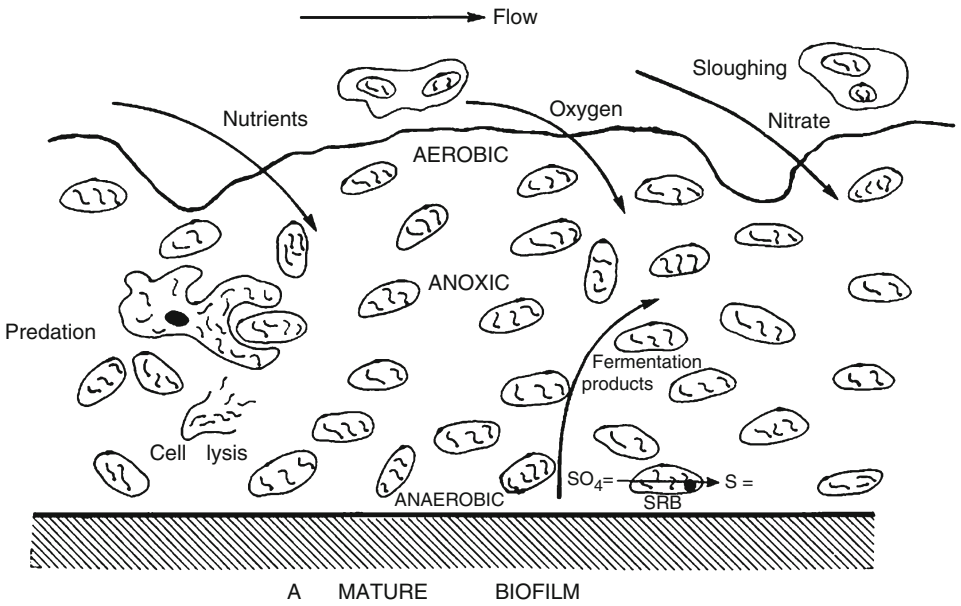


Fig. 11.3

Diagram of a section through a well-developed biofilm, showing bacteria embedded in an EPS matrix and the direction of decreasing gradients (*arrows*) of nutrients, oxygen, nitrate, and fermentation products. A predatory amoeba within the biofilm is shown at the left. SRB = sulfate-reducing bacterium

nonmotile diatoms and fungi, whereas the second stage, which appeared after a 5-week exposure, consisted of large, colonial, motile diatoms, other diatoms, flagellates, and ciliates. On the other hand, inhibitory substrata, such as copper-nickel alloys or brass, were slowly fouled by bacteria capable of secreting mucoid extracellular polymeric substances (EPS). Such substrata eventually developed a much less diverse biofilm community than inert ones.

Sequential establishment of sessile populations also occurs in freshwater streams (Geesey et al. 1977, 1978) and lakes (Paerl 1980); in soils where the complexity and variability of the solid matrix makes adequate study difficult (Marshall 1975; Stotzky 1986); in the oral cavity (Bowden et al. 1979; Newman 1980); in the gastrointestinal tract, where the normal sessile biota plays an important role in preventing colonization by bacterial pathogens (Lee 1980, 1985; Savage 1980, 1984); and in the colonization of prosthetic devices employed in human patients (Gristina 1987).

Biofilm Formation

The combined effects of continuous adhesion and both growth and reproduction at surfaces eventually give rise to a macroscopic slime, or biofilm (*Fig. 11.3*).

Biofilms are of considerable nuisance on artificial structures, such as ship hulls, hydroelectric pipelines, water reticulation systems, heat exchangers, oil rigs, and floating oceanographic equipment, but find useful applications in wastewater trickling-filter plants and other fixed-film systems, as well as in fluidized-bed fermenters.

The development of a biofilm on a surface subjected to high shear rates may be described by a sigmoid-shaped curve, where the phase of biomass increase is a function of growth of attached bacteria along with further accretion of cells to the developing biofilm. The plateau of the curve represents the point at which the film penetrates the boundary (or viscous) sublayer (Characklis 1981b). The final biofilm thickness is dependent on the magnitude of the fluid shear rate. Any protrusion of film irregularities above the viscous sublayer creates turbulence in the water flowing past the biofilm surface leading to frictional flow resistance.

The colonization of mucus excreted by higher organisms (e.g., the mucous blanket of the animal gastrointestinal tract (Lee 1985) and the mucigel of plant roots (Rovira et al. 1979)) leads to a partial or complete immobilization of cells in the mucus adjacent to the organism's tissue. The final product in this instance bears a superficial resemblance to a biofilm, but its mode of origin is entirely different. Certain organisms, particularly spiral bacteria (Phillips and Lee 1983), appear to have a selective advantage in penetrating and colonizing this viscous habitat.

Methods of Studying Sessile Prokaryotes

Because of the inherent difficulty in directly observing the behavior of microorganisms at surfaces, a wide range of semidirect and indirect techniques have been employed to study adhesion, growth, biofilm development, and detachment from surfaces. Because of the different techniques needed for different surfaces and ecosystems, no attempt will be made here

to give detailed instructions for the many techniques available, but, rather, references to the descriptions of the original techniques will be provided.

Microscopy

Many of the applications of various forms of microscopy in the study of sessile bacteria have been reviewed (Marshall 1986b). Most studies involve the use of transmitted or incident light microscopy or of transmission (TEM) or scanning electron microscopy (SEM). For transmitted light microscopy, the use of transparent substrata (glass, mica, cellophane, polystyrene, etc.) as test surfaces is essential. Epifluorescence microscopy is necessary for translucent and opaque substrata (Zvyagintsev 1962; Hobbie et al. 1977). Sessile bacteria may be observed by washing the exposed substratum to remove debris and loosely attached cells and then either staining, with conventional bacteriological stains or fluorescent dyes, or viewing directly with phase-contrast optics. The advantages and disadvantages of such techniques have been presented by Marshall (1986b).

Novel techniques involving light microscopy include the use of submerged microscopy (Staley 1971), capillary microscopy (Perfil'ev and Gabe 1969), computer-enhanced image analysis (Caldwell and Germida 1985), interference reflection microscopy (Fletcher 1988), dialysis microculture (Duxbury 1977), marked slides (Bott and Brock 1970), soil films (Harris 1972), transparent sections in tubular reactors to study biofilm development (Characklis 1980), and light section microscopy to measure biofilm thickness (Loeb 1980).

Other Methods of Study

During the early stages of colonization of surfaces, and particularly if glass, plastic, metal, or wooden slides are immersed in an aqueous phase, bacteria adhering firmly to the surface may be cultured by washing the slides or coupons to remove loosely adhering organisms and then smearing the slide or coupon over the surface of a suitable agar plate (Marshall et al. 1971a). If a distinct biofilm has formed on a surface, the biofilm may be scraped from the surface, suspended in a suitable diluent, and homogenized, a dilution series prepared, and aliquots of each dilution plated on an appropriate agar medium. Such methods suffer from the normal problems of selectiveness of the medium employed, and it is likely that some colonizing species (e.g., *Caulobacter*, *Hyphomicrobium*) are never obtained by such techniques. Often the use of special selective media is required in order to isolate particular organisms that may be obvious microscopically. In some cases, it may be necessary to resort to micromanipulation techniques to separate slow-growing or sensitive organisms from more aggressive or resistant species. The simple micromanipulation system devised by Skerman (1968) is especially recommended for this purpose.

A variety of other methods have been adapted to estimate numbers of microorganisms or the total biomass found in a sessile state at surfaces. These include measurement of radioactivity following the uptake of labeled substrates (Brock 1971; Lupton and Marshall 1981), autoradiography (Fletcher 1979; Bright and Fletcher 1983), ATP determinations for total biomass (La Motta 1976), muramic acid determinations for bacterial biomass (Moriarty 1977), bacterial growth rates using thymidine incorporation (Moriarty 1986), and determination of bacterial types at surfaces by phospholipid fatty acid signature analysis (Guckert et al. 1985) and by 16S rRNA sequence analysis (Pace et al. 1986; Weller and Ward 1989). Other techniques that may prove valuable in analyzing biofilm composition and function include the use of Fourier transform infrared spectrophotometry (Nichols et al. 1985) and the use of microelectrodes to measure various gradients with depth of biofilms (Revsbech and Jørgensen 1986) (*Fig. 11.3*).

Adaptation to the Sessile State

Are certain prokaryotes uniquely adapted to a sessile form of life? The answer to this question is not simple because of the very wide range of bacteria that can be found on various surfaces. Several examples of different modes of sessile behavior will be considered in order to illustrate the complexity that may be encountered in natural habitats.

Although many bacteria are capable of adhering to a wide variety of surfaces (nonspecific adhesion), the extent of adhesion on the various surfaces varies considerably. Some bacteria adhere best to hydrophobic surfaces (Fletcher and Loeb 1979), some adhere best to hydrophilic surfaces (Dexter et al. 1975), whereas others adhere best to surfaces of more intermediate surface-free-energy values (Pringle and Fletcher 1983). The conditions under which the bacteria are grown also modify the adhesive ability of various bacteria on a range of different surfaces (McEldowney and Fletcher 1986).

Many bacteria that require relatively high nutrient concentrations (copiotrophic bacteria) exist planktonically in oligotrophic waters in a state of starvation. These starvation-survival forms are characterized by a significant reduction in size and by lower endogenous respiration and heat output and are often more adhesive than actively growing cells (Morita 1982; Dawson et al. 1981; Humphrey and Marshall 1984). Adhesion to surfaces by these starvation-survival forms provides access to nutrients accumulated at the surfaces. The starved bacteria are able to scavenge these nutrients and metabolize them (Kefford et al. 1982; Kjelleberg et al. 1983), thereby leading to cellular growth and reproduction (Kjelleberg et al. 1982; Power and Marshall 1988; Szewzyk and Schink 1988). In many marine environments, it appears that such small, starved bacteria are the primary colonizers of freshly immersed surfaces (Marshall et al. 1971b).

Some copiotrophic bacteria seem unable to adhere firmly to surfaces, yet, under oligotrophic conditions, any starvation-survival forms approaching a surface are able to metabolize surface-bound substrates (Hermansson and Marshall 1985) and exhibit both cellular growth and reproduction

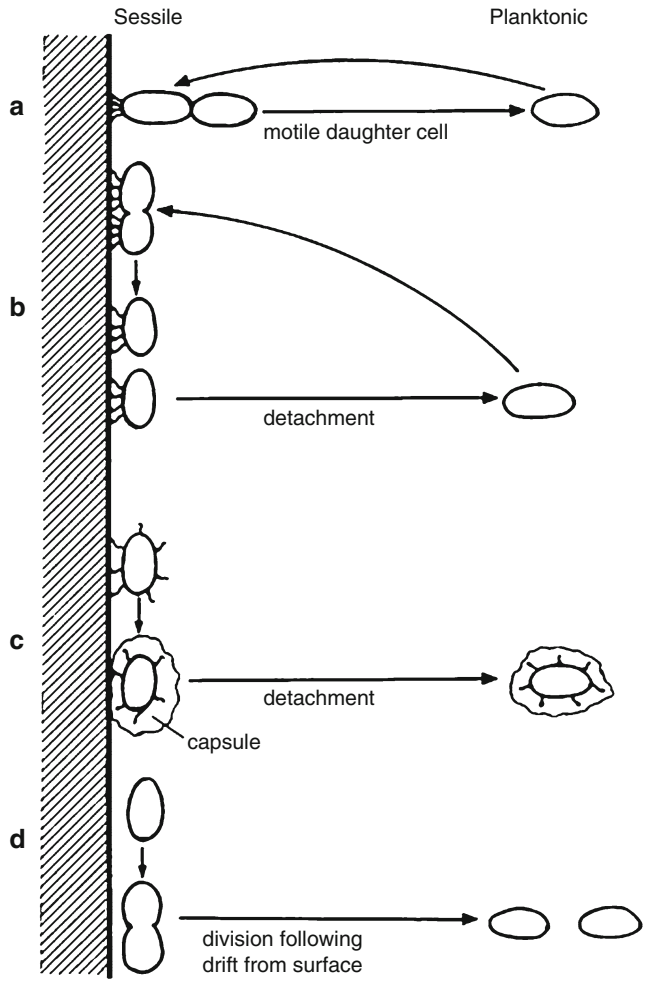


Fig. 11.4

Four mechanisms for alternating between the planktonic and sessile states: (a) a perpendicularly attached mother cell releases a motile daughter cell, as in *Vibrio* sp. DWI; (b) division of a cell adhering in a face-to-face manner and release of a cell on utilization of a bound hydrophobic substrate, as in *Pseudomonas* sp. JD8; (c) detachment of a fimbrial-attached organism following the production of a hydrophilic capsule, as in *Acinetobacter calcoaceticus*; and (d) growth of a reversibly adhering organism at a surface and completion of the division phase following drift of the cell from the surface, as in *Vibrio* sp. MH3

(Power and Marshall 1988). Thus, nonadhesive bacteria do exist in the planktonic state, but it is still possible for such organisms to benefit from association with surfaces.

A particularly effective adaptation to the sessile state is the ability of many bacteria in nature to adhere in an orientation perpendicular to the surface (Fig. 11.4a; see also Fig. 11.1). Such prokaryotes appear to have either a specialized holdfast (*Caulobacter*) or a particularly adhesive portion at one pole of the cell (*Hyphomicrobium*, *Flexibacter*, and *Leucothrix*). Such an orientation allows a very efficient contact with both the solid and the aqueous phases as well as provides an effective means of releasing daughter cells into the planktonic state. An examination of this mode of orientation at solid surfaces revealed that both *Hyphomicrobium* and *Flexibacter* exhibited the same perpendicular orientation at air-water and oil–water interfaces (Marshall and Cruickshank 1973). It was postulated that the pole of the cell approaching the interface was hydrophobic while the bulk of the cell was hydrophilic, and the hydrophobic pole was rejected from the water phase and aligned at the nonaqueous phase, regardless of whether it was solid, air, or oil (Marshall and Cruickshank 1973).

Some bacteria are adapted to growth at surfaces yet possess various mechanisms to ensure that some cells return to the planktonic state. For instance, cells of the marine species *Vibrio* DW1 adhered to a surface in a perpendicular manner (Fig. 11.4a), and, following cellular growth of the starved cells to normal size, motile daughter cells were released at regular intervals (approximately 57 min) from the attached mother cells (Kjelleberg et al. 1982). Cells of the marine *Pseudomonas* sp. JD8 adhered in a face-to-face manner (Fig. 11.4b), and, following cellular growth and one division cycle, the daughter cells slowly (about 0.15 μg/min) began to migrate away from each other while still adhering to the surface. After subsequent division cycles, similar migration patterns were observed, but, eventually, some of the daughter cells detached from the surface (Power and Marshall 1988). This slow migration was explained in terms of the cells being initially irreversibly attached to the hydrophobic stearic-acid-covered surface, but, upon utilization of the fatty acid in the microenvironment around the cell, the cells became reversibly attached to the underlying hydrophilic substratum (Busscher et al. 1986) and were capable of some form of movement. As soon as the cells moved a short distance, however, they encountered more hydrophobic stearic acid and adhered irreversibly again until that substrate was utilized, and the cycle was repeated. When the bound substrate was essentially exhausted, cells detached from the underlying hydrophilic surface (Power and Marshall 1988). Even the nonadhesive *Vibrio* MH3 (Fig. 11.4d) was able to grow from the small starvation-survival form to normal size and then begin the division cycle when exposed to surface-bound stearic acid (Power and Marshall 1988). The dividing cells drifted away from the surface and completed the division cycle in the planktonic state.

An interesting adaptation ensuring reversibility of the sessile state has been described in *Acinetobacter calcoaceticus*, which adheres reversibly to epithelial cells and oil by means of thin fimbriae (Fig. 11.4c). The adhesion of this bacterium is reversed as a result of the production of an excessive amount of extracellular emulsan that surrounds and thus masks the adhesive properties of the fimbriae (Rosenberg et al. 1983). Another example of reversible adhesion has been described in the cyanobacterium *Phormidium*, which in its sessile state possesses a hydrophobic surface but under certain conditions produces a hydrophilic capsule, thus allowing the organism to revert to the planktonic state (Fattom and Shilo 1984).

These studies emphasize the ability of some prokaryotes to take advantage of substrates adsorbed to surfaces as well as reveals a variety of strategies for releasing daughter cells from the sessile to the planktonic state. As pointed out by Pedros-Alio

and Brock (1983), a simple division into sessile and planktonic forms is overly simplistic. Different bacteria have a variety of mechanisms to attach at surfaces, but they also possess a range of mechanisms for detachment in order to return to a planktonic existence.

Advantages of the Sessile State

Nutrient Availability

When a clean surface is immersed into a natural habitat, a molecular film rapidly forms on the surface as a result of adsorption of macromolecules and smaller hydrophobic molecules. This film serves to "condition" the surface, causing alterations in surface charge (Neihof and Loeb 1974) and surface free energy (Baier 1980). One of the most obvious advantages of the sessile state is the increased probability of access to nutrients accumulating at surfaces, particularly in flowing, oligotrophic conditions. ZoBell (1943) was the first to suggest that complex macromolecules adsorbed at surfaces would serve as concentrated sources of nutrients for organisms adhering at those surfaces. It was clearly demonstrated by Jannasch (1958) that the beneficial effect of surfaces in the presence of added complex nutrients only occurred at very low nutrient concentrations, where the level of nutrient in the aqueous phase was negligible and the nutrients had adsorbed to the surfaces.

Many investigators comparing the activities of bacteria in the sessile and planktonic states have employed simple soluble substrates such as glucose and amino acids (Azam and Hodson 1977; Berman 1975; Berman and Stiller 1977; Campbell and Baker 1978; Ferguson and Palumbo 1979; Fletcher 1979, 1986; Hanson and Wiebe 1977; Kirchman and Mitchell 1982; Pedros-Alio and Brock 1983; Riemann 1978). In natural habitats, and particularly in low nutrient situations, such soluble substrates would be rapidly utilized by planktonic bacteria and would rarely encounter a substratum surface. Similarly, many of these low-molecular-weight substrates cannot adsorb to surfaces and would not be expected to concentrate these. If the substrates do adsorb, their availability for bacterial utilization is often reduced substantially (Gordon and Milero 1985). In many field studies, filtration has been used to separate sessile and attached bacteria, but filtration can lead to problems in that (1) shear forces involved in filtration are sufficient to remove some reversibly attached bacteria that are feeding at surfaces (Hermansson and Marshall 1985) and (2) such reversibly attached bacteria may have fed, grown, and reproduced at the surface and then returned to the aqueous phase at some time prior to filtration (Power and Marshall 1988).

A more logical method of studying the activity of bacteria at surfaces is to provide substrates such as macromolecules or lower-molecular-weight hydrophobic molecules that are likely to adsorb at surfaces. Using surface-bound stearic acid as a model substrate, Kefford et al. (1982) and Kjelleberg et al. (1983) clearly demonstrated that a range of bacteria were capable of scavenging ^{14}C-labeled stearic acid from a surface. In particular, a reversibly adhering *Leptospira* species rapidly utilized the labeled fatty acid, and ^{14}C-labeled bacteria were readily recovered from the planktonic state. A similar result was obtained with the nonadhesive marine *Vibrio* MH3 (Hermansson and Marshall 1985), a result that emphasizes the fact that bacteria do not need to firmly adhere to surfaces in order to utilize substrates adsorbed at the surface. Subsequent studies have shown that starved bacteria adhering to surfaces where nutrients have accumulated not only metabolize the nutrients but are capable of cellular growth and reproduction (Kjelleberg et al. 1982; Power and Marshall 1988; see also *Fig. 11.4a–d*).

Protection from Harmful Factors

Sessile bacteria appear to be more resistant to the inhibitory effects of antibacterial agents, such as antibiotics, chlorine, and heavy metals (Costerton et al. 1981). In relatively thick biofilms, this apparent resistance may be the result of the reaction of the agents with the outer layers of cells and, in the case of chlorine and heavy metals, reaction with the extracellular polymer that makes up the matrix of the biofilm. There is increasing evidence, however, that bacteria attached to surfaces are inherently more resistant to certain antibacterial agents than are planktonic forms, but the mechanism of this increased resistance is not understood. Bacteria below the biofilm-water interface are also protected from external grazing by protozoa and metazoa. In addition, association of prokaryotes with various sizes of particles or colloidal clays can provide a degree of protection from parasitism by bacteriophage and *Bdellovibrio* as well as from predation by amoebae and the lytic effects of certain gliding bacteria (Roper and Marshall 1974, 1978).

Disadvantages of the Sessile State

Sedimentation

Although bacteria attached to particle surfaces may gain an advantage by utilization of adsorbed nutrients or by the dissolution of organic particles, such bacteria would sink to the sediments and would be unable to colonize new particle surfaces if mechanisms did not exist for their release or the release of daughter cells from the particle surfaces. As seen above, such mechanisms are common among sessile forms of bacteria (*Fig. 11.4*). It is precisely these phenomena of bacterial attachment, nutrient utilization, recycling, and detachment that are continually occurring within "marine snow" in the pelagic zone of oceans (Alldredge 1989).

Grazing

Zooplankton are capable of ingesting planktonic bacteria, but detritus feeders have been found to consume the bacteria

growing on detritus particles rather than ingest the particles themselves (Fenchel and Jørgensen 1977). Fenchel (1986) reported that the flagellate *Bodo* sp. spends about 45 s ingesting a bacterium from a surface, during which time the flagellate does not move. *Bodo* normally slides over the substratum at a velocity of 3.5 μm/s and only detects and ingests bacteria lying in a 1.0-μm-wide band along the path of the flagellate. Zooplankton grazing on biofilm surfaces, however, may play a useful role in maintaining the bacteria near the biofilm surface in an active state of growth. Amoebae have been observed grazing well within the matrix of a biofilm (Mack et al. 1975) (see *Fig. 11.3*).

Gradients

Decreasing gradients of nutrient and oxygen availability develop with increasing depth of a biofilm (*Fig. 11.3*) (Christensen and Characklis 1990). Such gradients form as a result of diffusional resistance within the biofilm and of utilization of the nutrients and oxygen by microorganisms within the biofilm. Consequently, aerobic organisms near the biofilm-water interface tend to be actively growing and create anoxic conditions at greater depths within the biofilm. If nitrate is present, then some microorganisms at depth in the biofilm are capable of using the nitrate as an alternative to oxygen as an electron acceptor. Other aerobic organisms tend to be inactive, or even lyse, within the anoxic zone, whereas strict anaerobes and fermentative bacteria may be active in such sites. In biofilms developed on metallic surfaces, the activity of sulfate-reducing bacteria (SRB) has been implicated in corrosion processes (Little et al. 1990).

Physiological Responses by Bacteria at Surfaces

Observed Responses

Probably the most obvious physiological response observed in bacteria associated with surfaces is cellular growth and, in some instances, reproduction (Jannasch 1958; Bott and Brock 1970; Kjelleberg et al. 1982; Pedros-Alio and Brock 1983; Power and Marshall 1988). Another possible response in bacteria to the physical presence of a surface is the time-dependent appearance of firm adhesion, which may indicate the induction of suitable bridging polymer production by the surface-associated bacteria (Marshall et al. 1971a).

The best documented response to a surface is the change observed in certain marine vibrios from a single, sheathed, polar flagellum in the planktonic stage to the production of multiple, lateral flagella when plated on an agar surface (Golten and Scheffers 1975; de Boer et al. 1975; Belas and Colwell 1982). Other reported responses include a reduction in size and an increase in endogenous respiration and in heat output by starving marine bacteria at interfaces in the absence of exogenous nutrients (Kjelleberg et al. 1982, 1983; Humphrey et al. 1983; Humphrey and Marshall 1984). Also, attached bacteria show an increase in resistance to antibacterial substances (Costerton et al. 1981).

Control of Responses

Silverman et al. (1984) have described two possible control mechanisms regulating bacterial responses at surfaces, namely, "responsive" and "variable" control (*Fig. 11.5*). Essentially, responsive control involves information processing, whereby the bacterium senses some environmental signal and responds accordingly. In the case of *Vibrio parahaemolyticus*, the response to a shift from an aqueous medium to an agar surface is to deregulate lateral flagella production (*Fig. 11.5a*). In the case of variable control, a fraction of the cells are preadapted, for example, to adhere to a particular surface, and individuals within the population are constantly switching among a variety of forms. For instance, a portion of the population may produce fimbriae and attach to epithelial cells (*Fig. 11.5b*). Nonadhesive variants of these cells arise and detach to return to the aqueous phase. Such phase variation in certain salmonellae results from a rearrangement of the DNA structure involving the inversion of part of the molecule containing a transcriptional control element.

Physicochemical Triggering of Responses

Using *lux* gene fusion mutants, Belas et al. (1986) studied the responsive control of lateral gene expression when *Vibrio parahaemolyticus* was transferred from liquid to agar medium. They were able to show conclusively that the physicochemical factor triggering lateral flagella production was increased viscosity. Whether this surface effect was entirely the result of viscosity or whether it was also related to a reduction in water activity has not been tested.

Another important factor at surfaces that would result in metabolic, as well as cellular growth and reproduction, responses is the adsorption of organic nutrients at surfaces (Kefford et al. 1982; Kjelleberg et al. 1981; Hermansson and Marshall 1985; Power and Marshall 1988). Enhanced phosphorus uptake by attached bacteria has also been reported by Paerl and Merkel (1982). A further situation involving possible adsorption phenomena at surfaces is the finding by Humphrey and Marshall (1984) that changes in size, endogenous respiration, and heat output in starving marine bacteria at surfaces could be reproduced in the presence of surfactants and even when no surface was present. Many bacteria in nature produce surfactants, and these surfactants could adsorb to surfaces where they might trigger various responses in other bacteria adhering to the surfaces.

Other possible explanations for the triggering of physiological responses in bacteria at surfaces include alterations in the proton motive force on the face of the cell nearest the surface (Ellwood et al. 1982) and possible cell deformation near a surface (Fletcher 1984).

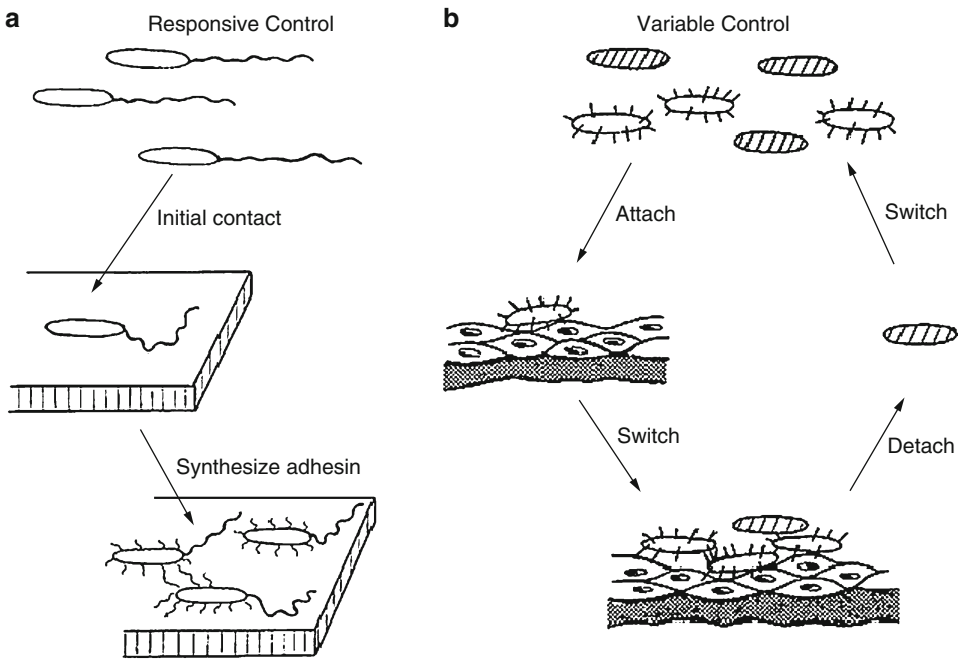


Fig. 11.5

Strategies for responsive and for variable control of adhesive substance expression. (a) Responsive control, as shown by a shift from polar to lateral flagella in *Vibrio parahaemolyticus*. (b) Variable control, in which a fraction of the cells are preadapted to the fimbriated state and attach to epithelial cells. Nonfimbriated variants detach and return to the aqueous phase (From Silverman et al. 1984)

Conclusions

Although the sessile state is very common in bacteria in natural habitats, it is not a state limited to particular groups of organisms. All sessile bacteria are derived from the planktonic state, and, in addition to active growth and metabolism at surfaces, these sessile organisms have also evolved a variety of methods to ensure that representatives of the population can return to the planktonic state. Such mechanisms include direct release of daughter cells, changes in the hydrophobicity of the sessile cells or of the substratum surface, exclusively reversible adhesion (subject to removal by gentle shear forces), and, possibly, enzymatic degradation of adhesive bridging polymers.

Planktonic bacteria, on the other hand, possess a wide range of mechanisms whereby they can adhere to a variety of surfaces. In some instances these bacteria possess preformed adhesive polymers, whereas in other cases the bacteria appear to produce appropriate polymers following association with the surface. There is increasing evidence for responsive control of a number of physiological functions evident only at surfaces, but more detailed investigations are required to elucidate the nature of these physicochemical triggering mechanisms.

References

Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW (1983) Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol 46:90–97

Alldredge AL (1989) The significance of suspended detrital aggregates of marine snow as microhabitats in the pelagic zone of the ocean. In: Hattori T, Ishida Y, Maruyama Y, Morita RY, Uchida A (eds) Recent advances in microbial ecology. Japan Scientific Societies Press, Tokyo, pp 108–112

Angles ML (1988) Microbial colonization of *Zostera capricorni* in Botany Bay. B.Sc. (honors) thesis, University of New South Wales

Azam F, Hodson RE (1977) Size distribution and activity of marine microheterotrophs. Limnol Oceanogr 22:492–501

Baier RE (1980) Substrate influence on adhesion of microorganisms and their resultant new surface properties. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 59–104

Beachey EH (ed) (1980) Bacterial adherence. Chapman and Hall, London

Belas MR, Colwell RR (1982) Adsorption kinetics of laterally and polarly flagellated ***Vibrio***. J Bacteriol 151:1568–1580

Belas R, Simon M, Silverman M (1986) Regulation of lateral flagella gene transcription in ***Vibrio parahaemolyticus***. J Bacteriol 167:210–218

Berman T (1975) Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake Mar. Biol 33:215–220

Berman T, Stiller M (1977) Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labeling and differential filtration. Microb Ecol 3:279–288

Bitton G, Marshall KC (eds) (1980) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York

Bott TL, Brock TD (1970) Growth and metabolism of periphytic bacteria: methodology. Limnol Oceanogr 15:333–342

Bowden GHW, Ellwood DC, Hamilton IR (1979) Microbial ecology of the oral cavity. Adv Microb Ecol 3:135–217

Bright JJ, Fletcher M (1983) Amino acid assimilation and electron transport system activity in attached and free-living marine bacteria. Appl Environ Microbiol 45:818–825

Brock TD (1971) Microbial growth rates in nature. Bacteriol Rev 35:39–58

Busscher HJ, Weerkamp AH (1987) Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiol Rev 46:165–173

Busscher HJ, Uyen MHMJC, Weerkamp AH, Postma AH, Arends J (1986) Reversibility of adhesion of oral streptococci to solids. FEMS Microbiol Lett 35:303–306

Caldwell DE, Germida JJ (1985) Evaluation of difference imagery for visualizing and quantitating microbial growth. Can J Microbiol 31:35–44

Campbell PGC, Baker JH (1978) Estimation of bacterial production in freshwaters by the simultaneous measurement of [^{35}S] sulfate and D-[^{3}H] glucose uptake in the dark. Can J Microbiol 24:939–946

Characklis WG (1980) Biofilm development and destruction, U.S. Report. Electric Power Research Institute, Palo Alto, CA, 902–1

Characklis WG (1981a) Fouling biofilm development: a process analysis. Biotechnol Bioeng 23:1923–1960

Characklis WG (1981b) Microbial fouling: a process analysis. In: Somerscales EFC, Knudsen JG (eds) Fouling of heat transfer equipment. Hemisphere, Washington, DC, pp 251–291

Characklis WG, Marshall KC (1990) Biofilms: a basis for an interdisciplinary approach. In: Characklis WG, Marshall KC (eds) Biofilms. Wiley-Interscience, New York, pp 3–15

Christensen BE, Characklis WG (1990) Physical and chemical properties of biofilms. In: Characklis WG, Marshall KC (eds) Biofilms. Wiley-Interscience, New York, pp 93–130

Corpe WA (1973) Microfouling: the role of primary film-forming bacteria. In: Acker RF, Brown BF, de Palma JR, Iverson WP (eds) Proceedings of the third international congress on marine corrosion and fouling. Northwestern University Press, Evanston, IL, pp 598–609

Corpe WA (1980) Microbial surface components involved in adsorption of microorganisms onto surfaces. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 105–144

Costerton JW, Irvin RJ, Cheng KJ (1981) The bacterial glycocalyx in nature and disease. Annu Rev Microbiol 35:299–324

Dawson MP, Humphrey BA, Marshall KC (1981) Adhesion, a tactic in the survival strategy of a marine vibrio during starvation. Curr Microbiol 6:195–198

De Boer WE, Golten C, Scheffers WA (1975) Effects of some physical factors on flagellation and swarming of *Vibrio alginolyticus*. Neth J Sea Res 9:197–213

Dempsey MJ (1981) Marine bacterial fouling: a scanning electron microscope study. Mar Biol 61:305–315

Dexter SC, Sullivan JD Jr, Williams J III, Watson SW (1975) Influence of substrate wettability on the attachment of marine bacteria to various surfaces. Appl Microbiol 30:298–308

Duxbury T (1977) A microperfusion chamber for studying the growth of bacterial cells. J Appl Bacteriol 42:247–251

Ellwood DC, Keevil CW, Marsh PD, Brown CM, Wardell JN (1982) Surface associated growth. Philos Trans R Soc Lond B297:517–532

Fattom A, Shilo M (1984) Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. Appl Environ Microbiol 47:135–143

Fenchel T (1986) The ecology of heterotrophic microflagellates. Adv Microb Ecol 9:57–97

Fenchel T, Jørgensen BB (1977) Detritus food chains of aquatic environments. Adv Microb Ecol 1:1–58

Ferguson RL, Palumbo AV (1979) Distribution of suspended bacteria in neritic waters south of Long Island during stratified conditions. Limnol Oceanogr 24:697–705

Fletcher M (1979) A microautoradiographic study of the activity of attached and free-living bacteria. Arch Microbiol 122:271–274

Fletcher M (1980) The question of passive versus active attachment mechanisms in non-specific bacterial adhesion. In: Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B (eds) Microbial adhesion to surfaces. Ellis Horwood, Chichester, pp 197–210

Fletcher M (1984) Comparative physiology of attached and free-living bacteria. In: Marshall KC (ed) Microbial adhesion and aggregation. Springer, Berlin, pp 223–232

Fletcher M (1986) Measurement of glucose utilization by ***Pseudomonas fluorescens*** that are free living and that are attached to surfaces. Appl Environ Microbiol 52:672–676

Fletcher M (1988) Attachment of ***Pseudomonas fluorescens*** to glass and influence of electrolytes on bacterium-substratum separation distance. J Bacteriol 170:2027–2030

Fletcher M, Floodgate GD (1973) An electron microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J Gen Microbiol 74:325–334

Fletcher M, Loeb GI (1979) Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. Appl Environ Microbiol 37:67–72

Geesey GG, Richardson WT, Yeomans HG, Irvin RT, Costerton JW (1977) Microscopic examination of natural sessile bacterial populations from an alpine stream. Can J Microbiol 23:1733–1736

Geesey GG, Mutch R, Costerton JW, Green RB (1978) Sessile bacteria: an important component of the microbial population in small mountain streams. Limnol Oceanogr 23:1214–1223

Gerchakov SM, Marszalek DS, Roth FJ, Udey LR (1977) Succession of periphytic microorganisms on metal and glass surfaces 203–211 V. In: Romanovsky I (ed) Proceedings of the 4th International Congress on Marine Corrosion and Fouling. Centre de Recherches et d'Etudes Oceangraphiques, Boulogne, France

Golten C, Scheffers WA (1975) Marine vibrios isolated from water along the Dutch coast. Neth J Sea Res 9:351–364

Gordon AS, Milero FJ (1985) Adsorption mediated decrease in the biodegradation rate of organic compounds. Microb Ecol 11:289–298

Gristina AG (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237:1588–1595

Guckert JB, Antworth CB, Nichols PD, White DC (1985) Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol Ecol 31:147–158

Hanson RB, Wiebe WJ (1977) Heterotrophic activity associated with particulate size fractions in a *Spartina alterniflora* salt-marsh estuary, Sapelo Island, Georgia, U.S.A., and the continental shelf waters. Mar Biol 42:321–330

Harris PJ (1972) Micro-organisms in surface films from soil crumbs. Soil Biol Biochem 4:105–106

Hermansson M, Marshall KC (1985) Utilization of surface localized substrate by non-adhesive marine bacteria. Microb Ecol 11:91–105

Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228

Humphrey BA, Marshall KC (1984) The triggering effect of surfaces and surfactants on heat output, oxygen consumption and size reduction of a starving marine ***Vibrio***. Arch Microbiol 140:166–170

Humphrey BA, Kjelleberg S, Marshall KC (1983) Responses of marine bacteria under starvation conditions at a solid-water interface. Appl Environ Microbiol 45:43–47

Jannasch HW (1958) Studies on planktonic bacteria by means of a direct membrane filter method. J Gen Microbiol 18:609–620

Jordan TL, Staley JT (1976) Electron microscopic study of succession in the periphyton communities of Lake Washington. Microb Ecol 2:241–251

Kefford B, Kjelleberg S, Marshall KC (1982) Bacterial scavenging: utilization of fatty acids localized at a solid–liquid interface. Arch Microbiol 133:257–260

Kirchman D, Mitchell R (1982) Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl Environ Microbiol 43:200–209

Kjelleberg S, Humphrey BA, Marshall KC (1982) The effect of interfaces on small starved marine bacteria. Appl Environ Microbiol 43:1166–1172

Kjelleberg S, Humphrey BA, Marshall KC (1983) Initial phases of starvation and activity of bacteria at surfaces. Appl Environ Microbiol 46:978–984

Kölbel-Boelke J, Hirsch P (1989) Comparative physiology of biofilm and suspended organisms in the groundwater environment. In: Characklis WG, Wilderer PA (eds) Structure and function of biofilms Dahlem Konferenzen. Wiley, New York, pp 221–238

La Motta EJ (1976) Kinetics of growth and substrate uptake in a biological film system. Appl Environ Microbiol 31:286–293

Lawrence JR, Caldwell DE (1987) Behavior of bacterial stream populations within the hydrodynamic boundary layers of surface microenvironments. Microb Ecol 14:15–27

Lee A (1980) Normal flora of animal intestinal surfaces. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 145–173

Lee A (1985) Neglected niches: the microbial ecology of the gastrointestinal tract. Adv Microb Ecol 8:115–162

Little BJ, Wagner PA, Characklis WG, Lee W (1990) Microbial corrosion. In: Characklis WG, Marshall KC (eds) Biofilms. Wiley-Interscience, New York, pp 635–670

Loeb GI (1980) Measurement of microbial marine fouling films by light section microscopy. Mar Tech Soc J 14:17–30

Lupton FS, Marshall KC (1981) Specific adhesion of bacteria to heterocysts of *Anabaena* spp. and its ecological significance. Appl Environ Microbiol 42:1085–1092

Mack WN, Mack JP, Ackerson AO (1975) Microbial film development in a trickling filter. Microb Ecol 2:215–226

Marshall KC (1975) Clay mineralogy in relation to survival of soil bacteria. Ann Rev Phytopathol 13:357–373

Marshall KC (1976) Interfaces in microbial ecology. Harvard University Press, Cambridge, MA

Marshall KC (ed) (1984) Microbial adhesion and aggregation. Springer, Berlin

Marshall KC (1985) Mechanisms of bacterial adhesion at solid-water interfaces. In: Savage DC, Fletcher M (eds) Bacterial adhesion: mechanisms and physiological significance. Plenum Press, New York, pp 131–161

Marshall KC (1986a) Adsorption and adhesion processes in microbial growth at interfaces. Adv Colloid Interface Sci 25:59–86

Marshall KC (1986b) Microscopic methods for the study of bacterial behavior at inert surfaces. J Microbiol Methods 4:217–227

Marshall KC, Cruickshank RH (1973) Cell surface hydrophobicity and the orientation of certain bacteria at interfaces. Arch Mikrobiol 91:29–40

Marshall KC, Stout R, Mitchell R (1971a) Mechanism of the initial events in the sorption of marine bacteria to surfaces. J Gen Microbiol 68:337–348

Marshall KC, Stout R, Mitchell R (1971b) Selective sorption of bacteria from seawater. Can J Microbiol 17:1413–1416

Marszalek DS, Gerchakov SM, Udey LR (1979) Influence of substrate composition on marine microfouling. Appl Environ Microbiol 38:987–995

McEldowney S, Fletcher M (1986) Effect of growth conditions and surface characteristics of aquatic bacteria on their attachment to solid surfaces. J Gen Microbiol 132:513–523

Moriarty DJW (1977) Improved method using muramic acid to estimate biomass of bacteria in sediments. Oecolgia 26:317–323

Moriarty DJW (1986) Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv Microb Ecol 9:245–292

Morita RY (1982) Starvation-survival of heterotrophs in the marine environment. Adv Microb Ecol 6:171–198

Neihof R, Loeb G (1974) Dissolved organic matter in seawater and the electric charge of immersed surfaces. J Mar Res 32:5–12

Newman HN (1980) Retention of bacteria on oral surfaces. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 207–251

Nichols PD, Henson JM, Guckert JB, Nivens DE, White DC (1985) Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria polymer mixtures and biofilms. J Microbiol Methods 4:79–94

Pace NR, Stahl DA, Lane DJ, Olsen GJ (1986) The analysis of natural microbial populations by ribosomal RNA sequences. Adv Microb Ecol 9:1–55

Paerl HW (1980) Attachment of microorganisms to living and detrital surfaces in freshwater systems. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 375–402

Paerl HW, Merkel SM (1982) Differential phosphorus assimilation in attached vs. unattached microorganisms. Arch Hydrobiol 93:125–134

Pedros-Alio C, Brock TD (1983) The importance of attachment to particles for planktonic bacteria. Arch Hydrobiol 98:354–379

Perfil'ev BV, Gabe DR (1969) Capillary methods of investigating micro-organisms. University of Toronto Press, Toronto (translated from Russian by J. M. Shewan)

Pethica BA (1980) Microbial and cell adhesion. In: Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B (eds) Microbial adhesion to surfaces. Ellis Horwood, Chichester, pp 19–45

Phillips MW, Lee A (1983) Isolation and characterization of a spiral bacterium from the crypts of rodent gastrointestinal tracts. Appl Environ Microbiol 45:675–683

Power K, Marshall KC (1988) Cellular growth and reproduction of marine bacteria on surface-bound substrate. Biofouling 1:163–174

Pringle JH, Fletcher M (1983) Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces Appl. Environ Microbiol 45:811–817

Revsbech NP, Jørgensen BB (1986) Microelectrodes: their use in microbial ecology. Adv Microb Ecol 9:252–293

Riemann B (1978) Differentiation between heterotrophic and photosynthetic plankton by size fractionation, glucose uptake ATP, and chlorophyll content. Oikos 31:358–367

Roper MM, Marshall KC (1974) Modification of the interaction between ***Escherichia coli*** and bacteriophage in saline sediment. Microb Ecol 1:1–14

Roper MM, Marshall KC (1978) Effects of a clay mineral on microbial predation and parasitism on ***Escherichia coli***. Microb Ecol 4:279–289

Rosenberg E, Gottlieb A, Rosenberg M (1983) Inhibition of bacterial adherence to epithelial cells and hydrocarbons by emulsan. Infect Immun 39:1024–1028

Rovira AD, Foster RD, Martin JK (1979) Note on terminology: Origin, nature and nomenclature of the organic materials in the rhizosphere. In: Harley JL, Russell RS (eds) The soil root interface. Academic, London, pp 1–4

Rutter PR, Vincent B (1980) The adhesion of microorganisms to surfaces: physico-chemical aspects. In: Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B (eds) Microbial adhesion to surfaces. Ellis Horwood, Chichester, pp 79–93

Savage DC (1980) Colonization by and survival of pathogenic bacteria on intestinal mucosal surfaces. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley-Interscience, New York, pp 175–206

Savage DC (1984) Activities of microorganisms attached to living surfaces. In: Marshall KC (ed) Microbial adhesion and aggregation Dahlem Konferenzen. Springer, Berlin, pp 233–249

Savage DC, Fletcher MM (eds) (1985) Bacterial adhesion: mechanisms and physiological significance. Plenum Press, New York

Silverman M, Belas R, Simon M (1984) Genetic control of bacterial adhesion. In: Marshall KC (ed) Microbial adhesion and aggregation. Springer, Berlin, pp 95–107

Skerman VBD (1968) A new type of micromanipulator and microforge. J Gen Microbiol 54:287–297

Staley JT (1971) Growth rates of algae determined in situ using an immersed microscope. J Phycol 7:13–17

Stotzky G (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang PM, Schnitzer M (eds) Interactions of soil minerals with natural organics and microbes, Soil Science Society of America Special Publication No. 17. Soil Science Society of America, Madison, WI, pp 305–428

Switalski L, Höök M, Beachey E (eds) (1989) Molecular mechanisms of microbial adhesion. Springer, New York

Szewzyk U, Schink B (1988) Surface colonization by and life cycle of *Pelobacter acidigallici* studied in a continuous flow microchamber. J Gen Microbiol 134:183–190

Weller R, Ward DM (1989) Selective recovery of 16SrRNA sequences from natural microbial communities in the form of cDNA. Appl Environ Microbiol 55:1818–1822

ZoBell CE (1943) The effect of solid surfaces upon bacterial activity. J Bacteriol 46:39–56

Zvyagintsev DG (1962) Adsorption of microorganisms by soil particles. Soviet Soil Sci 140–144

12 The Phototrophic Way of Life

Jörg Overmann[1] · *Ferran Garcia-Pichel*[2]
[1]Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany
[2]School of Life Sciences, Arizona State University, Tempe, AZ, USA

Introduction

Photosynthesis is the utilization of radiant energy for the synthesis of complex organic molecules. The phototrophic way of life implies the capture of electromagnetic energy (see section ❯ "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter), its conversion into chemical energy (see section ❯ "Conversion of Light into Chemical Energy" in this chapter), and its use for cellular maintenance and growth (see section ❯ "Efficiency of Growth and Maintenance Energy Requirements" in this chapter). The mode of growth in which photosynthesis is coupled to the reduction of carbon dioxide into organic molecules is defined as photoautotrophy. The solar electromagnetic energy reaching the Earth's surface (a flux of 163 $W \cdot m^{-2}$; see section ❯ "Light Energy and the Spectral Distribution of Radiation") represents 48 % of that reaching the top of the atmospher (known as the solar constant = 1.361 $kW \cdot m^{-2}$, corresponding to a flux of 340 $W \cdot m^{-2}$, and representing a total incoming power of 173 PW; Kopp and Lean 2011). Despite its losses in the atmosphere due to reflection, absorption/reflection, or evaporation, the electromagnetic energy available still surpasses the energy contributed by all other sources by at least 4–5 orders of magnitude. Thus, present-day geothermal energy derived from stored heat and heat produced by radioactive decay is $\leq$0.08 $W \cdot m^{-2}$ or 47 TW total (Davies and Davies 2010; for primordial Earth the estimate is ~0.0062 $W \cdot m^{-2}$, and was

This chapter is dedicated to the memory of Prof. Dr. Dr. h.c. Norbert Pfennig (July 8, 1925—February 11, 2008), one of the great pioneers in the research field of photosynthetic bacteria and other anaerobes.

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_51,

delivered by electric discharge, radioactivity, volcanism, or meteoritic impacts; Mauzerall 1992).

At present, the flux of electromagnetic energy supports a total primary production of 104.9×10^9 t C·year^{-1} (205.7 g C·m^{-2}·year^{-1}) based on satellite-derived estimates of the terrestrial vegetation cover and of sea-surface chlorophyll for the oceans (Field et al. 1998). This value exceeds an earlier estimate (172.5×10^9 t dry weight·year^{-1}, equivalent to 168 g C·m^{-2}·year^{-1}; Whittaker and Likens 1975). If global primary production is converted to energy units (39.9 kJ·g C^{-1}, assuming that all photosynthetic products are carbohydrate), just 0.26 W·m^{-2} (total, 133 TW) only 0.16 % of the solar energy flux available on the Earth's surface are converted into chemical energy. And yet, even at this low efficiency, the chemical energy stored in organic carbon significantly exceeds geothermal energy. As a consequence, photosynthesis directly or indirectly drives the biogeochemical cycles in all extant ecosystems of the planet. Even hydrothermal vent communities, which use inorganic electron donors of geothermal origin and assimilate CO_2 by chemolithoautotrophy (rather than photoautotrophy), still depend on the molecular O_2 generated by oxygenic phototrophs outside of these systems (Jannasch 1989).

Global net primary production has been estimated to amount to 65.5×10^9 t C·year^{-1} (Haberl et al. 2007), equivalent to 83 TW. The current energy consumption of humankind amounts to 10 TW. This demand is largely (i.e., by 85 %) furnished by fossil energy sources. By contrast, overall consumption of global net primary production by humans amounts to 23.8 % (Haberl et al. 2007) and hence 19.8 TW. Given (1) the increasing human appropriation of photosynthetic production, (2) the low global efficiency of energy conversion by photosynthesis and (3) its significant role as driver of global biogeochemical cycles, the understanding of the architecture and functioning of photosynthetic systems is not only of principle interest for basic research but has direct relevance for the our future global food and energy supplies.

Several lines of evidence indicate that in the early stages of biosphere evolution, prokaryotic organisms were once responsible for the entire global photosynthetic carbon fixation. Today, terrestrial higher plants account for the vast majority of photosynthetic biomass; the chlorophyll bound in light-harvesting complex LHCII of green chloroplasts alone represents 50 % of the total chlorophyll on Earth (Sidler 1994), even if microbial biosynthetic biomass can locally contribute a significant proportion of ecosystem biomass in the topsoils of arid and polar lands, where plant cover is restricted. In stark contrast to terrestial systems, the biomass of primary producers in the marine realm is very low (0.2 % of the global value). However, the biomass turnover of marine photosynthetic microorganisms is some 700 times faster than that of terrestrial higher plants. Thus, marine photosynthetic organisms contribute significantly to total primary productivity ($55 \cdot 10^9$ t dry weight·year^{-1}, or 44 % of the global primary production). Because the biomass of cyanobacterial picoplankton (see section ❯ "Habitats of Phototrophic Prokaryotes" in this chapter) can amount to 67 % of the oceanic plankton, and their photosynthesis up to 80 % in the marine environment (Campbell et al. 1994; Goericke and Welschmeyer 1993; Liu et al. 1997; Waterbury et al. 1986), prokaryotic primary production is still significant on a global scale. A single monophyletic group of marine unicellular cyanobacterial strains encompassing the genera *Prochloroccoccus* and *Synechococcus* with a global biomass in the order of a billion of metric tons (Garcia-Pichel 1999) may be responsible for the fixation of as much as 10–25 % of the global primary productivity. Additionally, prokaryotic (cyanobacterial) photosynthesis is still locally very important in other habitats such as cold (Friedmann and Ocampo 1976) and hot deserts (Garcia-Pichel and Belnap 1996) and hypertrophic lakes.

Today, the significance of anoxygenic photosynthesis for global carbon fixation is limited for two reasons. On the one hand, phototrophic sulfur bacteria (the dominant anoxygenic phototrophs in natural ecosystems) form dense accumulations only in certain lacustrine environments and in intertidal sandflats. The fraction of lakes and intertidal salt marshes that harbor anoxygenic phototrophic bacteria is unknown, but these ecosystems altogether contribute only 4 % to global primary production (Whittaker and Likens 1975). In those lakes harboring phototrophic sulfur bacteria, an average of 28.7 % of the primary production is anoxygenic (Overmann 1997). Consequently, the amount of CO_2 fixed by anoxygenic photosynthesis must contribute much less than 1 % to global primary production. On the other hand, anoxygenic photosynthesis depends largely on reduced inorganic compounds that originate in the anaerobic degradation of organic carbon. Since this carbon was already fixed by oxygenic photosynthesis, the CO_2-fixation of anoxygenic phototrophic bacteria does not lead to a net increase in organic carbon available to higher trophic levels. For this reason, the CO_2-assimilation by anoxygenic phototrophic bacteria has been termed "secondary primary production" (Pfennig 1978). Therefore, capture of light energy by anoxygenic photosynthesis merely compensates for the degradation of organic carbon in the anaerobic food chain. Geothermal sulfur springs are perhaps the only exception since their electron donors (like sulfides) are of abiotic origin. However, because sulfur springs are rather scarce, anoxygenic photosynthetic carbon fixation of these ecosystems also appears to be of minor significance on a global scale.

The scientific interest in anoxygenic phototropic bacteria stems from (1) the simple molecular architecture and variety of their photosystems, which makes anoxygenic phototrophic bacteria suitable models for the biochemical and biophysical study of photosynthetic mechanisms; (2) the considerable diversity of anoxygenic phototrophic bacteria, which has implications for reconstructing the evolution of photosynthesis; and (3) the biogeochemical and ecological consequences for the cycles of carbon and sulfur as mediated by the dense populations of phototrophic bacteria in natural ecosystems.

All known microorganisms use two functional principles for the conversion of light into chemical energy. Chlorophyll-based systems are widespread among members of the domain Bacteria and consist of a light-harvesting antenna and reaction centers. In the latter, excitation energy is converted into a redox gradient across the membrane. A second system, the retinal-based

bacteriorhodopsin system is found in members of a monophyletic group within the domain Archaea and in a variety of bacteria. These prokaryotes lack an antenna system and use light energy for the direct translocation of protons across the cytoplasmic membrane.

The advent of modern genetic and biochemical methods has led to a considerable gain in knowledge of the molecular biology of phototrophic prokaryotes. At the same time, microbial ecologists have found these microorganisms of considerable interest and now frequently use molecular methods to investigate natural populations. The present chapter is limited to the discussion of phototrophic bacteria and attempts to link the physiology, ecology, and evolution of phototrophic bacteria to a molecular basis. Emphasis is placed on those molecular structures or functions that have evident adaptive value. This integrative view may provide a more solid foundation for understanding the biology of photosynthetic prokaryotes.

Taxonomy of Phototrophic Prokaryotes

The capacity for chlorophyll-based photosynthetic energy conversion so far has been found in six of the 35 (Cole et al. 2009; *Table 12.1*) currently recognized bacterial phyla (based on 16S rRNA gene phylogenies some 100 phyla are recognized; Achtman and Wagner 2008). These are: the *Chloroflexus* subgroup, the green sulfur bacteria (*Chlorobi*), the *Proteobacteria*, the *Acidobacteria*, the *Cyanobacteria*, and the Firmicutes (*Heliobacteriaceae*). With the exception of the *Cyanobacteria*, phototrophic bacteria perform anoxygenic photosynthesis, a process that is not accompanied by photochemical cleavage of water and that therefore does not lead to the formation of molecular oxygen. Based on their phenotypic characters, anoxygenic phototrophic bacteria had been divided previously into the five families *Rhodospirillaceae*, *Chromatiaceae*, *Ectothiorhodospiraceae*, *Chlorobiaceae*, and *Chloroflexaceae* (Trüper and Pfennig 1981). However, 16S rRNA oligonucleotide cataloguing and 16S rRNA sequence comparisons have revealed that the *Proteobacteria* and the *Chloroflexus* subgroup both contain nonphototrophic representatives (Woese 1987; *Table 12.1*). Recently, a chemotrophic member was also isolated for the phylum *Chlorobi* (Iino et al. 2010) and its genome was sequenced (Liu et al. 2012a). Nonphototrophic representatives of the cyanobacterial lineage are so far unknown whereas the currently recognized percentage of phototrophic species in the five other phyla ranges from 93 % in *Chlorobi*, over 26 % in *Chloroflexi* and 3.7 % and 3.5 % in *Proteobacteria* and *Acidobacteria*, to 0.5 % in the *Firmicutes* (*Table 12.1*). Based on these considerations, the use of light as an energy source for growth is not limited to a phylogenetically coherent group of bacteria.

Phototrophic *Chloroflexi* are Gram-negative, filamentous bacteria which exhibit gliding motility. Cells of *Oscillochloris chrysea* are an exception since they stain Gram-positive. Within the filaments, cells are uniseriately arranged. Cell division occurs by simple fission and branching of filaments is not observed. The chemical features of the *Chloroflexi* cell wall resemble those of Gram-positive bacteria, however, since the peptidoglycan contains L-ornithine as diamino acid, forms a complex with polysaccharide, and lacks lipopolysaccharide-containing outer membrane as well as lipoproteins (Castenholz 2001b). Within the *Chloroflexaceae*, four different species (*Chloroflexus aurantiacus*, *Chloroflexus aggregans*, *Heliothrix oregonensis*, *Roseiflexus castenholzii*) of filamentous multicellular phototrophs have been described. All four are thermophilic and grow photoorganoheterotrophically. In addition four mesophilic species (*Oscillochloris chrysea*, *Oscillochloris trichoides*, *Chloronema giganteum*, *Chloronema spiroideum*) have been affiliated with the *Chloroflexi* based on their multicellular filaments, gliding motility, and the presence of chlorosomes containing bacteriochlorophylls *c* or *d* (Pfennig and Trüper 1989). *Oscillochloris* spp. lack a sheath and form BChl *c*. *Oscillochloris trichoides* differs from members of the *Chloroflexaceae* by employing the Calvin cycle for autotrophic CO_2-fixation, being unable to grow chemoheterotrophically, by its phylogenetic divergence and its low DNA-DNA similarity. Consequently, the family *Oscillochloridae* was proposed to accomodate *O. trichoides* and relatives (Keppen et al. 2000). *Chloronema* spp. are surrounded by a sheath and contain BChl *d* as the main photosynthetic pigment. *Chloronema*-type green trichomes often constitute a major fraction of all phototrophic bacterial cells in the chemocline of freshwater lakes (Overmann and Tilzer 1989). A strain of *Chloronema* was later shown to group within the *Chloroflexaceae* (Gich et al. 2001). With the exceptions of *Heliothrix oregonensis* and *Roseiflexus castenholzii*, all species mentioned contain chlorosomes as distinct light-harvesting structures (*Fig. 12.1*). The genera *Chloroflexus*, *Chloronema*, *Roseiflexus*, *Heliothrix*, and *Oscillochloris* constitute a natural group which is also named the "filamentous anoxygenic phototrophic (FAP) bacteria."

A considerable number of additional 16S rRNA gene sequence types of *Chloroflexi* have been discovered (RDP database; Cole et al. 2009), including not only thermophilic relatives of *Roseiflexus castenholzii* (Boomer et al. 2002) but also *Chloroflexaceae* sequence types in mesophilic marine and hypersaline microbial mats (Klappenbach and Pierson 2004; Ley et al. 2006). Yet to be cultivated axenically, nonthermophilic "*Chloroflexus*-like" organisms are also known from intertidal benthic environments (Pierson et al. 1994) and from cold freshwater sulfidic springs (F. Garcia-Pichel, unpublished observation). "*Candidatus* Chlorothrix halophila" is a mesophilic halophile that phylogenetically branches deeply within the FAB (Klappenbach and Pierson 2004). *Oscillochloris trichoides* (Keppen et al. 1994) was isolated from freshwater sediments. A systematic and culture-independent analysis of *Chloroflexi* in nine North American stratified freshwater lakes yielded 10 additional sequence types that were phylogenetically related to the *Chloroflexaceae* (Gich et al. 2001). Together, these data indicate a larger diversity and more widespread occurrence of the "filamentous anoxygenic phototrophic bacteria" than was previously recognized (Overmann 2008).

Green sulfur bacteria represent a coherent and isolated group within the domain Bacteria. Until recently, isolates of

Table 12.1

Comparative analysis of the numbers of 16S rRNA gene sequences affiliated with the currently recognized bacterial phyla based on the Hierarchy Browser of the Ribosomal Database Project (Cole et al. 2009) release 10, the number of type strains described for each phylum based on database entries in the Living Tree Project (Munoz et al. 2011) release LTPs106, the number of validly described species based on the Nomenclature-up-to-date database (DSMZ 2012), and the number of validly described species of anoxygenic or oxygenic phototrophs. The number of available strains of phototrophs listed in the DSMZ database and RDP is provided as well. Note that the category "phylum" is not covered by the Rules of the Bacteriological Code; hence, some of the phyla listed are not recognized in LTP (Data as of May 2012)

Bacterial phylum	No. of 16S rRNA gene sequences				
	Total	Type strains (LTP)	Species		Phototr strains[a]
			Total	Phototroph[a]	
Acidobacteria	53,582	12	28	1[b]	1[b]
Actinobacteria	244,053	2,260	2,448		
Aquificae	1,806	28	28		
Armatimonadetes	2,409	2	2		
Bacteroidetes	268,318	722	842		
Caldiserica	347	1	1		
Chlamydiae	991	13	13		
Chlorobi	2,714	11	15	14	104[b]
Chloroflexi	33,833	19	25	7	18
Chrysiogenetes	11	4	4		
Cyanobacteria/Chloroplast	53,352	7	7	7 (>1,000[b])	(>750[b])
Deferribacteres	1,214	12	12		
Deinococcus-Thermus	3,239	66	73		
Dictyoglomi	55	2	2		
Elusimicrobia	359	1	1		
Fibrobacteres	969	3	3		
Firmicutes	618,718	1,701	1,911	9	23
Fusobacteria	8,698	38	38		
Gemmatimonadetes	3,537	1	1		
Lentisphaerae	2,191	2	2		
Nitrospira	5,789	8	10		
Planctomycetes	19,092	12	17		
Proteobacteria	720,640	3,193	3,664	137	544
Spirochaetes	15,614	72	115		
Synergistetes	2,262	16	19		
Tenericutes	6,060	184	205		
Thermodesulfobacteria	179	7	7		
Thermotogae	907	35	39		
Verrucomicrobia	18,937	32	34		
BRC1	553	0	0		
OD1	616	0	0		
OP11	267	0	0		
SR1	922	0	0		
TM7	3,307	0	0		
WS3	916	0	0		

[a]Only true phototrophs, and not aerobic anoxygenic phototrophs or chloroplasts, are listed

[b]Based on additional literature searches, alleged species included

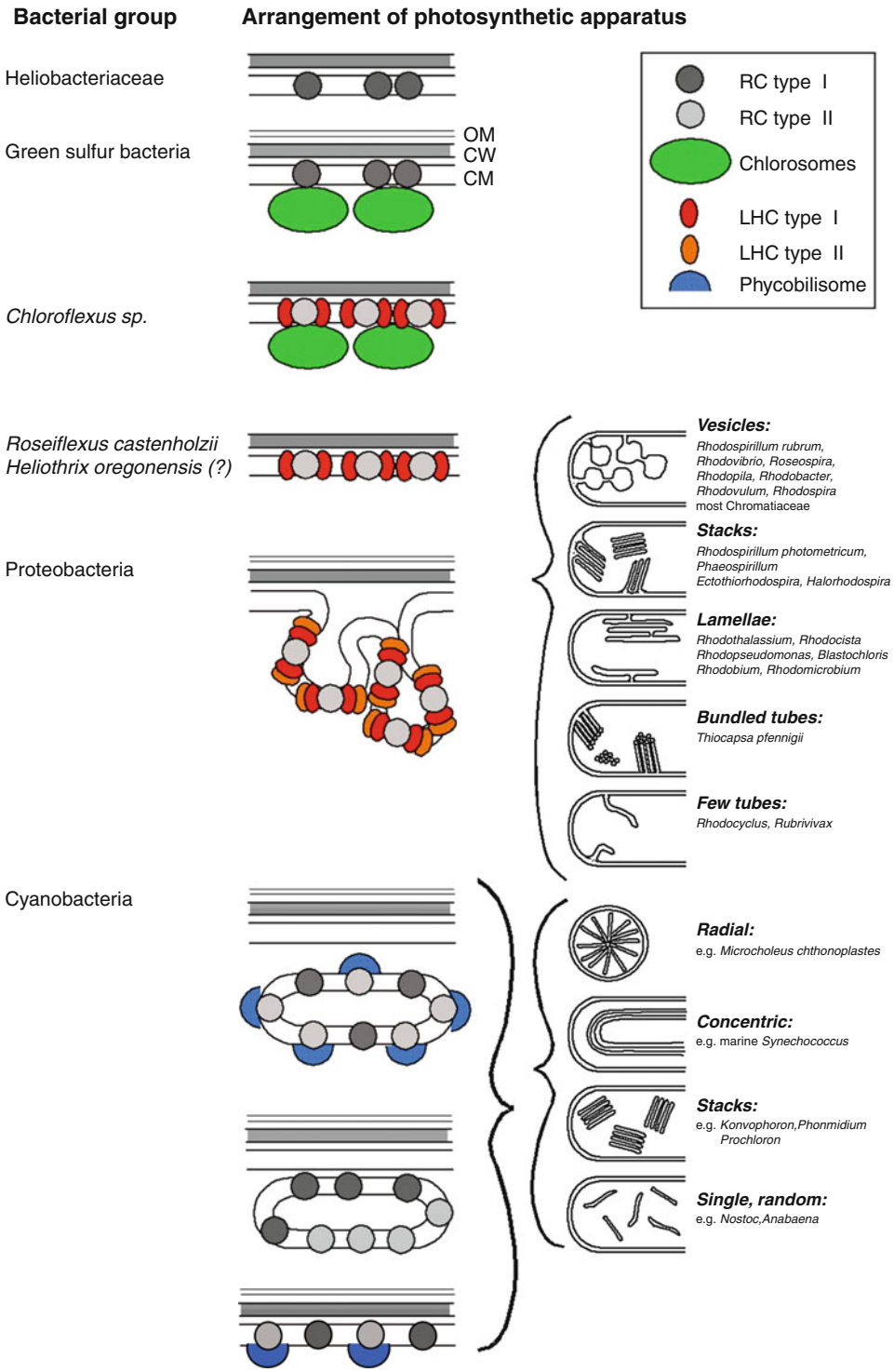


Fig. 12.1
Organization of the phototrophic apparatus in different groups of phototrophic bacteria. *OM* outer membrane, *CW* cell wall, *CM* cytoplasmic membrane, *RC* reaction center, *LHC* light-harvesting complex. *Question marks* indicate that the organization of the cell envelope and the organization of the photosynthetic apparatus in *Heliothrix oregonensis* is not exactly known

the phylum were known as strict photolithotrophs and contained chlorosomes (*Fig. 12.2a*). During the oxidation of sulfide, elemental sulfur is deposited extracellularly. Another typical feature of this group is the very limited physiological flexibility. The taxonomy of the *Chlorobiaceae* has been revised based on 16S rRNA and *fmo* gene sequence comparisons (Imhoff 2003). Recently, the perception of green sulfur bacterial diversity and evolution has changed

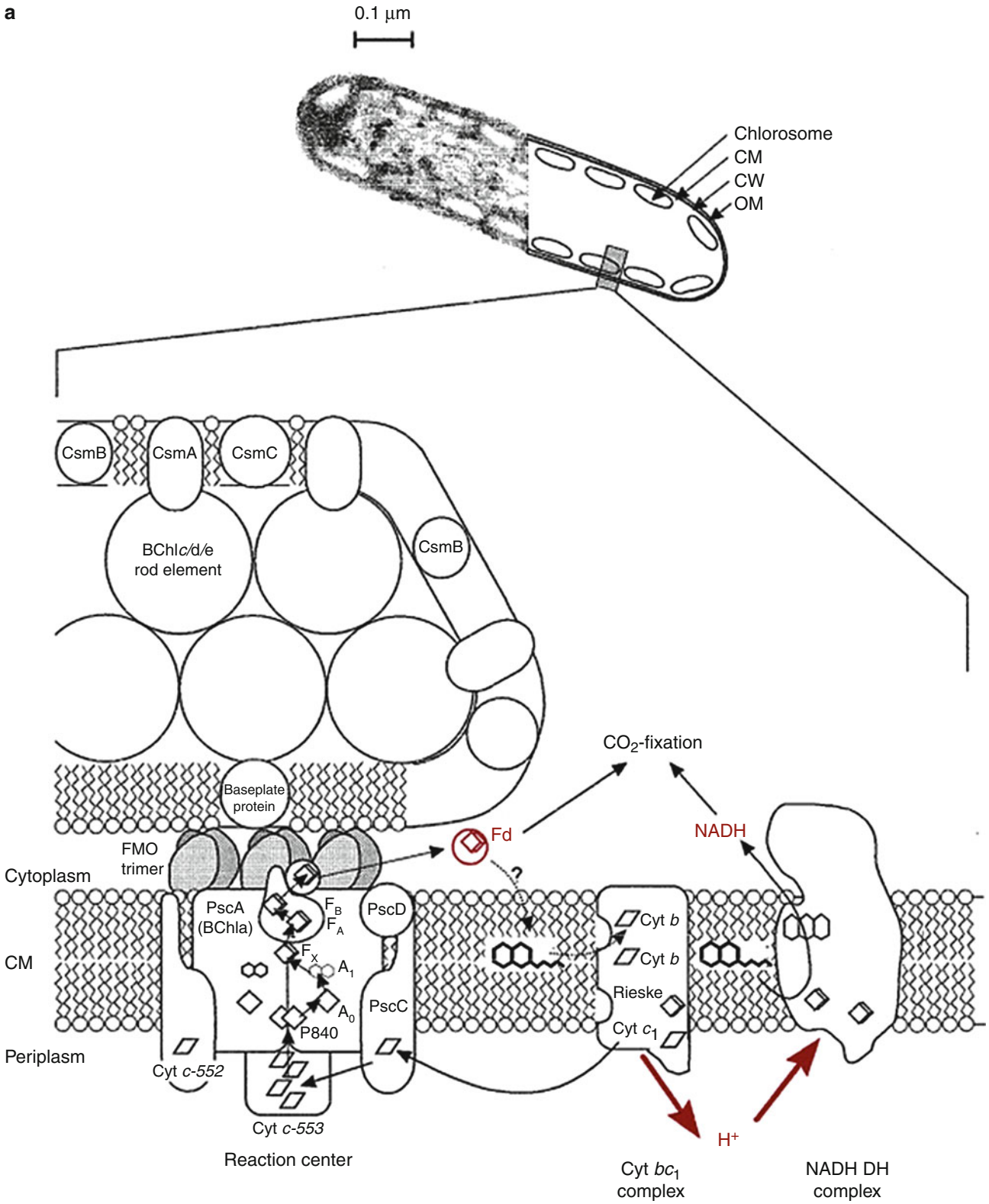


Fig. 12.2
(continued)

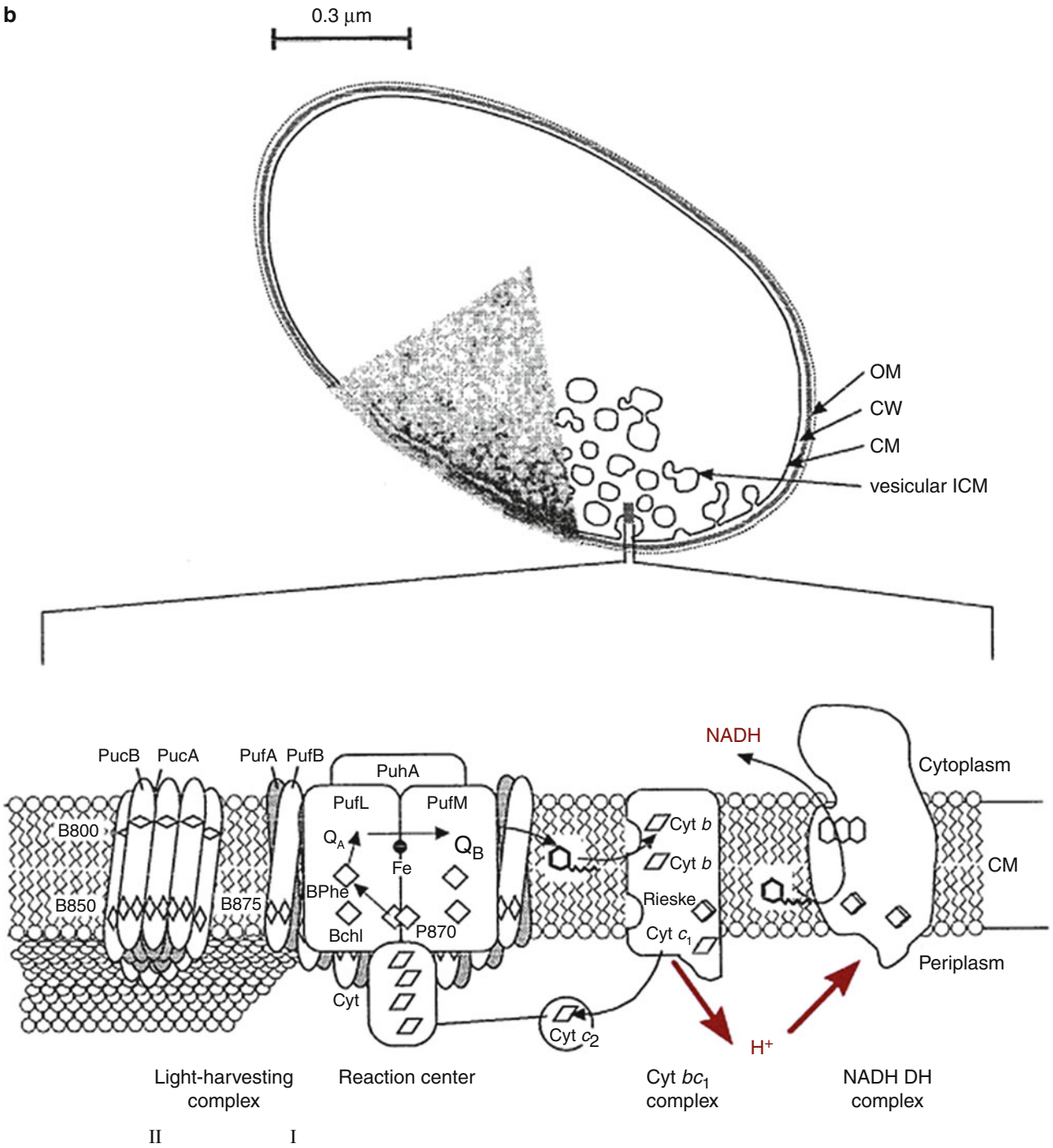


Fig. 12.2 (continued)

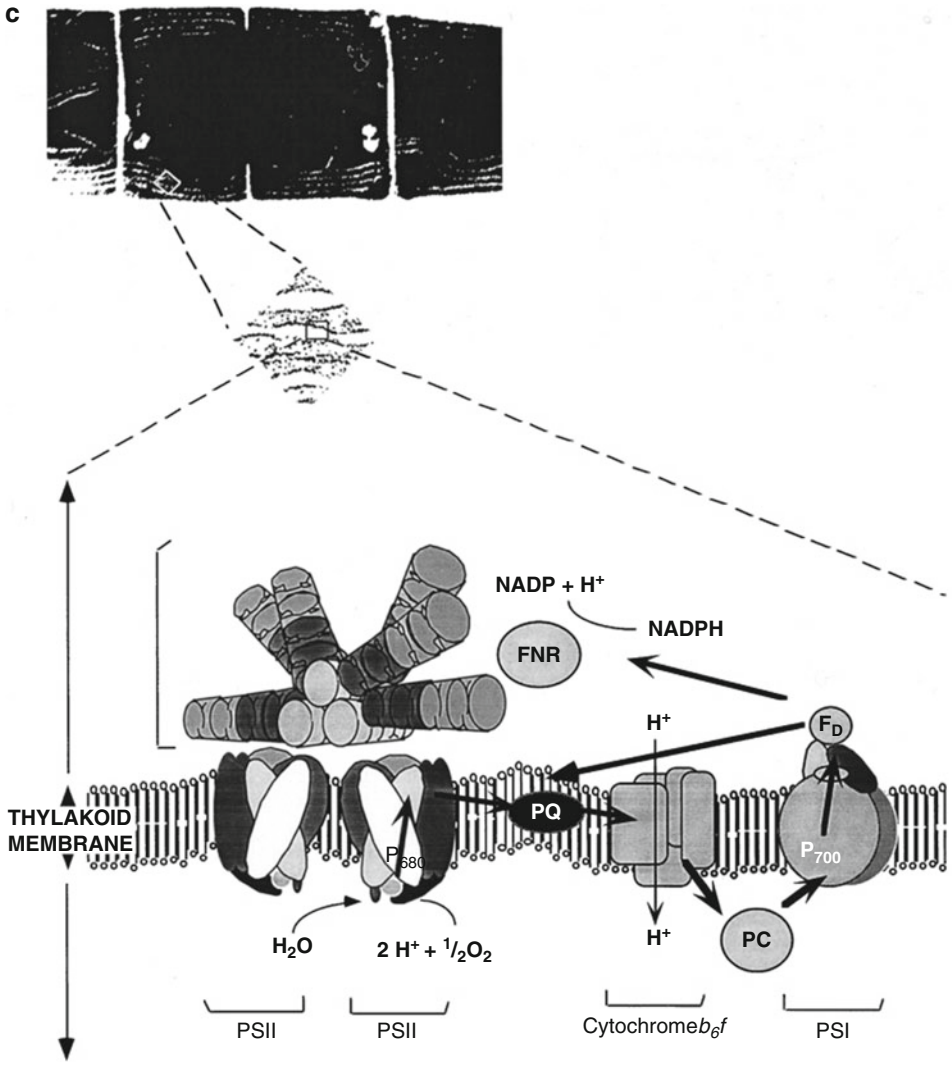


Fig. 12.2

Localization and organization of the photosynthetic apparatus in three major groups of phototrophic bacteria. Electron-donating enzyme systems, like flavocytochrome or sulfide quinone reductase, and ATP formation by the membrane-bound ATP synthase are not shown. (a) Green sulfur bacteria (Chlorobiaceae). (b) Purple nonsulfur bacteria and Chromatiaceae. (c) Cyanobacteria. *OM* outer membrane; *CW* cell wall; *CM* cytoplasmic membrane; *Cyt* cytochrome; *P840* and *P870* reaction center special pair primary electron donor; *B800*, *B850*, *B875* bacteriochlorophyll molecules bound to light-harvesting complexes II and I; A_0 primary electron acceptor in green sulfur bacteria Chl *a*; A_1 secondary electron acceptor in green sulfur bacteria menaquinone; Q_A, Q_B ubiquinone; F_X, F_A, F_B FeS-clusters bound to the reaction center; *Fd* ferredoxin; *FMO* Fenna-Matthews-Olson protein; *FNR* ferredoxin $NADP^+$ reductase; *PQ* plastoquinone; *PC* plastocyanin; *PS* photosystem

considerably following the isolation of *Ignavibacterium album* from microbial mats of a sulfidic hot spring (Iino et al. 2010). This bacterium represents a deep phylogenetic branch within the *Chlorobi*, branches outside the class *Chlorobia*, and, hence, is only distantly related to members of the *Chlorobiaceae*. *I. album* is an aerobic or anaerobic chemoorganotroph that lacks photosynthesis genes and genes for sulfur oxidation but has a full complement of genes encoding flagella and chemotaxis (Liu et al. 2012a). A recent metagenomic study of microbial mats colonizing alkaline siliceous hot springs of Yellowstone National Park (Liu et al. 2012b) indicated the presence of a novel member of the *Chlorobi* that contains type-1 reaction centers and chlorosomes but at the same time is incapable to use sulfide as an electron donor and to fix CO_2 autotrophically. This putative aerobic photoheterotroph has provisionally been named "*Candidatus* Thermochlorobacter aerophilum." The discovery of aerobic deep branching lineages in the *Chlorobi* may actually suggest that the anoxygenic phototrophic members of the phylum developed from aerobic chemoheterotrophic ancestors.

When comparing the number of recognized species with the number of available strains for each group of anoxygenic phototrophs, the *Chlorobiaceae* comprise by far more strains per species (7.4) than all other groups (*Chloroflexi*, 2.6;

Table 12.2
Groups of photosynthetic prokaryotes and their characteristics

Taxon		Preferred growth mode	Light harvesting	Photochemical reaction
Class *Chloroflexi*	(7)[a]	Anoxygenic photoorganoheterotroph(cls);	cls; BChl *c* or *d*, BChl *a*, car	Type II reaction center
		Aerobic chemoorganoheterotroph	—	—
Class *Chlorobia*	(14)	Anoxygenic photolithoautotroph	cls; BChl *c,d* or *e*, car	Type I reaction center
Alphaproteobacteria	(73)	Anoxygenic photoorganoheterotroph	icm; BChl *a* or *b*, car	Type II reaction center
		Aerobic chemoorganoheterotroph	—	—
Alphaproteobacteria (aerobic photosynthetic)	(77)	Aerobic chemoorganoheterotroph	BChl *a*	Type II reaction center
Betaproteobacteria	(5)	Anoxygenic photoorganoheterotroph	icm; BChl *a*, car	Type II reaction center
		Aerobic chemoorganoheterotroph	—	—
Betaproteobacteria (aerobic photosynthetic)	(3)	Aerobic chemoorganoheterotroph	BChl *a*	Type II reaction center
Chromatiaceae	(54)	Anoxygenic photolithoautotroph	icm; BChl *a* or *b*, car	Type II reaction center
Ectothiorhodospiraceae	(12)	Anoxygenic photolithoautotroph	icm; BChl *alb*, car	Type II reaction center
Gammaproteobacteria (aerobic photosynthetic)	(5)	Aerobic chemoorganoheterotroph	BChl *a*	Type II reaction center
Heliobacteriaceae	(9)	Anoxygenic photoorganoheterotroph	BChl *g*, car	Type I reaction center
Cyanobacteria	(>> 1,000)	Oxygenic photolithoautotroph	thy; Chl *a* + PBS or Chl *b*, or Chl *d*; car	Type I + II reaction center
Acidobacteria	(1)	Aerobic photoheterotroph	cls; BChl *c*	Type I reaction center
Halobacteria	(3)	Aerobic chemoorganoheterotroph	Purple membrane; bacteriorhodopsin	Bacteriorhodopsin

[a]The numbers of photosynthetic species described for each taxon are given in parenthesis
BChl bacteriochlorophyll, *car* carotenoids, *Chl* chlorophyll, *cls* chlorosomes, *icm* intracellular membranes, *PBS* phycobilisomes, *thy* thylacoids

Firmicutes, 2.6; *Proteobacteria*, 4.0; ❯ *Table 12.1*). Theoretically, this could indicate that most existing species of the *Chlorobiaceae* have already been recovered. However, recent diversity estimates based on the analysis of the 323 available high-quality 16S rRNA gene sequences of *Chlorobiaceae* from the databases suggest that about 50 species (based on a conservative cutoff level of 97 % nucleotide similarity) are to be expected in total, but that 3,500 different 16S rRNA gene sequence types exist (J. Müller and J. Overmann, in preparation). Thus the delineation of species of *Chlorobiaceae* and the evolutionary significance of the 16S rRNA microdiversity requires further investigations.

In the *Proteobacteria*, the Alpha- and Betaproteobacteria comprise photosynthetic representatives (often also called the purple nonsulfur bacteria), which do not form separate phylogenetic clusters but are highly intermixed with various other metabolic phenotypes. Characteristically, members of these two groups exhibit a high metabolic versatility and are capable of photoorganotrophic, photolithoautotrophic, and chemoorganotrophic growth. Photosynthetic pigments are bacteriochlorophyll *a* or b and a variety of carotenoids. Light-harvesting complexes, reaction centers, and the components of the electron transport chain are located in intracellular membrane systems of species-specific architecture (❯ *Fig. 12.1*; see section ❯ "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter). Over the past decade, the number of validly described alphaproteobacterial anoxygenic phototrophs has more than doubled and now amounts to 73 (❯ *Table 12.2*). In contrast, only one

additional species of anoxygenic phototroph has been isolated and described for the *Betaproteobacteria* during the same time period.

As of May 2012, 77 Alphaproteobacteria species, 3 Betaproteobacteria species (*Methyloversatilis universalis*, *Roseateles depolymerans*, *R. terrae*), and 5 Gammaproteobacteria species (*Chromatocurvus halotolerans*, *Congregibacter litoralis*, *Congregibacter sp. Rap1red*, *Haliea rubra*, *Luminiphilus syltensis*) are capable of bacteriochlorophyll *a* synthesis but cannot grow by anoxygenic photosynthesis (*Table 12.2*). This physiological group has therefore been designated "aerobic anoxygenic phototrophic bacteria" (Shimada 1995; Yurkov and Beatty 1998), "aerobic phototrophic bacteria" (Shiba 1989), or "quasi-photosynthetic bacteria" (Gest 1993) and comprises a considerable number of species. Since the initial description of the marine genera *Erythrobacter* and *Roseobacter* and the six freshwater genera *Acidiphilium*, *Erythromonas*, *Erythromicrobium*, *Porphyrobacter*, *Roseococcus*, and *Sandarcinobacter* (Yurkov and Beatty 1998), some 35 additional genera have been described over the past decade (combined searches of the RDP database; Cole et al. 2009; the *International Journal of Systematic and Evolutionary Microbiology*, and LPSN 2012) (compare Rathgeber et al. 2004; Yurkov and Csotonyi 2009). Many isolates fall in the so-called *Roseobacter* clade of the *Alphaproteobacteria* (Brinkhoff et al. 2008). Additional isolates that might represent novel species have been reported (Csotonyi et al. 2008). The results of studies of environmental *pufM* gene sequences from marine samples suggest an even higher diversity (Béjà et al. 2002). The aerobic anoxygenic phototrophs also include some aerobic facultatively methylotrophic bacteria of the genus *Methylobacterium* and a *Rhizobium* (strain BTAi1; Evans et al. 1990; Shimada 1995; Urakami and Komagata 1984).

The oxidation of organic carbon compounds is the principal source of metabolic energy of aerobic anoxygenic phototrophic bacteria. Photophosphorylation can be used as a supplementary source of energy, with a transient enhancement of aerobic growth following a shift from darkness to illumination (Harashima et al. 1978; Shiba and Harashima 1986). These bacteria harbor a photosynthetic apparatus very similar to type II photosystem II of anoxygenic phototrophic Proteobacteria (Yurkov and Beatty 1998). Photochemically active reaction centers and light-harvesting complexes are present, as are the components of cyclic electron transport (e.g., a cytochrome *c* bound to the reaction center and soluble cytochrome c_2). In contrast to anoxygenic phototrophic bacteria, however, the aerobic phototrophic bacteria seem to not grow autotrophically. Intracellular photosynthetic membrane systems that are typical for anoxygenic phototrophic *Proteobacteria* are absent in most aerobic photosynthetic bacteria, *Rhizobium* BTAi1 being a possible exception (Fleischman et al. 1995). The presence of highly polar carotenoid sulfates and C_{30} carotenoid glycosides is a unique property of this group.

The *Gammaproteobacteria* comprises two families of anoxygenic phototrophic species, the *Chromatiaceae* and *Ectothiorhodospiraceae* (also called purple sulfur bacteria). *Chromatiaceae* accumulate sulfur globules within the cells and represent a conspicuous microscopic feature of these bacteria. With one notable exception (*Thiococcus pfennigii*), the intracellular membrane system is of the vesicular type (*Figs. 12.2* and *12.3b*). In contrast, members of the *Ectothiorhodospiraceae* deposit elemental sulfur outside of the cells and contain lamellar intracellular membrane systems. Like their relatives of the Alpha- and Betaproteobacteria, the purple sulfur bacteria contain bacteriochlorophylls *a* and *b*, and all components of the photosynthetic apparatus are located in the intracellular membrane.

No photosynthetic species have been described for the *Delta-* or *Epsilonproteobacteria*.

Heliobacteriaceae differ from other anoxygenic phototrophic bacteria by their unique light-harvesting and reaction center pigment, bacteriochlorophyll g, the presence of an unusual C30 carotenoid (4,4′-diaponeurosporene; Takaishi et al. 1997) or OH-diaponeurosporene glucoside esters (Takaishi et al. 2003) and by their phylogenetic affiliation (*Fig. 12.1*). The first member of this group, *Heliobacterium chlorum* was described in 1983 by Gest and Favinger (Gest and Favinger 1983). Based on peptidoglycan structure studies (Beer-Romero et al. 1988), their high proportion of branched-chain fatty acids (Beck et al. 1990) and 16S rRNA sequencing, the *Heliobacteriaceae* belong to the Gram-positive low GC lineage. A close relatedness can also be deduced from the capability of *Heliobacterium modesticaldum* and *Heliobacterium gestii* to form endospores. However, a detailed phylogenetic analysis also indicated a close relatedness of *Heliobacteriaceae* to the *Cyanobacteria* (Vermaas 1994). *Heliobacteriaceae* harbor the simplest photosynthetic complexes of all known phototrophs; they do not contain distinct intracellular structures of the photosynthetic apparatus and the reaction centers are located in the cytoplasmic membrane. Bacteriochlorophyll *g* confers to the cells a near infrared absorption maximum at 788 nm, which is unique among photosynthetic organisms. The known species of *Heliobacteriaceae* all grow photoheterotrophically and are strict anaerobes. *Heliobacterium modesticaldum* can also grow chemotrophically in the dark by fermentation of pyruvate (Tang et al. 2012). With one possible exception, all *Heliobacteriaceae* are capable of nitrogen fixation (Asao et al. 2012).

More recently, an anoxygenic phototrophic representative of the *Acidobacteria* was enriched from phototrophic bacterial mats of hydrothermal springs of Yellowstone National Park and characterized by metagenomic sequencing (Bryant et al. 2007). The cells of "*Candidatus* Chloracidobacterium thermophilum" synthesize bacteriochlorophylls *a* and *c* under oxic conditions. The photosynthetic apparatus consists of chlorosomes, Fenna-Matthews-Olsen (FMO) proteins, and type I homodimeric reaction centers with the associated Fe/S protein and hence resembles that of the green sulfur bacteria (*Table 12.2*). The novel type of anoxygenic phototroph thrives at temperatures between 50 °C and 66 °C. The genome contains genes of the respiratory chain whereas genes of carbon fixation pathways and sulfur oxidation are absent. This, together with growth experiments, indicates that Cab. thermophilum likely grows as an aerobic photoorganoheterotroph using organic carbon sources, especially fermentation end products (Garcia Costas et al. 2012a).

Oxygenic photosynthesis is only found in members of a single bacterial lineage out of the six that contain phototrophs

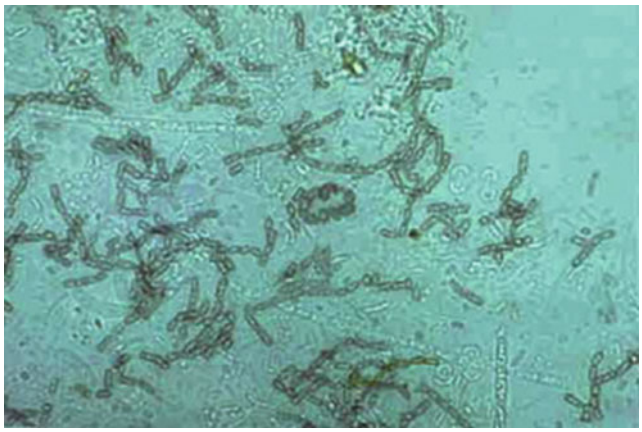


Fig. 12.3
Bright-field photomicrograph of the bacterioplankton community thriving in the chemocline of the meromictic Buchensee (near Radolfzell, Germany) during autumn. The dominant anoxygenic phototroph at this time of the year is the green sulfur bacterium *Pelodictyon phaeoclathratiforme* (brown cells, which appear in chains or netlike colonies). In addition, phototrophic consortia ("*Pelochromatium roseum*," one consortium in the center) are found. Similar to *Pld. phaeoclathratiforme*, most of the colorless bacterial cells found in the chemocline contain gas vesicles as is evident from their highly refractile appearance in the bright field

(Table 12.1). The *Cyanobacteria* by far comprise the largest number of isolated strains and described species (Table 12.2). The *Cyanobacteria* (= oxyphotobacteria) are defined by their ability to carry out oxygenic photosynthesis (water-oxidizing, oxygen-evolving, plant-like photosynthesis) based on the coordinated work of two photosystems (Fig. 12.2c). Phylogenetically, they constitute a coherent phylum that contains the plastids of all eukaryotic phototrophs. They all synthesize chlorophyll *a* as a photosynthetic pigment, and most types contain phycobiliproteins as light-harvesting pigments. These multimeric proteinaceous structures are found on the cytoplasmic face of the intracellular thylakoid membranes and contain phycobilins as light-harvesting pigments. All cultivated *Cyanobacteria* are able to grow using CO_2 as the sole source of carbon, which they fix using primarily the reductive pentose phosphate pathway (see section "Carbon Metabolism of Phototrophic Prokaryotes" in this chapter). Their chemoorganotrophic potential typically is restricted to the mobilization of reserve polymers (mainly starch but also polyhydroxyalkanoates) during dark periods, although some strains are known to grow chemoorganotrophically in the dark at the expense of external sugars. Recently, metagenomic studies have revealed the presence in the open ocean of abundant microbial populations of unicellular cyanobacteria that lack genes necessary for oxygen evolution and carbon fixation (Zehr et al. 2008). These are yet to be studied in culture but would represent the only exception to the general physiological definition of the *Cyanobacteria*. They likely represent forms symbiotic with eukaryotic plankton and lead a photoheterotrophic lifestyle.

Owing to their ecological role, in many cases indistinguishable from that of eukaryotic microalgae, the cyanobacteria had been studied originally by botanists. The epithets "blue-green algae," "Cyanophyceae," "Cyanophyta," "Myxophyceae," and "Schizophyceae" all apply to the cyanobacteria. Two main taxonomic treatments of the *Cyanobacteria* exist, and are widely used, which divide them into major groups (orders) on the basis of morphological and life-history traits. The botanical system (Geitler 1932) recognized 3 orders, 145 genera, and some 1,300 species, but it has recently been modernized (Anagnostidis and Komárek 1988; Komárek and Anagnostidis 1989). The bacteriological system (Stanier 1977; Rippka et al. 1979; Castenholz 2001a) relies on the study of cultured axenic strains. It recognizes five larger groups or orders, separated on the basis of morphological characters. Genetic (i.e., mol% GC, DNA-DNA hybridization) as well as physiological traits have been used to separate genera in problematic cases.

Several groups of cyanobacteria contain photopigment complements diverging from the norm. Some lack phycobiliproteins (Fig. 12.1) and contain chlorophyll *b*, such as *Prochloron* (Lewin 1981). *Prochlorococcus marinus*, arguably the most widespread cyanobacterium, contains divinyl-Chl a and divinyl-Chl b, and may or may not express phycobilins. Strains of *Acaryochloris* are Chl *d*-containing, lack phycobilisomes and can be found in a variety of habitats marine and freshwater habitats (Miyashita et al. 1996; Miller et al. 2005).

According to phylogenetic analysis of 16S rRNA sequences, and those from an increasing number of fully sequenced cyanobacterial genomes (Hess 2008), the *Cyanobacteria* are a diverse phylum of organisms within the bacterial radiation, well separated from their closest relatives (Giovanonni et al. 1988; Turner 1887; Garcia-Pichel 1999). These analyses support clearly the endosymbiotic theory for the origin of plant chloroplasts, as they place plastids (from all eukaryotic algae and higher plants investigated) in a diverse, but monophyletic, deep-branching cluster (Nelissen et al. 1995). Phylogenetic reconstructions show that the present taxonomic treatments of the cyanobacteria diverge considerably from a natural system that reflects their evolutionary relationships. For example, separation of the orders *Chroococcales* and *Oscillatoriales* (Nelissen et al. 1995; Reeves 1996), and perhaps also the *Pleurocapsales* (Turner 1887; Garcia-Pichel et al. 1998), is not supported by phylogenetic analysis. The heterocystous cyanobacteria (comprising the two orders *Nostocales* and *Stigonematales*) together form a monophyletic group, with relatively low sequence divergence, as low as that presented by the single accepted genus *Spirulina* (Nübel 1999). A grouping not corresponding to any official genus, the *Halothece* cluster, gathers unicellular strains of diverse morphology that are extremely tolerant to high salt and stem from hypersaline environments (Garcia-Pichel et al. 1998). A second grouping, bringing together very small unicellular open-ocean cyanobacteria (picoplankton) includes only marine picoplanktonic members of the genera *Synechococcus* and all *Prochlorococcus*. Several other statistically well-supported groups of strains that may or may not correspond to presently defined taxa can be distinguished. The botanical genus "*Microcystis*" of

unicellular colonial freshwater plankton species is very well supported by phylogenetic reconstruction, as is the genus *Trichodesmium* of filamentous, nonheterocystous nitrogen-fixing species typical from oligotrophic marine plankton of the tropics. Strains from diverse origins and habitats containing Chl *d* nicely fall together into a single monophyletic clade around *Acaryochloris marina* (Miller et al. 2005). The picture that emerges from these studies is that sufficient knowledge of ecological and physiological characteristics can lead to a taxonomic system that is largely congruent to the 16S rRNA phylogeny. But formal taxonomic work has not been keeping up with molecular developments.

Whereas minimal standards for the description of new species of anoxygenic phototropic bacteria have been recommended some time ago (Imhoff and Caumette 2004), the nomenclature of cyanobacteria has not been resolved so far (International committee on Systematics of Prokaryotes 2007; Oren 2004). These problems arise due to the treatment of cyanobacteria in both the Botanical and Bacteriological Codes of Nomenclature, leading, e.g., to different types of reference materials (nonliving type specimen versus living type strains). Accordingly, a "list of approved names of cyanobacteria," as well as standards recommended for the description of cyanobacteria are still not available (Oren 2004).

A different principle of conversion of light energy into chemical energy is found in membrane systems containing rhodopsins, initially discovered in the haloarchaea *Halobacterium halobium*, *H. salinarium*, and *H. sodomense* (Oesterhelt and Stoeckenius 1973). These archaea are largely confined to surface layers of hypersaline aquatic environments and grow predominantly by chemoorganoheterotrophy with amino or organic acids as electron donors and carbon substrates, generating ATP by respiration of molecular oxygen. At limiting concentrations of oxygen bacteriorhodopsin, a chromoprotein containing a covalently bound retinal is expressed. Bacteriorhodopsin is incorporated in discrete patches in the cytoplasmic membrane ("purple membrane"). The protein consists of a single amino-acid chain that folds into seven transmembrane helices. Its light-sensitive component is the retinal cofactor that forms a Schiff base to a conserved lysine residue of the protein and that isomerizes from *all-trans* to the 13-*cis* form upon absorption of a photon (Grote and O'Malley 2011). This isomerization causes conformational changes of the protein. Ultimately, the photocycle results in ion transport across the cytoplasmic membrane. Bacteriorhodopsin acts as a light-driven proton pump and leads to a proton extrusion out of the cell. Consequently, energy conversion in these systems does not involve electron transport. However, Haloarchaea have only a very limited capability of light-dependent growth. Only slow growth and one to two cell doublings could be demonstrated experimentally (Hartmann et al. 1980; Oesterhelt and Krippahl 1983). Halorhodopsin is a light-driven chloride importer that is involved in osmotic homeostasis. Finally, sensory rhodopsins mediate phototactic responses through protein-protein interactions. Since rhodopsin-based photosynthesis had originally been found exclusively in the phylogenetically tight group of Haloarchaea, it had been concluded that, because of its lower efficiency, this type of light utilization might be of selective advantage only under specific (and extreme) environmental conditions. More recently, rhodopsins have been detected also in Bacteria, green algae, and Fungi. Metagenomic studies of marine bacterioplankton revealed the presence of rhodopsins in Proteobacteria (so-called proteorhodopsins) that serve a bioenergetic function (Béjà et al. 2000). Proteorhodopsins also occur in freshwater *Actinobacteria* (Sharma et al. 2008). The type I rhodopsins described above share their principle structure with photosensitive receptor proteins of animal retinas (type II rhodopsins). The latter are visual pigments that are coupled to G-proteins and, in contrast to their microbial counterparts, release their cofactor from the opsin. At present it remains unclear whether both types of rhodopsins share a common ancestor or arose by convergent evolution (Grote and O'Malley 2011).

During the past decades, culture-independent 16S rRNA-gene-based and high-throughput sequencing methods have been used for the investigation of the composition of natural communities of phototrophic prokaryotes (Ley et al. 2006; Garcia-Pichel 2008). These studies have provided evidence that more than one genotype of *Chloroflexus* occur in one hot spring microbial mat and that four previously unknown sequences of cyanobacteria dominate in the same environment (Ferris et al. 1996; Ruff-Roberts et al. 1994; Weller et al. 1992). A hypersaline microbial mat was found to contain approximately 100 different 16S rRNA gene sequences associated with the *Chloroflexus* group (Ley et al. 2006). Nine different partial 16S rRNA gene sequences of *Chromatiaceae* and green sulfur bacteria, which differed from all sequences previously known, were retrieved from just two lakes and one intertidal marine sediment (Coolen and Overmann 1998; Overmann et al. 1999a).

While so-far-uncultured sequence types are frequently recovered from natural samples, 16S RNA signatures from natural populations were indistinguishable from those of cultured strains in other cases. This is true for cyanobacteria with conspicuous morphologies, such as the cosmopolitan *Microcoleus chthonoplastes* (Garcia-Pichel et al. 1996) from intertidal and hypersaline microbial mats or *Microcoleus vaginatus* from desert soils (Garcia-Pichel et al. 2001). In a similar manner, the 16S rRNA sequence of an isolated strain of *Amoebobacter purpureus* (*Chromatiaceae*) was found to be identical to the environmental sequence dominating in the chemocline of a meromictic salt lake (Coolen and Overmann 1998; Overmann et al. 1999a). Obviously, the limited number of isolated and characterized bacterial strains rather than an alleged "nonculturability," often accounts for our inability to assign ecophysiological properties to certain 16S rRNA sequence types. This point is illustrated for extremely halotolerant unicellular cyanobacteria by the fact that only after a physiologically coherent group of strains was defined on the basis of newly characterized isolates (Garcia-Pichel et al. 1998) could the molecular signatures retrieved from field samples be assigned correctly. Similarly, efforts in cultivation could essentially find matches for all cyanobacterial nitrogenase sequences obtained from desert soils (Yeager et al. 2007).

It has to be concluded that (1) the numbers of species listed in Table 12.1 do not reflect the full phylogenetic breadth at least in the four groups of anoxygenic phototrophic prokaryotes as well as in morphologically simple *Cyanobacteria* and (2) the physiology and ecology of those species of phototrophic prokaryotes that are dominant in the natural environment in some cases may differ considerably from those of known type strains.

Habitats of Phototrophic Prokaryotes

Bacteria of the *Chloroflexus*-subgroup form dense microbial mats in geothermal springs, often in close association with cyanobacteria. *Chloroflexus aurantiacus* is a thermophilic bacterium that grows optimally between 52 °C and 60 °C and thrives in neutral to alkaline hot springs up to 70–72 °C. Of all anoxygenic phototrophic bacteria isolated so far, only *Chloroflexus aurantiacus* is capable of growth up to 74 °C. In contrast to the domain Archaea, no hyperthermophilic species are known from the domain Bacteria. *Heliothrix oregonensis* is phylogenetically related to *Chloroflexus* and grows optimally between 50 °C and 55 °C and is abundant as a flocculant surface layer in a few alkaline springs in Oregon. *Roseiflexus castenholzii* was isolated from a bacterial mat in a Japanese hot spring (Hanada et al. 2002) but also detected at a considerable diversity in alkaline hot spring mats (35–60 °C, pH 7–9) in Yellowstone National Park (Boomer et al. 2002). Hydrothermal springs of 56–66 °C, which contain sulfide of geothermal origin, are dominated by a surface layer or a "unispecific" mat of *Chloroflexus* (Castenholz and Pierson 1995). Because of the absence of cyanobacteria in some of these systems, *Chloroflexus* presumably grows autotrophically (Pierson and Castenholz 1995). In the presence of O_2, the mats exhibit an orange color whereas they are green under anoxic conditions (Castenholz and Pierson 1995). The orange color is the result of the enhanced carotenoid biosynthesis under oxic conditions (see section "Chemotrophic Growth with O_2" in this chapter). In the absence of sulfide, *Chloroflexus* is present as a distinct orange layer beneath a surface layer of cyanobacteria and may utilize their exudates or the fermentation products generated during decomposition of cyanobacteria. Molecular oxygen represses bacteriochlorophyll synthesis in *Chloroflexus* and often is present at saturation levels in the orange layers. Since bacteriochlorophylls *a* and *c* are still present in this layer, however, it must be assumed that bacteriochlorophylls are synthesized at anoxic conditions during nightime (Castenholz and Pierson 1995).

Green and purple sulfur bacteria often form conspicuous blooms in nonthermal aquatic ecosystems (Figs. 12.4 and 12.5a, b), although moderately thermophilic members of the genera *Chromatium* and *Chlorobium* have been described from hot spring mats (Castenholz et al. 1990). *Chlorobium tepidum* occurs in only a few New Zealand hot springs at pH values of 4.3 and 6.2 and temperatures up to 56 °C. *Chromatium tepidum* was found in several hot springs of western North America at temperatures up to 58 °C and might represent the most thermophilic proteobacterium (Castenholz and Pierson 1995). In a recent compilation (van Gemerden and Mas 1995), 63 different lakes and 7 sediment ecosystems harboring phototrophic sulfur bacteria were listed. Cell densities between 10^4 and $10^7 \cdot ml^{-1}$ and biomass concentrations between 10 and 1,000 µg bacteriochlorophyll·l^{-1} are common in pelagic habitats. Of the purple sulfur bacteria, *Chromatiaceae* are typically found in freshwater and marine environments (Fig. 12.4a, b) whereas *Ectothiorhodospiraceae* inhabit hypersaline waters. The phototrophic sulfur bacteria grow preferentially by photolithoautotrophic oxidation of reduced sulfur compounds and are therefore limited to those environments where light reaches anoxic, sulfide-containing bottom layers. Because light and sulfide occur in opposing gradients, growth of phototrophic sulfur bacteria is confined to a narrow zone of overlap and is only possible if the chemical gradient of sulfide is stabilized against vertical mixing. In pelagic environments like lakes or lagoons, chemical gradients are stabilized by density differences between the oxic and anoxic water layers. Such density differences are either the result of thermal stratification and mostly transient (as in holomictic lakes) or are caused by high salt concentrations of the bottom water layers, in which case stratification is permanent (meromictic lakes). Pelagic layers of phototrophic sulfur bacteria extend over a vertical distance of 10 cm (van Gemerden and Mas 1995; Overmann et al. 1991a) up to 30 m (Repeta et al. 1989) and reach biomass concentrations of 28 mg bacteriochlorophyll·l^{-1} (Overmann et al. 1994).

Littoral sediments represent the second type of habitat of phototrophic sulfur bacteria. In these systems, turbulent mixing is largely prevented by the sediment matrix, and diffusion is the only means of mass transport. Gradients of light and sulfide are much steeper, and the fluxes of sulfide much larger compared to the pelagic environment. These conditions allow layers of phototrophic sulfur bacteria in sediments to reach much higher biomass densities (up to 900 mg bacteriochlorophyll·dm^{-3}; van Gemerden et al. 1989) than in lakes. At the same time, the layers are very narrow (1.3–5 mm; van Gemerden and Mas 1995; Fig. 12.4a). This vertical distribution of anoxygenic phototrophic biomass ultimately determines the significance of microbial sulfide oxidation for the sulfur cycle in these ecosystems (see section "Significance of Anoxygenic Photosynthesis for the Pelagic Carbon and Sulfur Cycles" in this chapter). The spectral composition of light available for anoxygenic photosynthesis is considerably different between pelagic and benthic habitats (Fig. 12.5) and selects for different species of anoxygenic phototrophic bacteria. Whereas light of the blue to yellow-green wavelength bands dominates the depths of most lakes, infrared light is an important source of energy in benthic microbial mats (see section "Light Energy and the Spectral Distribution of Radiation" in this chapter).

The dominance of certain species of green sulfur bacteria (Fig. 12.3) or *Chromatiaceae* in pelagic environments in many cases can be explained by their specific light-harvesting capabilities (see sections "Light Absorption and Excitation Transfer in Prokaryotes" and "Competition for Light" in this chapter) and other phenotypic traits. Typically, those species that have been isolated from natural blooms in lakes are obligately

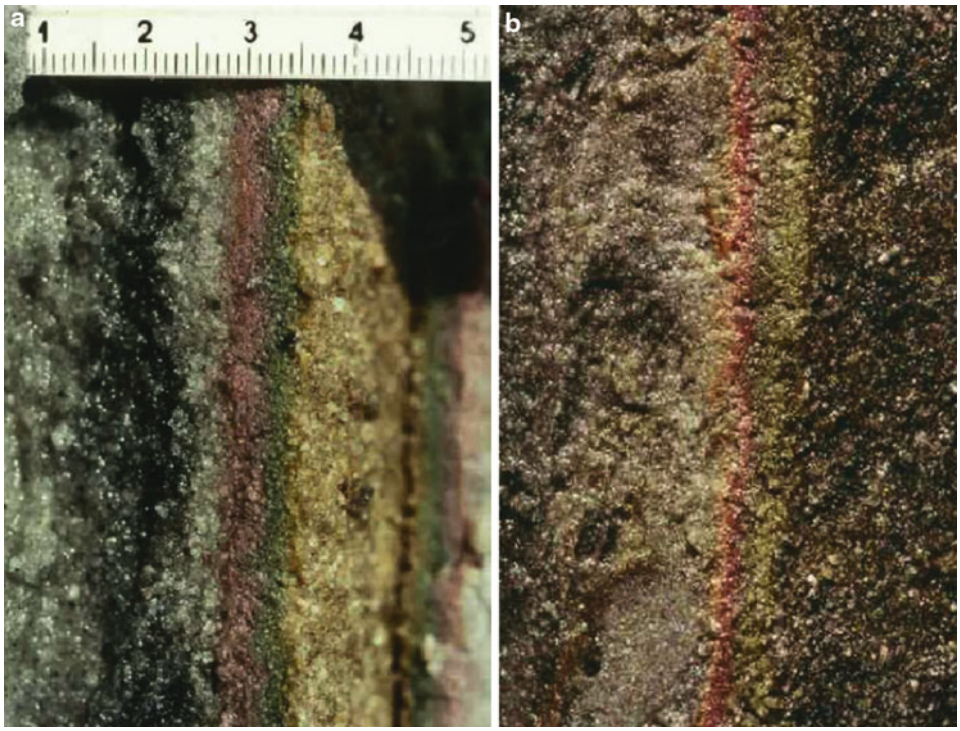


Fig. 12.4
Multilayered microbial mat as it is regularly found in the sandflats of Great Sippewissett Salt Marsh (Cape Cod, Massachusetts, USA). (a) In most instances, the mats consist of a *top green* layer, an intermediate *purple* layer, and a *grayish* to *blackish bottom* layer. (b) Fully developed microbial mats consist (from *top*) of an *olive-green* layer of diatoms and cyanobacteria, a *green layer* consisting mostly of cyanobacteria, a *purple* layer of purple sulfur bacteria, a peach-colored layer formed by BChl *b*-containing purple sulfur bacteria (morphologically similar to *Thiocapsa pfennigii*), and a *grayish* to *blackish bottom* layer

photolithotrophic, lack assimilatory sulfate reduction, cannot reduce nitrate, and assimilate only few organic carbon sources (see section "Carbon Metabolism of Phototrophic Prokaryotes" in this chapter). This applies not only to all green sulfur bacteria but also to the dominant species of *Chromatiaceae*. Obviously, in the chemocline of lakes, the metabolic versatile *Chromatiaceae* species have no selective advantage. As judged from the physiological characteristics of strains of phototrophic sulfur bacteria isolated from sediments, the pronounced diurnal variations in oxygen concentrations and salinity, together with the different light quality, select for different species composition in benthic microbial mats. The purple sulfur bacterium *Allochromatium* (and the multicellular gliding colorless sulfur bacterium *Beggiatoa*) are found in many microbial mats and exhibit diurnal vertical migrations in response to the recurrent changes in environmental conditions (Jørgensen 1982; Jørgensen and Des Marais 1986). Microbial mats of intertidal sediments are typically colonized by the immotile purple sulfur bacterium *Thiocapsa roseopersicina* and small motile thiobacilli (van den Ende et al. 1996).

In contrast to the phototrophic members of the *Gammaproteobacteria*, purple nonsulfur bacteria of the Alpha- and Beta-subclasses of *Proteobacteria* do not appear to form dense accumulations under natural conditions (Biebl and Drews 1969; Swoager and Lindstrom 1971; Steenbergen and Korthals 1982). However, purple nonsulfur bacteria can be readily isolated from a wide variety of marine, lacustrine, and even terrestrial environments (Imhoff and Trüper 1989; J. Overmann, unpublished observation). While comprehensive comparative quantitation of the ecological importance of purple nonsulfur bacteria is still lacking, as many as ca. 10^6 c.f.u. of purple nonsulfur bacteria could be cultivated per cm^3 of sediment in coastal eutrophic settings (Guyoneaud et al. 1996). However, members of the genera *Rhodobacter* and *Rhodopseudomonas* are known to sometimes form dense microbial mats in wastewater (e.g., Okubo et al. 2006) which is attributed to the availability of high concentrations of short-chain fatty acids. Purple nonsulfur bacteria could also be enriched from permanently frozen lakes in Antarctica (Karr et al. 2003).

Originally, aerobic phototrophic bacteria were thought to thrive in eutrophic marine environments. Obligately aerobic bacteria containing bacteriochlorophyll *a* had been isolated from beach sand and seaweeds (thalli of *Enteromorpha linza* and *Sargassum horneri*; Shiba et al. 1979), and in some cases also from freshwater ponds and microbial mats. At least some of the aerobic phototrophic bacteria apparently can survive in situ temperatures of up to 54 °C (Yurkov and Beatty 1998). Aerobic phototrophic bacteria were isolated from hydrothermal plume

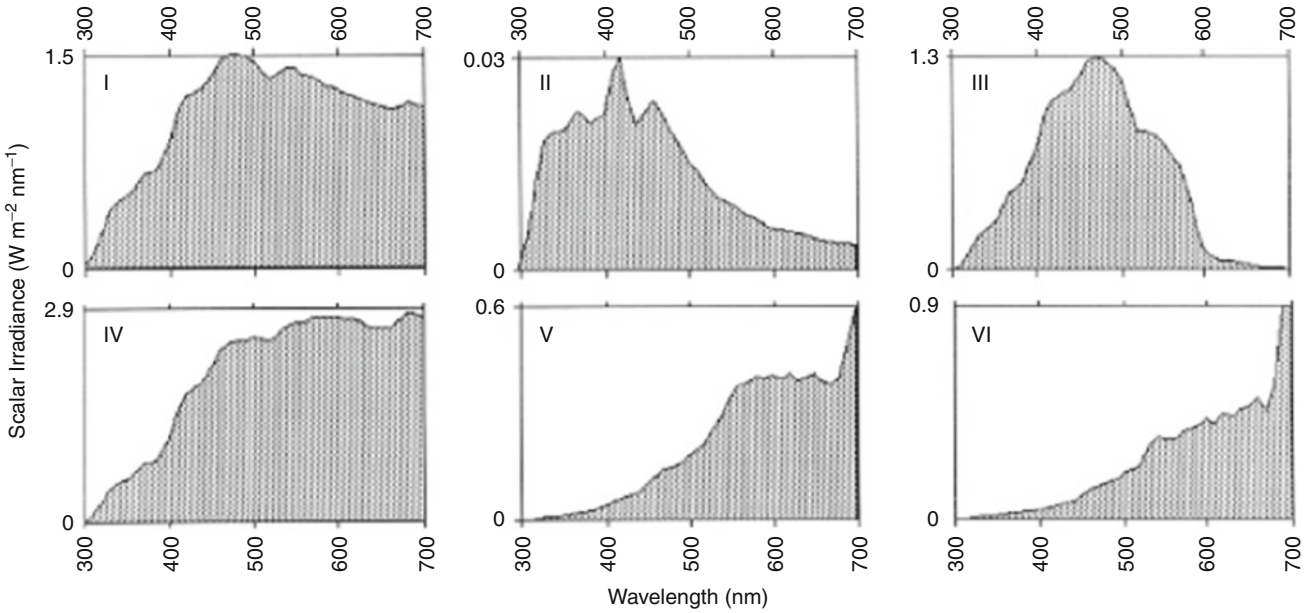


Fig. 12.5

Effects of the habitat on the physical exposure of cyanobacteria. The spectral scalar irradiance (sun and sky radiation) incident at ground level at noon in a clear midsummer day at 41 °N is plotted in Plate I. The rest of the plates depict the in situ scalar irradiance experienced by cyanobacterial cells thriving in several habitats exposed to the incident fluxes in plate I (note different scales). Plate II: a "strong shade" habitat (north-facing surface illuminated by extremely diffuse sky radiation only), where scalar irradiance is very low but the relative importance of UV is enhanced. Plate III: a planktonic habitat (under 1 m of clear open-ocean water), where all fluxes remain fairly high, and UVB and visible are more strongly attenuated than UVA. Plate IV: the surface of beach (quartz, feldspar) sand, where all UVB, UVA, and visible are higher than incident (by 120 %, 150 %, and 205 %, respectively) due to light trapping effects. Plate V: 300-m deep in a wet topsoil, where UVB and U VA have been attenuated below 5 % of incident but ca. 20 % of the visible light remains. Plate VI: scalar irradiance within the thallus of the terrestrial cyanobacterial lichen *Collema* sp. (Modified from Castenholz and Garcia-Pichel (1999), after data from the following sources: F. Garcia-Pichel (unpublished observation); Garcia-Pichel (1995), Büdel et al. (1997), and Smith and Baker (1981))

water of a black smoker 2,000 m below ocean surface (Yurkov and Beatty 1998); acidophilic strains could be isolated from acidic mine drainage. More recent analysis of marine bacterioplankton revealed that aerobic anoxygenic phototrophs can account for up to 16 % of all prokaryotic cells and exhibited clear abundance maxima in the photic zone (Cotrell et al. 2006). In some cases, the pigment content of AAP bacteria approaches that of the oxygenic phototrophic cyanobacterium *Prochlorococcus*. Typically, *Methylobacterium* species are isolated from foods, soils, and leaf surfaces (Shimada 1995). Photosynthetic *Rhizobium* strains are widely distributed in nitrogen-fixing stem nodules of the tropical legume *Aeschynomene* spp. where they are present as symbiosomes. Similar strains have also been found in root and hypocotyl nodules of *Lotononis bainesii* (Fabaceae). These photosynthetic rhizobial and regular symbiosomes differ in that the former contains only one large spherical bacteroid. The photosynthesis of these endosymbionts may provide energy for nitrogen fixation and permit a more efficient growth of the host plant, since up to half of the photosynthate produced by legumes is allocated to nitrogen fixation (Fleischman et al. 1995).

Heliobacteriaceae appear to be primarily soil bacteria but have also been isolated from dry paddy fields and hot springs, lakeshore muds, or from sediments of soda lakes (Amesz 1995; Madigan and Ormerod 1995; Asao and Madigan 2010; Asao et al. 2012). Bacteria of this family may even represent the dominant anoxygenic phototrophic bacteria in anoxic soils (Madigan 1992). These conclusions have exclusively been deduced from successful isolation of *Heliobacteriaceae* from the different natural samples. However, molecular signatures for these organisms do not constitute part of the phyloypes recovered from most soils in molecular surveys. *Heliobacterium modesticaldum* is a thermophile that grows up to 56 °C (Kimble et al. 1995). Spore formation may offer a selective advantage to *Heliobacterium modesticaldum*, *Heliophilum fasciatum*, and *Heliobacterium gestii* in their main habitat (rice field soil), which undergoes periodic drying and concomitantly becomes oxidized (Madigan 1992). During growth of the rice plants, organic compounds excreted by their roots could provide sufficient substrates for photoheterotrophic growth of the *Heliobacteriaceae*. In turn, the bacteria could provide fixed nitrogen to the plant. Members of the phylogenetic clade comprising the genera *Heliobacterium*, *Heliobacillus*, and *Heliophilum* are

neutrophilic, whereas species of *Heliorestis* are alkaliphilic, exhibit growth optima at pH 8–9, and are isolated from shoreline sediments and soils of soda lakes (Asao and Madigan 2010).

Cyanobacteria as a group exhibit the widest range of habitats of all phototrophic prokaryotes due to the ubiquity of water, their preferred electron donor for the reduction of CO_2. In principle, cyanobacteria can thrive in any environment that has, at least temporarily, liquid water and sunlight. They are known from Antarctic endolithic habitats and from hot springs. More than 20 species of cyanobacteria (Castenholz and Pierson 1995) are thermophilic. Effectively, however, cyanobacteria cannot tolerate acidic environments (below pH 4.5) and competition with eukaryotic microalgae or higher plants may restrict their growth in other environments. Cyanobacteria are found in the plankton of coastal and open oceans, as well as in freshwater and saline inland lakes. They thrive in the benthos of marine intertidal (Fig. 12.4b), lacustrine, and fluvial waters and in a large variety of terrestrial habitats (soils, rocks, trees). Symbiotic associations are common. The total estinated biomass of cyanobacteria reaches some 3×10^{14} g C globally, from which approximately 1/2 correspond to the marine picoplankters, and approximately 1/4 is found in terrestrial soils and rocks of arid lands, while freshwater plankton makes up only about 1 % of the total (Garcia-Pichel et al. 2003).

In the marine plankton, the picoplanktonic genera *Procholoroccoccus* (Campbell and Vaulot 1993; Chisholm et al. 1988; Olson et al. 1990b) and *Synechococcus* (Waterbury et al. 1986), in this order, represent a major fraction of all primary producers. Compared with the high number of cyanobacterial species found in freshwater plankton, intertidal areas, and hypersaline environments, the diversity of this group is very limited in the open ocean (Carr and Mann 1994). While the two genera of marine picoplankters groups likely share a phylogenetic not so distant origin, there has been considerable evolutionary diversification. *Synechococcus* consists of small (<2 m) mostly nonmotile, non-nitrogen-fixing single cells contain phycoerythrin as accessory photopigment, which confers an orange autofluorescence on the cells and an ability to utilize light fields enriched in blue light in the deep ocean. *Prochloroccus* are yet smaller, and have for the most part lost the ability to use phycobilins, using divynil chlorophyll a/b antenna pigments instead. *Prochloroccoccus* have some of the more minimalistic genomes among cyanobacteria (Hess 2008). An important component of the phytoplankton in tropical and subtropical oceans are the filamentous *Trichodesmium* spp., with an estimated biomass of 10×10^{12} g C (Garcia-Pichel et al. 2003). The bundle and aggregate-forming *Trichodesmium* typically develop into blooms that can extend for kilometers and that can be detected on the surface of oligotrophic tropical and subtropical oceans with the naked eye or with satellite imagery from space. The success of *Trichodesmium* can be mainly traced to the highly efficient nitrogen-fixing capacity of these nonheterocystous cyanobacteria. Their activities attain global magnitude for the nitrogen cycle (Capone et al. 1977).

Edaphic cyanobacteria are also distributed worldwide, especially in soils of basic pH; sheathed rope-forming oscillatorian forms (*Microcoleus vaginatus*, *M.steentrupii*) are the pioneers and most abundant (Garcia-Pichel and Wojciechowski 2009), and along with heterocystous ones (*Nostoc*, *Scytonema*, *Tolypothrix*; Yeager et al. 2007) are of major ecological relevance in arid and semiarid regions where growth of higher plants is restricted. In such environments, cyanobacteria adopt a life strategy of resistance to desiccation (Potts 1994) making use of the few occasions in which liquid water is available from rain or dew. Very intense productivity spurts occur in a matter of minutes after wetting (Garcia-Pichel and Belnap 1996). The so-called cyanobacterial desert crusts contribute significantly to the biogeochemistry and to the physical stability of arid soils. Other important terrestrial habitats of cyanobacteria are the surface or subsurface of rocks: extensive endolithic cyanobacterial communities, usually dominated by members of the genus *Chroococcidiopsis*, have been described from tropical, desert, and polar environments (Friedmann 1982; Wessels and Büdel 1995).

Heterocystous, nitrogen-fixing cyanobacteria of the genera *Nodularia*, *Anabaena*, and *Aphanizomenon* bloom in mesotrophic and eutrophic fresh and brackish waters. Together with the blooms of the nonheterocystous colonial genus *Microcystis* and the filamentous *Planktothrix*, these cyanobacteria have become a serious environmental concern, not only because of their effects of overall water quality but also because of their ability to produce toxins, which are known to have caused the deaths of humans and cattle. In the chemocline of stratified lakes, deep blooms of cyanobacteria occur frequently (Hudnell 2008).

In the course of evolution, cyanobacteria have entered into symbiotic associations with a multitude of organisms. These have reached a wide range in the degree of interdependence between partners (see section "Symbioses Between Phototrophic Bacteria and Eukaryotes" in this chapter).

Principles and Prerequisites of Photosynthesis

Principle Reactions

Bacterial photosynthesis can be divided into two different types of reactions: (1) the light reaction, in which light energy is trapped and converted into ATP (via a proton-motive force ΔP) and a reduced redox carrier $R_{red} \cdot H^+$, and (2) the so-called dark reaction of biosynthetic carbon reduction.

$$\text{Light reaction}: 2H_2A + 4R_{ox} + \text{light} \rightarrow 2A + 4R_{red} \cdot H + \Delta P$$
$$nADP + nP_i + \Delta P \rightarrow nATP + mH_2O$$

$$\text{Dark reaction}: CO_2 + nATP + 4R_{red} \cdot H + (m-1)H_2O \rightarrow <CH_2O> + nADP + nP_i + 4R_{ox}$$

$$\text{Sum}: CO_2 + 2H_2A + \text{light} \rightarrow <CH_2O> + H_2O + 2A$$

(van Niel equation)

Microorganisms have found different ways to accomplish these two tasks.

Light Energy and the Spectral Distribution of Radiation

The present day solar irradiance at the average distance of Earth to the sun and outside the atmosphere (the so-called *solar constant*) is 1,361 W m^{-2}, corresponding to 340 W·m^{-2} per surface of the Earth and to a total incoming energy of 173 PW (Kopp and Lean 2011). The spectral energy distribution of this solar radiation approximates that of a black body at 6,000°K (the surface temperature of the Sun). According to Wien's Law, a black body at this temperature has a maximum emission of electromagnetic energy at about 480 nm. The actual spectral energy distribution of solar radiation exhibits minima that reflect the absorption bands of hydrogen in the outer atmosphere of the Sun (◉ *Fig. 12.7*). The total light energy received by the Earth is $5.46 \cdot 10^{24}$ J·$year^{-1}$, which would correspond to 339.4 W·m^{-2}. The actual solar (time- and space-averaged) irradiance reaching the surface of the Earth amounts only to 160 W·m^{-2} (Gates 1962; Dietrich et al. 1975). This large reduction is due to Raleigh scattering by air molecules and dust particles, and of light absorption by water vapor, O_2, O_3, and CO_2 during the passage of radiation through the Earth's atmosphere. Concomitantly, the spectral distribution of solar irradiance is changed especially because water vapor absorbs infrared light (◉ *Fig. 12.6*). At sea level, light of the wavelength regions 400–700 nm (PAR, photosynthetically available radiation) constitutes 50 % of this irradiance (Kirk 1983).

Based on estimates for global primary productivity, only 0.16 % of the flux of solar energy reaching the surface of the Earth is converted into chemical energy by photosynthesis (see section ◉ "Introduction" in this chapter). Under natural conditions, photosynthesis of the various groups of phototrophic prokaryotes is limited by different environmental factors including light, reduced sulfur compounds, organic carbon substrates, oxygen, and temperature. The physical characteristics of the medium have, through processes of absorption and scattering, a large influence on the available radiation (see section ◉ "Competition for Light" in this chapter). As a second major limiting factor, the availability of nutrients limits the growth of phototrophic bacteria and as a consequence, photosynthetic energy conversion.

Surface environments exposed to sky radiation (as in strong shades) may be enriched in blue and UV radiation (◉ *Fig. 12.5*). Water is the major light-absorbing component only in very clear open ocean and inland lakes. It strongly absorbs the light of the ultraviolet (UV), red, and especially infrared (wavelengths around 745 and 960 nm). As a consequence, tens of meters below the surface of clear waters the spectrum is enriched in blue wavelengths. Several meters below coastal or most lacustrine water surfaces, the spectrum is enriched toward the green wavelengths, and deep (several millimeters) in the photic zones of sediments and soils, infrared wavelengths dominate. Yellow substance in lakes is mostly of terrestrial origin and particularly absorbs light of the ultraviolet and blue portion of the spectrum (Kirk 1983). In dystrophic lakes in which high concentrations of humic compounds are the major light-absorbing components,

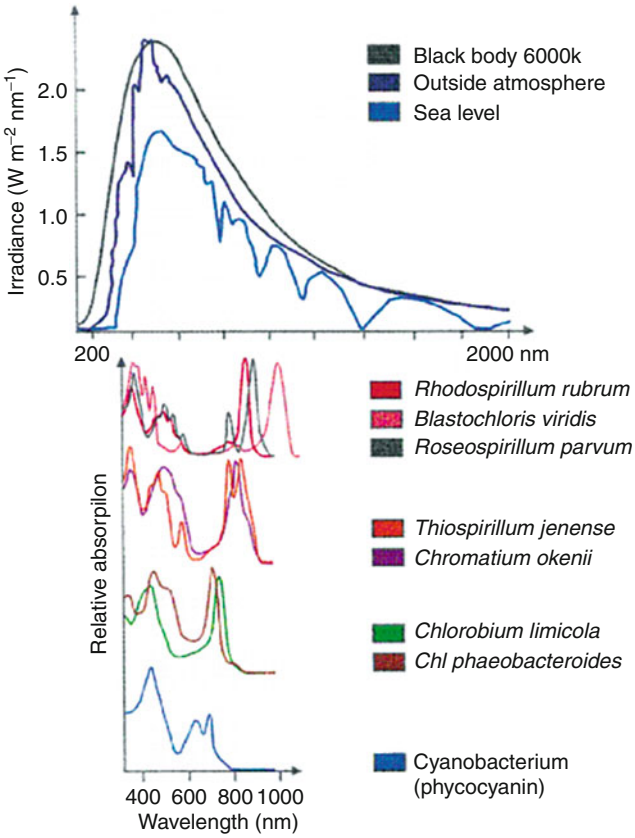


Fig. 12.6
Spectral energy distribution of solar radiation outside the atmosphere and at sea level as compared to the absorption spectra of various phototrophic bacteria. Absorption spectra of the purple nonsulfur bacterium *Rhodospirillum rubrum* (containing BChl *a*, spirilloxanthin), *Blastochloris viridis* (BChl *b*, 1,2-dihydroneurosporene), and *Roseospirillum parvum* (BChl *a*, spirilloxanthin, lycopenal); of the Chromatiaceae species *Thiospirillum jenense* (BChl *a*,lycopene, rhodopin) and *Chromatium okenii* (BChl *a*, okenone); of the Chlorobiaceae species *Chlorobium limicola* (BChl *c*, chlorobactene) and *Chlorobium phaeobacteroides* (BChl *e*, isorenieratene); and of a cyanobacterium (Chl *a*, phycocyanin) are depicted. A recently described BChl*a*-containing purple sulfur bacterium that exhibits a long wavelength absorption maximum at 963 nm (Permentier et al. 2001; Rücker et al. 2012) is not depicted

light of the red wavelength range prevails such that green-colored species of green sulfur bacteria have a selective advantage over their brown-colored counterparts or purple sulfur bacteria (Parkin and Brock 1980a).

In benthic and soil ecosystems, light quality differs fundamentally from that in the pelagic environment. In the visible wavelength range, radiation is strongly attenuated by mineral and biogenic particles. In sandy sediments, light attenuation occurs preferentially in the wavelength range of blue light due to the reflection by sand grains (Jørgensen and Des Marais 1986; Kühl and Jørgensen 1992). The presence of iron minerals results

in an enhanced attenuation of UV and blue wavelengths (Garcia-Pichel and Belnap 1996). In contrast, absorption of infrared light by sediment particles is low and absorption by water is negligible due to the short optical pathlength. As a consequence of the optical properties of the sediment particulates, the red and infrared portion of the spectrum penetrates to the deepest levels. Multiple scattering causes the light fields to become rapidly diffuse, so that bacteria thriving within these environments receive light from all directions. The parameter measuring light received at a point in space from all directions is called scalar irradiance (E_0, or photon fluence rate). A third important, but counterintuitive, phenomenon is the presence of maximum irradiance values close to the surface, which are even larger than the incident scalar irradiance (*Fig. 12.5*). Below this surficial zone where the E_0 maximum occurs, E_0 attenuates exponentially (Jørgensen and Des Marais 1986, 1988; Kühl and Jørgensen 1992; Lassen et al. 1992). For visible light, the measured photic depths (depths where E_0 is attenuated to 1 % of the incident) varied between 3.1 mm for quartz sand and 0.45 mm for silty muds (Garcia-Pichel and Bebout 1996). In the ultraviolet (UV) at 310 nm, the corresponding depths were only 1.25 and 0.23 mm.

Besides solar photic environments, geothermal vents have been suggested to represent another potential, but extraordinary type of environment that may provide sufficient electromagnetic energy to support the growth of phototrophic microorganisms. Although the evidence for the emission of electromagnetic radiation in the visible and near infrared wavelength range by hydrothermal vents is debated, a novel phylotype of green sulfur bacterium could be enriched and isolated from samples taken from the vicinity of a black smoker on the East Pacific Rise at a water depth of 2,391 m (Beatty et al. 2005).

Principles of Light Absorption

The chlorophyll-based photosystems of bacteria convert electromagnetic energy into a redox gradient. The redox reactions are initiated by absorption of electromagnetic energy, leading to a transition of specific molecules into an excited electronic state. An increase in the electronic energy of a molecule requires more energy than changes in vibrational or rotational states. Since the energy of light quanta is inversely related to their wavelength (Planck's Law), molecules absorb electromagnetic radiation of short wavelengths (ultraviolet and visible light) during changes in electronic energy, and longer wavelengths during changes in vibrational (near infrared radiation) and rotational energy (far infrared radiation and microwaves). Changes in the electronic state of molecules, and thus photochemically driven redox reactions by light absorption, can only occur by absorption of quanta of wavelengths $<$1,240 nm (i.e., an energy larger than 1 eV per electron). This fact obviously limits the wavelength range that is usable for photochemical reactions. The major fraction of solar energy is present in the wavelength range between 400 and 750 nm. These wavelengths can only be harvested by organic molecules that contain delocalized π-electrons in conjugated double bonds (*Fig. 12.6*). Such molecules are called pigments.

Light Absorption and Excitation Transfer in Prokaryotes

Pigments and Light-Harvesting Complexes

To capture light, phototrophic organisms employ four classes of pigment molecules: magnesium porphyrins (chlorophylls and bacteriochlorophylls, also called chlorins), open-chain tetrapyrrole bilin pigments (phycobilins), carotenoids, and retinal. Besides the linear tetrapyrroles in bacteriophytochromes (Davis et al. 1999), other types of chromophores may be used in non-phototrophic light-harvesting, as is the case of the flavins and pterines of DNA-photolyase (Tanada et al. 1997) or the photoisomerizable *p*-hydroxycinnamic acid in bacterial photoactive yellow protein (Essen and Oesterhelt 1998). Until recently, it appeared that only the magnesium-containing chlorin molecules were employed as the major photosynthetic pigment. The aerobic photosynthetic bacterium *Acidiphilium rubrum* was the first photosynthetic organism known to employ zinc-containing bacteriochlorophyll *a* as the photochemically active pigment (Wakao et al. 1996).

When pigments capture a photon, they reach an excited state of high energy. Free molecules remain in the excited singlet state for as little as 10^{-8} to 10^{-9} s and rapidly return to the ground state. Through the multiplicity of vibrational and rotational states associated with each electronic energy level, two different electronic energy states may overlap. In such molecules the lowermost electronic energy level (the lowest excited singlet state) is reached in a rapid series of radiationless transitions with a concomitant small decrease in free energy. The photons emitted during the subsequent return of the electron to the ground state are therefore less energetic (of longer wavelength) than those that were absorbed (Stokes shift) (Hoppe et al. 1983). Chlorophylls and bacteriochlorophylls exhibit two major absorption bands (*Table 12.3*) and, when excited in the dissolved state, a corresponding red (685 nm for chlorophyll a) or infrared (786 nm for bacteriochlorophyll *a*) fluorescence. In photosynthetically active cells, however, only about 1 % of the absorbed light energy is lost by fluorescence. It is a characteristic of the photosynthetic apparatus of living organisms that fluorescence (hence loss of already absorbed energy) is minimized. Most of the energy absorbed by the antenna pigments is channeled by vectorial and radiationless inductive dipole resonance toward the reaction centers, where it drives the photochemical redox reactions. The specific coordination of pigment molecules in photosynthetic organisms favors inductive resonance and photochemical reactions over fluorescence. Within the photosynthetic antenna, a fine modulation of the absorption properties of the pigments occurs because of differences in their binding to the antenna proteins, so that a vectorial excitation cascade is thermodynamically favored (i.e., in a sequence involving pigments with progressively longer absorption maxima).

Table 12.3
Major absorption maxima of chlorins in whole cells and in the dissolved state and fluorescence maxima of whole cells of phototrophic prokaryotes

Chlorin	Absorption maxima (nm) Whole cells	Absorption maxima (nm) Acetone extracts	Fluorescence maxima (nm) Whole cells
Chl *a*	670–675	435, 663	680–685
Chl *b*	n.d.	455, 645	(in acetone 652)
Chl *d*	714–718	400, 697	(in acetone 745)
Chl *f*	n.d.	406, 706 (methanol)	(in methanol 722)
BChl *a*	375, 590, 805, 830–911	358, 579, 771	907–963
BChl *b*	400, 605, 835–850, 986–1,035	368, 407, 582, 795	1,040
BChl *c*	457–460, 745–755	433, 663	775
BChl *d*	450, 715–745	425, 654	763
BChl *e*	460–462, 710–725	459, 648	738
BChl *g*[a]	375, 419, 575, 788	365, 405, 566, 762	n.d.

[a]Bacteriochlorophyll *g* of the Heliobacteriaceae shows structural relationships to chlorophyll *a* because it contains a vinyl group on tetrapyrrole ring I. Like in bacteriochlorophylls *a* and *b*, pyrrole ring II is reduced, however, and the esterifying alcohol is famesol as in bacteriochlorophylls of green sulfur bacteria. As for bacteriochlorophyll *a* or *b*, the reduced state of ring II in bacteriochlorophyll *g* causes an additional though smaller absorption maximum, the Q_x band at about 567 nm. *n.d.* not determined

The resulting small differences in the energy level of antenna pigments direct the transfer of excitation energy centripetally to the reaction center.

A second consequence of the molecular interactions between pigments and proteins is a shift in the absorption peaks of the former toward wavelengths longer than one would find in the free pigments. These interactions mostly affect the energy level of the lowermost excited state and hence the long wavelengths absorption maxima. In the case of chlorophyll *a*, the shift in the position of the long wavelength absorption maximum is comparatively small while it is larger in bacteriochlorophyll-protein complexes (up to ~200 and ~250 nm in bacteriochlorophyll *a* and *b*-containing phototrophic bacteria, respectively; *Table 12.3*). The shift for most carotenoids in association with proteins is as small as for chlorophyll *a*. In intact cells, carotenoids absorb mainly in the 420–550 nm wavelength region. In contrast, binding of one type of porphyrin pigment (bacteriochlorophyll *a*) by different apoproteins has led to a considerable diversification of the long-wavelength absorption maxima in purple sulfur and nonsulfur bacteria (*Fig. 12.6*). Obviously the role of proteins in pigment-protein-complexes is not confined to the proper steric organization of pigment molecules but can also represent a means to exploit wavelength regions not utilized by other phototrophic organisms. Especially in intertidal microbial mats, variations in the fine structure of the pigment-protein complexes is a means of ecological niche separation (see sections "Interactions with Other Microorganisms" and "Competition for Light" and *Fig. 12.4* in this chapter). The absorption spectra of whole cells of phototrophic bacteria seem to have evolved in such a way that almost the entire electromagnetic spectrum suitable for electrochemical reactions can be exploited (*Fig. 12.6*). Analyses of the amino acid sequences of LH 1 polypeptides of *Roseospirillum parvum* yielded five candidate amino acid substitutions (most notably cysteine residues) that appear to be located closely to the BChl*a*-ligating histidine and might be involved in the unusually large red-shift of the long wavelength absorption (Q_y) band of this species (Tuschak et al. 2004). In the *Chromatiaceae* strain 970 that exhibits the most extreme red shift of the Q_y of all known $BChl_a$-containing bacteria, an amino acid deletion and a replacement (of_lysine by histidine) may cause the highly unusual absorption properties in vivo (Rücker et al. 2011).

The first step of porphyrin synthesis is the formation of 5-amino levulinic acid (δ-ALA). In *Chloroflexus aurantiacus*, Beta- and Gammaproteobacteria, *Cyanobacteria*, *Heliobacteriaceae*, and *Chlorobiaceae*, δ-ALA is synthesized from glutamate (C5-pathway), which appears to represent the more ancestral pathway. In contrast, Alphaproteobacteria as well as yeasts, fungi, and animals form δ-ALA by the ALA synthase-mediated condensation of glycine with succinyl-CoA (Beale 1995; Oh-Hama 1989; Oh-Hama et al. 1991).

All (bacterio)chlorophylls exhibit two major absorption bands (*Table 12.3*), leaving a considerably wide gap in the absorption spectrum, which is partially complemented by the absorption spectrum of carotenoids found in all phototrophic bacteria, and by a range of phycobiliproteins in most cyanobacteria. Owing to the linear sequence of the up to 15 conjugated double bonds, carotenoids absorb light at the short wavelength end of the visible range.

The light-harvesting antenna complexes of green sulfur bacteria, "*Candidatus* Chloracidobacterium thermophilum," as well as *Chloroflexus* and relatives, are extramembranous ovoid organelles, so-called chlorosomes, which are attached to the inner surface of the cytoplasmic membrane and contain bacteriochlorophylls *c*, *d*, or (in green sulfur bacteria) BChl *e*. In green sulfur bacteria and Cab. thermophilum, chlorosomes are linked and transfer excitation energy to the reaction center via the Fenna-Matthews-Olson (FMO) protein, a trimeric water-soluble antenna protein binding seven or eight BChl *a* molecules (Tronrud et al. 2009). Chlorosomes are exceptional in that proteins do not seem to be involved as ligands for most of the antenna bacteriochlorophyll molecules. Instead, interactions between the bacteriochlorophylls themselves govern the absorptive properties of the photosynthetic antenna in green sulfur bacteria (Blankenship et al. 1995; *Fig. 12.2a*). Here, the bacteriochlorophylls self-assemble in *syn-anti* stacks and thereby form tubular-shaped elements with a diameter >10 nm. Other, conflicting models have been proposed (e.g., Psencik et al. 2004). The close packing of the tetrapyrrols involves π-π stacking and

hydrogen bonding and results in ultrafast, long-distance transmission of excitation energy (Ganapathy et al. 2009). In all other phototrophic prokaryotes studied, chlorins and carotenoid molecules occur in complexes with proteins. The antenna bacteriochlorophylls of green sulfur bacteria reprent a mixture of a large number of different homologs. Thus, a detailed study of just five strains of brown-colored *Chlorobium* spp. yielded a total number of 23 different BChl*e* structures (Glaeser et al. 2002). The relative abundance of individual homologs differed between the strains and for a single strain varied with the physiological state (exponential or the stationary phase of growth) or with incubation light intensities (compare section ❯ "Adaptations to Low Light Intensities" in this chapter).

Chlorins in pigment-protein complexes are noncovalently bound by histidine imidazole residues, which ligate the central magnesium atom of the porphyrin (Drews and Golecki 1995). In some cases (e.g., heliobacterial reaction center protein; Vermaas 1994), the histidine residues are replaced by asparagine, glutamine, or arginine, which may function as ligands. Noncovalent binding of carotenoids seems to be mediated largely by hydrophobic interactions. In the purple nonsulfur bacteria, the *Chromatiaceae*, and *Ectothiorhodospiraceae*, all antenna complexes (and reaction centers) are located within intracytoplasmic membranes that are differentiated from, but contiguous to, the cytoplasmic membrane of the cell. In purple nonsulfur bacteria, *Chromatiaceae*, and *Ectothiorhodospiraceae*, intracellular membranes occur as vesicles, stacks, lamellae, or tubules (❯ *Figs. 12.1* and ❯ *12.2b*). Most photosynthetic species of the *Alphaproteobacteria* (*Rhodocyclus purpureus*, *Rhodocyclus tenuis*, *Rubrivivax gelatinosus*) do not form extensive intracellular membrane systems. The photochemical apparatus of purple nonsulfur bacteria is confined to the intracellular membrane system, whereas the enzyme complexes of the respiratory chain and transport systems are located in the cytoplasmic membrane (Bowyer et al. 1985). This functional differentiation does not seem to exist in purple sulfur bacteria (*Allochromatium vinosum*, *Ectothiorhodospira mobilis*; Drews and Golecki 1995). With one known exception, the photosynthetic apparatus in *Cyanobacteria* is located on specialized intracellular membranes (thylakoids). Thylakoids may be either single or stacked and are distributed concentrically (parallel to the cytoplasmatic membrane), radially, or randomly (❯ *Fig. 12.1*). In *Heliothrix*, *Roseiflexus castenholzii*, the *Heliobacteriaceae*, some purple nonsulfur bacteria (e.g., *Rhodocyclus tenuis*; Wakim and Oelze 1980), and one cyanobacterium (*Gloeobacter violaceus*), the photosynthetic apparatus is located in the cytoplasmic membrane but not on intracellular membrane invaginations or thylakoids.

The light-harvesting antenna complexes of purple nonsulfur and purple sulfur bacteria are composed of two small membrane-spanning α- and β-polypeptides (encoded by the *pufA* and *B* genes) to which bacteriochlorophyll *a* or *b*, and carotenoids are noncovalently bound. The polypeptide monomers aggregate within the membrane to form ring structures of 15–16 (LHI) or 9 (LHII) subunits, respectively (❯ *Fig. 12.2b*). Based on the crystal structure of the reaction center–light harvesting I core complex of *Rhodopseudomonas palustris*, the reaction center is surrounded by an oval LHI complex consisting of 15 pairs of transmembrane helical α- and β-polypeptides that harbor the antenna BChl*a*. A gap in this LHI array is located next to the binding site of the electron-accepting ubiquinone in the reaction center and possibly allows ubiquinone to leave the complex and donate electrons to cytochrome bc_1 (Roszak et al. 2003). The photosynthetic gene cluster of *Allochromatium vinosum* harbors three sets of *pufA* and *B* genes that are transcribed (Nagashima et al. 2002). Similarly, another purple sulfur bacterium ("*Amoebobacter purpureus*" ML1) has an operon structure of the genes $pufB_1A_1LMCB_2A_2B_3A_3$ which appear to be co-transcribed, forming a 5.5-long transcript (Tuschak et al. 2005). In addition, the purple sulfur bacterium strain 970 contains a second *pufBA* copy downstream of *pufC*, but in this case only *pufB1A1* is transcribed (Rücker et al. 2012). So far, the implications of the multiple antenna genes have remained obscure but potentially could be involved in the adaptation to different light intensities. Several LHII-aggregates transfer energy to this supercomplex. The structure of the peripheral LHII complex from *Rhodospeudomonas acidophila* has been analyzed at high resolution (Papiz et al. 2003). It consists of a ring of nine α-/β-polypeptides and, correspondingly, nine BChl*a* dimers that are strongly coupled and hence absorb at 857 nm. In addition, nine well-separated BChl*a* molecules (that absorb at 800 nm) reside between the β-polypeptides and close to the cytoplasmic surface. In contrast, the peripheral antenna of *Phaeospirillum molischianum* contains only eight dimers.

In *Cyanobacteria*, light-harvesting chlorophyll *a* is present in two different types of protein complexes. The CP43 and CP47 are Chl a-bound protein core-antenna complexes tightly associated with photosystem II (Barry et al. 1994). Within photosystem I, however, antenna chlorophylls are an integral part of the reaction center itself (Golbeck 1994; ❯ *Fig. 12.2c*).

A third class of light-harvesting antenna complexes are phycobilisomes. They occur in the division *Cyanobacteria* (and in the plastids of red algae and some other groups of eukaryotic algae), and in most species represent the main light-harvesting antenna structures of these bacteria. Under the electron microscope, phycobilisomes appear as hemidiscoidal to cylindrical particles attached to the cytoplasmic side of the thylacoids. In *Gloeobacter violaceus*, the cytoplasmic membrane is underlain by a continuous subcortical layer containing the phycobilisomes. Light energy absorbed by phycobilisomes is transferred preferentially to photosystem II, with intrinsic chlorophyll *a* serving as an antenna for photosystem I. However, short-term or partial spillover may occur, as the phycobilisomes are quite mobile (van Thor et al. 1998). While the blue and red wavelength range is absorbed mainly by chlorophyll; the phycobilisomes harvest the blue-green, yellow, and orange regions (450–655 nm) of the light spectrum, thereby extending the spectral range of photosynthetic light harvesting considerably (❯ *Fig. 12.6*). The capacity of forming phycobilisomes is of selective advantage for the colonization of low-light aquatic habitats. Most (80 %) of the phycobilisome mass are water-soluble phycobiliproteins, which contain covalently bound open-chain tetrapyrrole chromophores (the phycobilins). Four

types of phycobilins are known: the blue-colored phycocyanobilin (PCB), red-colored phycoerythrobilin (PEB), yellow-colored phycourobilin (PUB), and purple-colored phycobiliviolin (PXB, also sometimes abbreviated CV). They are found in various molar ratios and form part of four recognized types of phycobiliproteins: allophycocyanin (APC), phycocyanin (PC), phycoerythrocyanin (PEC), and phycoerythrin (PE). In contrast to (bacterio)chlorophylls, the chromophores are covalently bound by thioether linkages to cysteine residues of the apoproteins. Up to three chromophores may be bound to a single α- or β-polypeptide. The phycobiliproteins are heteromonomers forming $(\alpha\beta)_3$ trimeric disks. Together with chromophore-free linker polypeptides, these disks are assembled in aggregates, the phycobilisomes, which are attached to the cytoplasmic side of photosystem II (*Fig. 12.2c*). Peripheral rod elements consisting of phycoerythrin (which harbors PEB, and sometimes also PUB), or phycoerythrocyanin (with PCB and PXB), and phycocyanin (with PCB, and in some cases small amounts of PEB) are arranged in a hemidiscoidal fashion around a core substructure consisting largely of allophycocyanin (with PCB). The different absorption properties of the phycobilins are the result of differences in the number of conjugated double-bonds (the conjugated π-electron system is shorter for PEB and PUB), in the side chains of the tetrapyrrole prosthetic groups, including also chemically distinct chromophore-protein linkages, and in the protein environments of the chromophores (Sidler 1994). Light energy is absorbed mainly by the peripheral rods, and transferred rapidly by radiation-less downhill energy transfer from phycoerythrin (absorption maximum 495–575 nm) or phycoerythrocyanin (575 nm) to phycocyanin (615–640 nm). Finally, allophycocyanin (650–655 nm) transfers the energy to photosystem II. Not all cyanobacteria possess all of these different phycobiliproteins. Those synthesizing exclusively APC and PC appear blue-green. Many heterocystous cyanobacteria also produce PEC in addition to APC and PC (Bryant 1982); these strains never produce PE. Dark-colored strains of many benthic genera contain large amounts of PC and PE. Red cyanobacteria, typical for deep lacustrine and marine waters produce large amounts of PE, and only small amounts of PC. Marine open ocean cyanobacteria (*Synechoccus*, *Trichodesmium*) contain large amounts of a PUB-rich PE, with absorbance maxima around 495–500 nm.

Some cyanobacteria make use of chlorin-based antenna complexes intrinsic to the membrane instead of phycobilisomes. These complexes are made up of a diverse class of proteins with six transmembrane domains: the Pcb family. The Pcb proteins are related to CP43 of PSII by homology. They bind Chl *a* and Chl *b* in *Prochloron didemni* and *Phrochlorothrix hollandica*, divinyl Chl *a* and divinyl Chl *b* in *Prochlorococcus*, and Chl *d* in *Acaryochloris marina* (De Ruyter and Fromme 2008). The switch in antenna is thought to give them an advantage in using particular spectral ranges unused by other phototrophs (see section "Competition for Light" in this chapter). Chlorophyll *b* represents only a minor fraction of the photosynthetic pigments. In *Prochloron*, the ratio of chlorophyll *a*/chlorophyll *b* is between 2.6 and 12.0 (Thorne et al. 1977); this ratio is even higher in *Prochlorothrix* (10–18), in which the ratio of PSI to PSII is <3:1. In *Prochlorothrix hollandica*, cells grown at low light intensities exhibit the lowest chlorophyll *a*/chlorophyll *b* ratios (Matthijs et al. 1994). *Acaryochloris marina*, where Chl *d* is the major antenna chlorin (2 % of the dry weight, whereas Chl *a* is only 0.1 %) harvests light for both photosystems (Schiller et al. 1997). *A. marina* also contains traces of a Chl *c*-like pigment in addition to more typically cyanobacterial carotenoids (α-carotene—found also in *Prochlororococcus*, and zeaxanthine—found in many cyanobacteria) and phycobiliproteins (APC and PC; Miyachi et al. 1997). Most recently, a novel type of red-shifted chlorophyll, chlorophyll *f*, has been discovered in Western Australian stromatolites formed by cyanobacteria (Chen et al. 2010). Chemically, Chl*f* was identified as (2-formyl)-Chl*a*.

In purple bacteria, the size of the photosynthetic antenna is in the range of 20–200 bacteriochlorophyll *a* per reaction center (Zuber and Cogdell 1995). The specific bacteriochlorophyll *a* content of aerobic bacteriochlorophyll-containing bacteria reaches only 5–10 % of that of anoxygenic phototrophic bacteria (Yurkov and Beatty 1998). At least in one strain (*Rhizobium* BTAi1), the size of the photosynthetic unit is similar to that of anoxygenic phototrophic bacteria (Evans et al. 1990), indicating that the low pigment content is due to a low number of reaction centers. In PSII of cyanobacteria, the antenna comprises 300–800 phycobilin chromophores and 47 chlorophyll *a* molecules (Sidler 1994; Matthijs et al. 1994), whereas the reaction center protein PsaA of PSI binds 110 chlorophyll *a* molecules (Golbeck 1994). The photosynthetic antenna of green sulfur bacteria is significantly larger than that of other anoxygenic phototrophs. In *Chlorobaculum tepidum*, each chlorosome harbors up to 200,000 antenna bacteriochlorophylls (Montaño et al. 2003) and may be connected via up to 200 FMO proteins to 25–40 reaction centers in the cytoplasmic membrane. Hence up to 8,000 bacteriochlorophyll molecules transfer their excitation energy to a single reaction center (Frigaard et al. 2003). The tubular organization of bacteriochlorophylls within the chlorosomes is optimal for an efficient excitation transfer within a very large antenna. These features appear to be one major reason for the competitive success of green sulfur bacteria in low-light environments (see section "Competition for Light" in this chapter). Antenna size is smaller in *Chloroflexus* (Olson 1998). About 35 molecules of bacteriochlorophyll g are associated with one reaction center in *Heliobacteriaceae* (Amesz 1995).

Efficiency of Light Harvesting

The light absorption capabilities of photosynthetic prokaryotes can be judged best by calculating which fraction *f* of the light impinging on a single cell is actually absorbed. This fraction is considerable for purple sulfur and other bacteria. The highest bacteriochlorophyll-specific attenuation coefficient k_B has been determined for a population of *Amoebobacter purpureus* (0.050 $m^2 \cdot (mg\ BChl\ a)^{-1}$; Overmann et al. 1991a).

For comparison *Prochlorococcus* has a chlorophyll-specific attenuation coefficient of 0.0147–0.0232 $m^2 \cdot (mg\ Chl\ a^{-1})$ (Moore et al. 1998). For *Amoebobacter*, *f* is 0.36, or 36 %, as calculated from Beer's Law and using the value of k_B, the intracellular concentration of light-harvesting pigments C (10.3×10^6 mg $BChl \cdot m^{-3}$), calculated from a content of 85 µg BChl·(mg protein)$^{-1}$ (van Gemerden and Mas 1995; Watson et al. 1977) and the average optical pathlength d of a cell (2 µm):

$$f = 100 \times \exp(-k_B \times C \times d)$$

Of the photosynthetic pigments that absorb this high fraction of incident light, the majority (typically >97 %) serve in light-harvesting and transfer excitation energy to the photochemical reaction centers. The combination of antenna complexes with one reaction center constitutes the photosynthetic unit. The efficiency of energy transfer within the photosynthetic unit and its size determine the fraction of the quantum flux that is harvested.

Large concentrations of pigments result in self-shading and thus a reduced efficiency of light absorption per mole of pigment. At the cell size and intracellular pigment concentrations typical of most prokaryotic phototrophs, this decrease in efficiency is not very important (Garcia-Pichel 1994), but it might be significant in some extremely low-light adapted anoxygenic phototrophs like the green sulfur bacterial strain isolated from the Black Sea chemocline (Overmann et al. 1991a).

Close proximity of photosynthetic pigments enables an efficient transfer of excitation energy but at the same time also causes a so-called package effect (Kirk 1983) by which self-shading of the pigment molecules exceeds that predicted by the Lambert-Beer law. The package effect is seen clearly in a flattening of absorption peaks, commonly observed when recording absorption spectra of whole cells. Because the energy requirement for biosynthesis of additional antenna structures is rather constant, the net energy gain for a photosynthetic cell must decrease at higher intracellular pigment concentrations, which restricts the amount of light-harvesting structures a photosynthetic cell can synthesize. Polypeptides of the photosynthetic machinery (a significant fraction of the total cell protein) amount to 20 % in purple nonsulfur bacteria and >50 % in phycobiliprotein-containing cyanobacteria. Interestingly, the total protein content of cyanobacterial cells is comparable to other phototrophic bacteria. Possibly, cyanobacteria contain reduced levels of proteins involved in nonphotosynthetic processes to compensate for the high energy and nitrogen expenditure of the antenna proteins.

The biosynthesis of proteins requires a major fraction of the energy expenditure of the bacterial cell (Gottschalk 1986). In chlorosomes, the mass ratio of protein:bacteriochlorophyll is significantly lower than in other light-harvesting complexes (*Table 12.4*) (Vassilieva et al. 2002). Probably this is one major reason for the larger antenna size and the lower light energy requirements of green sulfur bacteria as compared to their purple and cyanobacterial counterparts (see section "Interactions with Other Microorganisms" and "Competition for Light" in this chapter), and might help explain the competitive advantage gained by *Prochlorococcus* over their close relatives *Synechococcus* in the open oceans.

Table 12.4
Pigment: protein ratio in different photosynthetic antenna complexes

Antenna complex type	Protein:pigment	
	Mass ratio	Per pigment molecule (in Da)
Chlorosomes	0.5–2.2	420–1,840
B806-866 complex[a]	3.9–5.8	3,550–5,290
B800-850 LHII	4.4	4,000
B820 LHI	6.7	6,100
Phycobilisomes	~22.4	~12,300

[a]*Chloroflexus aurantiacus*
Data from Olson (1998) or calculated from Sidler (1994), Loach and Parkes-Loach (1995), Zuber and Cogdell (1995). Carotenoids have been neglected in these calculations because of their lower numbers as compared to bacteriochlorophylls (B800-850 LHII), their absence in phycobilisomes, and the controversy concerning their functional significance in light-harvesting (chlorosomes). Only antenna complexes which are separate entities from reaction centers were considered. Photosystem I does not contain a distinct antenna structure; the PsaA protein of the reaction center binds 110 chlorophyll *a* molecules

Conversion of Light into Chemical Energy

Principle

The unifying principle of bacterial and archaeal phototrophy is the light-driven generation of a proton-motive force (PMF). The PMF is subsequently used by ATP synthase to form ATP, or for active transport and motility.

In chlorophyll-based photosynthesis, redox reactions and charge separation precede the establishment of the PMF. In addition, reducing power (NAD(P)H + H^+) is generated as a primary product of the light reaction in *Cyanobacteria*. In the photochemical reaction, only the energy of the lowest excited singlet state (see section "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter) of the chlorophylls is used. Consequently, all absorbed light quanta have the same effect irrespective of their original energy (wavelength). Therefore, when comparing the light energy available in different habitats, or the light adaptation of different phototrophic bacteria, it is more meaningful to express irradiances in units of mol quanta$\cdot m^{-2} \cdot s^{-1}$ rather than $W \cdot m^{-2}$ (see section "Competition for Light" in this chapter).

The standard free energy for the reduction of CO_2 depends on the redox potential of the electron donor employed (*Table 12.5*, *Fig. 12.7*). If this energy requirement for electron transfer is compared with the energy available after absorption of photons of different wavelengths and when accounting

Table 12.5
Standard redox potentials of different electron donors of the photosynthetic light reaction[a]

Electron donor	E_o' [mV]
$^1/_2O_2/H_2O$	+820
NO_3^-/NO_2^-	+430
$Fe(OH)_3 + HCO_3^-/FeCO_3$	+200
Fumarate/succinate	+33
HSO_3/S^{0-}	−38
SO_4^{2-}/S^0	−200
SO_4^{2-}/HS^-	−218
$Fe(OH)_3/Fe^{2-}$	−236
S^0/HS^-	−278
HCO_3^-/acetate	−350
$S_2O_3^{2-}/HS^- + HSO_3^-$	−402
$H^+/^1/_2H_2$	−414
Electron acceptor	E_o' [mV]
$CO_2/<CH_2O>$	

[a]Taken from Brune (1989), Widdel et al. (1993), Thauer et al. (1977), Zehnder and Stumm (1988), Griffin et al. (2007)

for the energetic efficiency of glucose formation from ATP and NADH (32 %) that renders the process thermodynamically irreversible, it becomes clear that oxygenic photosynthesis is not feasible at a one-photon-per-electron stoichiometry in photosystems containing the known types of chlorin pigments and that it requires the absorption of two photons per electron (*Fig. 12.7*).

The biological conversion of light into chemical energy has been found to be remarkably efficient: the number of charge separation events per absorbed photon is 1.0 (Kok 1973; Wraight and Clayton 1973) and the efficiency of the entire photoconversion process of a red photon to chemical energy by oxygenic photosynthetic organisms is 43 % (Golbeck 1994). Whereas the efficiency of energy transfer between antenna bacteriochlorophyll and the reaction center in most cases is close to 100 % (Amesz 1995), the transfer between antenna carotenoids and the reaction center can be significantly lower, 70 % in *Heliobacteriaceae* (Amesz 1995) and even 20 % in a purple nonsulfur bacterium (Angerhofer et al. 1986). When carotenoids serve as the only light-harvesting pigments, 2.5 times higher irradiances are required by *Rhodopseudomonas acidophila* to attain the same growth rates as compared to light-absorption by bacteriochlorophyll (Göbel 1978). In aerobic phototrophic bacteria, most of the highly diverse carotenoids do not function as light-harvesting molecules but might serve in quenching of toxic oxygen radicals (Noguchi et al. 1992; Yurkov et al. 1994). In chlorosomes of green sulfur bacteria, the efficiency of energy transfer between carotenoids and BChl may range between 15 % and 70 % (Psencik et al. 2002). Here, carotenoids are probably involved in the protection against photo-oxidation and in structural stabilization of BChl*a* in the chlorosome baseplate (Frigaard et al. 2004).

One prerequisite for the photoconversion process is the presence of a membrane that is impermeable to protons and separates two different cell compartments. Three integral membrane multisubunit protein complexes participate in the generation of ATP in all phototrophic bacteria: the photosynthetic reaction center, a cytochrome complex, and an ATP synthase. All three are highly conserved within the bacterial radiation. Reaction centers have a dimeric core and consist of two closely associated integral membrane polypeptides plus additional proteins (*Fig. 12.3*). The special protein environment of the reaction center stabilizes the excited state and prevents back reaction after charge separation by enforcing ultrafast electron transfer to other electron acceptors nearby. The transfer of excitation energy from the antenna complexes to the reaction center initiates a charge separation at a special bacteriochlorophyll dimer (special pair), which is located on the periplasmic (or lumen) side of the photosynthetic membrane. It is this endergonic process of charge separation that is ultimately driven by light energy; all the following redox reactions are exergonic. An electric potential is established across the membrane (inside negative). In its excited state, the special pair becomes a powerful reductant and ultimately reduces a quinone (in pheophytin-type reaction centers) or ferredoxin (in FeS-type reaction centers) on the cytoplasmic side of the photosynthetic membrane. The quinol or reduced ferredoxin leaves the reaction center complex and in turn donates electrons to a membrane-bound cytochrome complex or NADH dehydrogenase. A series of redox reactions results in the establishment of a proton-motive force across the photosynthetic membrane. Finally, the PMF is converted to ATP by ATPase.

In contrast to the (bacterio)chlorophyll-based systems of bacteria, light energy conversion of Halobacteria does not involve redox reactions and is limited to a vectorial transport of protons by bacteriorhodopsin. Upon excitation by light, the prosthetic retinal undergoes a series of reversible photochemical transformations (an isomerization from the all-*trans* to the 13-*cis* form) and releases a proton into the extracellular space. The PMF thus generated is used for ATP synthesis by ATPase. Due to its low solubility, O_2 in the concentrated salt solution is present in a significantly lower amount than in freshwater. Rhodopsin-mediated formation of ATP may become the sole source of energy for growth under anaerobic conditions in the light (Oesterhelt and Krippahl 1983) and has therefore been viewed as an adaptation to the natural brine habitat of Halobacteria. More recently, rhodopsins (so-called proteorhodopsins) have also been detected in Bacteria, green algae, and Fungi. Based on metagenomic studies and genomic studies of cultivated bacteria, it occurs in marine planktonic Proteobacteria (Béjà et al. 2000), in *Flavobacteriaceae* (Gomez-Consarnau et al. 2007) but also in freshwater *Actinobacteria* (Sharma et al. 2008) where they serve a bioenergetic function. Spectrally distinct variants occur in the marine environment. Their different absorption spectra of proteorhodopsins correlate with the wavelength of underwater light that prevails in their

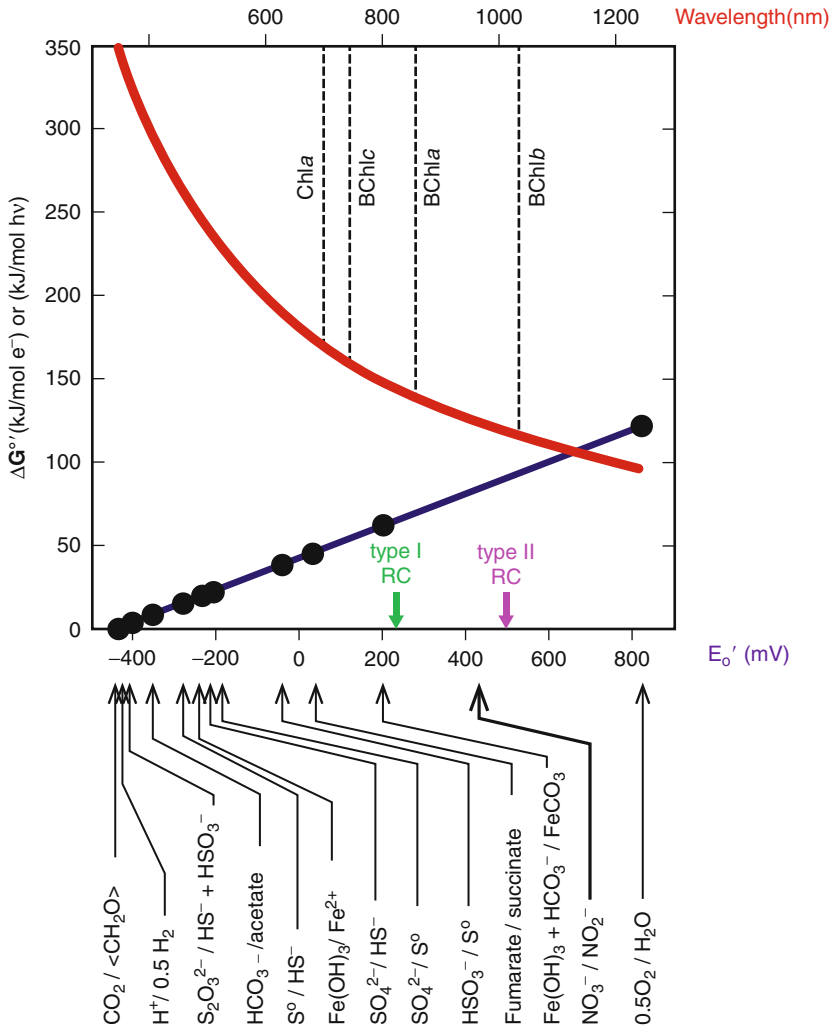


Fig. 12.7

Free energy of one mol quanta as a function of wavelength (*red line*), calculated from Planck's constant h (6.63×10^{-34} J·s), the speed of light c (2.99×10^{8} m·s^{-1}), the wavelength of light λ, and the Avogadro constant $N_A = 6.023 \times 10^{23}$ mol^{-1} according to $\Delta G^{o'}_{h\nu} = N_A \cdot h \cdot c \cdot \lambda^{-1}$. Free energy required for the transfer of 1 mole of electrons to CO_2 (*black line*) as a function of the standard redox potential $E_o'_d$ of the electron donor used (see *Table 12.5*) calculated according to $\Delta G^{o'}_{el} = -F \cdot (-470 - E_o'_d)$ using the Faraday constant F (96.5 kJ·V^{-1}·mol^{-1}). Dashed vertical lines indicate the energy that is available after absorption of light by the long wavelength Q_y absorption bands of different photosynthetic pigments. The standard redox potentials of type I and type II reaction centers that receive the electrons from the external electron donors are indicated

particular habitats (Béjà et al. 2001). Because of its distinct mechanism, this type of "photosynthesis" is not discussed in further detail in the present section. Additional information can be found in chapters to be published later (under the section "Archaea" in a volume tentatively titled "Other Major Lineages of Bacteria and the Archaea").

Molecular Architecture of the Reaction Center

All bacteria that perform anoxygenic photosynthesis employ a single photosystem. The decrease in redox potential that a single photosystem can undergo upon excitation appears to be limited (Blankenship 1992, compare *Fig. 12.7*). A combination of two different photosystems is required for the thermodynamically unfavorable utilization of water as an electron donor for photosynthesis (*Fig. 12.2c*). With the relatively simple architecture of their photosystems, all anoxygenic phototrophic bacteria depend on electron donors that exhibit standard redox potentials more negative than water (e.g., H_2S, H_2, acetate; *Table 12.5*). This molecular feature is one major reason for the narrow ecological niche of anoxygenic phototrophic bacteria in extant ecosystems (see section "Habitats of Phototrophic Prokaryotes" in this chapter).

Two different types of reaction centers occur in photosynthetic bacteria. Based on the chemical nature of the early electron acceptors, a pheophytin/quinone-type reaction center and a FeS-type reaction center are distinguished (Blankenship 1992; Homann-Marriott and Blankenship 2011; *Fig. 12.2a, b*). The first type is found in green gliding *Chloroflexus* species, phototrophic members of the *Alpha-* and *Betaproteobacteria*, *Chromatiaceae*, *Ectothiorhodospiraceae*, and in the PSII of *Cyanobacteria*. The reaction center of *Proteobacteria* consists of three protein subunits (L, M, H) which bind four bacteriochlorophylls, two bacteriopheophytins, two quinones, and one high-spin nonheme Fe^{2+} (Lancaster and Michel 1996; *Fig. 12.2b*). Many species (e.g., *Chloroflexus aurantiacus*, *Blastochloris viridis*, and *Allochromatium vinosum*) contain an additional tetraheme cytochrome c polypeptide attached to the periplasmic side of the reaction center.

Following the transfer of the electrons by ubiquinol or plastoquinol, the redox reactions at the cytochrome bc_1 (or b_6f) complex drive proton transport across the cytoplasmic membrane. Protons are translocated either into the extracellular space (anoxygenic phototrophic bacteria) or the intrathylacoidal space (cyanobacteria). The ratio of protons translocated to electrons transferred (H^+/e^- ratio) is 2. The reaction center and cytochrome bc_1 in pheophytin-type reaction centers of *Proteobacteria* and *Chloroflexus* are functionally linked by two diffusible electron carriers, ubiquinone in the hydrophobic domain of the membrane and cytochrome c_2 or auracyanin (Meyer and Donohue 1995) in the periplasmic space. The liberated electron is transferred back to the special pair via quinone, the cytochrome bc_1 complex and soluble periplasmic soluble electron carrier (often cytochrome c_2). Owing to this cyclic electron transport, the only primary product of photosynthesis is the proton-motive force, and the reduced pyridine nucleotide required for photosynthetic CO_2 fixation is generated by energy-dependent reverse electron flow (*Fig. 12.2*).

In oxygenic phototrophic bacteria, plastoquinone is the electron acceptor of PSII and donates electrons to the cytochrome b_6f-complex. The special pair is reduced by the manganese-containing water-splitting system located at the lumenal side of the transmembrane PSII complex (*Fig. 12.2c*).

In the pheophytin-type reaction centers of aerobic phototrophic bacteria, photoinduced charge separation occurs only in the presence of O_2 (Okamura et al. 1985). It has been proposed (Yurkov and Beatty 1998) that oxic conditions are required for photochemical activity because the primary acceptor ubiquinone has a significantly higher midpoint redox potential than in anoxygenic photosynthetic bacteria (65–120 mV more positive). The primary acceptor therefore may stay in its oxidized, electron-accepting state only in the presence of O_2.

The second type of reaction center contains iron-sulfur clusters as early electron acceptors and occurs in green sulfur bacteria (*Fig. 12.2a*), *Heliobacteriaceae*, and in the photosystem I of *Cyanobacteria*. Functionally, the reaction centers of green sulfur bacteria, *Heliobacteriaceae*, and PSI of *Cyanobacteria* are therefore similar. However, the former two are homodimeric and only one reaction center gene has been detected, whereas the reaction center of PSI of cyanobacteria and green plants contains two nonidentical, but similar, subunits (PS I-A and PS I-B; Vermaas 1994). In the heliobacterial reaction center, 8^1-hydroxy-chlorophyll *a* esterified with farnesol (8^1-OH-Chl a_F) serves as the primary electron acceptor of the special pair (Tang et al. 2010). Interestingly, the reaction center of the phylogenetically deeply branching *Gloebacter violaceus* contains menaquinone instead of phylloquinone that is present in other cyanobacteria. In FeS-type reaction centers, the redox potential of the special pair in its reduced state (P^*) is sufficiently low to permit a transfer of electrons to ferredoxin. $NAD(P)^+$ can therefore be directly reduced by noncyclic electron flow and does not require further energy expenditure as in purple bacteria. In green sulfur bacteria, a thioredoxin reductase-like protein may exert the necessary ferredoxin: $NADP^+$ oxidoreductase activity (Eisen et al. 2002).

Notably, ubiquitous unicellular small-celled and free-living cyanobacteria related to *Cyanothece* sp. have been found to be genetically incapable of oxygenic photosynthesis as their genome lacks photosystem II and carbon fixation genes (Zehr et al. 2008). It was concluded that this newly discovered type grows as a photoheterotroph.

Electron Donors

Anoxygenic phototrophic bacteria of the *Alpha-* and *Betaproteobacteria* use a wide variety of reduced organic carbon compounds as electron-donating substrates (see section "Carbon Metabolism of Phototrophic Prokaryotes" in this chapter; *Table 12.5*; *Fig. 12.7*). Most phototrophic sulfur bacteria are capable of using sulfide as photosynthetic electron donor. Other inorganic electron donors utilized include H_2, polysulfides, elemental sulfur, thiosulfate, sulfite, iron, and nitrite (Widdel et al. 1993; Griffin et al. 2007; Schott et al. 2010). Sulfide is oxidized to zero-valent sulfur, which in *Chromatiaceae* appears to be deposited as polysulfides or polythionates rather than in the form of S_8 rings (Steudel 1989; Steudel et al. 1990). In addition, thiosulfate is formed as an oxidation product by some species (Steudel et al. 1990). The photosynthetic sulfide oxidation rates of purple sulfur bacteria are higher than required for growth and remain constant at all growth rates. As a result, storage of sulfur is at maximum at low growth rates (van Gemerden and Mas 1995). Zero-valent sulfur is further oxidized to sulfate. In *Chlorobiaceae*, sulfide is oxidized by sulfide:quinone oxidoreductase. Except for *Chloroherpeton thalassium*, the oxidation product is subsequently oxidized to sulfite by the dissimilatory sulfite reductase. The final step is conducted by two different systems, adenosine-5′-phosphosulfate reductase and polysulfide reductase-like complex 3 (Gregersen et al. 2011). In microbial mats, polysulfides and organic sulfur compounds may be significant as photosynthetic electron donor. Polysulfide oxidation has been reported for *Chlorobium limicola* f.sp. *thiosulfatophilum*,

Allochromatium vinosum, *Thiocapsa roseopersicina*, while dimethylsulfide is utilized and oxidized to dimethylsulfoxide by the two purple sulfur bacteria *Thiocystis* sp. and *Thiocapsa roseopersicina* (van Gemerden and Mas 1995). In addition to reduced sulfur compounds, hydrogen serves as an electron donor in the majority of green sulfur bacteria, and in the metabolically more versatile species of purple sulfur bacteria (such as *Allochromatium vinosum* and *Thiocapsa roseopersicina*). In green sulfur bacteria, a reduced sulfur source is required during growth with molecular hydrogen since these bacteria lack assimilatory sulfate reduction. A few species of purple nonsulfur bacteria, of *Chromatiaceae*, and of the green sulfur bacteria have been found to utilize ferrous iron as photosynthetic electron donor (Widdel et al. 1993; Heising et al. 1999). This mode of anoxygenic photosynthesis may actually sustain a population of brown-colored *Chlorobiaceae* at a water depth >100 m in Indonesian Lake Matano (Crowe et al. 2008). Finally, nitrite has recently been shown to serve as electron donor in purple sulfur bacteria (*Thiocapsa* sp. strain KS1, *Tca. roseopersicina* strains DSM 217 and DSM 221) as well as a purple nonsulfur bacterium (*Rhodopseudomonas* sp. strain LQ17) (Griffin et al. 2007; Schott et al. 2010). Nitrite represents the electron donor for anoxygenic photosynthesis with the highest standard redox potential (◉ *Table 12.5*). Due to its toxicity, nitrite has to be supplied at concentrations <2 mM.

Sulfide acts as a strong poison of PSII activity in many algae and cyanobacteria. The ability of some *Cyanobacteria* to conduct anoxygenic photosynthesis with sulfide as an electron donor to PSI (Cohen et al. 1975; Padan 1979; Padan and Cohen 1982), or to continue oxygenic photosynthesis in the presence of sulfide (Cohen et al. 1986), may be one of the key traits that extend the habitat of sulfide-utilizing cyanobacteria into the temporarily anoxic, sulfide-containing, layers of hot springs (Castenholz and Utkilen 1984), marine microbial mats (De Wit and van Gemerden 1987a; De Wit et al. 1988), and the chemoclines of meromictic lakes (Jørgensen et al. 1979; Camacho et al. 1996). Sulfide is an inhibitor of PSII and induces the synthesis of a sulfide-oxidizing enzyme system. In contrast to phototrophic sulfur bacteria, cyanobacteria oxidize sulfide to elemental sulfur or thiosulfate but do not form sulfate (De Wit and van Gemerden 1987b). However, the use of sulfide by cyanobacteria in anoxygenic photosynthesis must be regarded as a detoxification mechanism, since their low affinity for sulfide (De Wit and van Gemerden 1987b; Garcia-Pichel and Castenholz 1990) renders them unable to compete with purple or green sulfur bacteria for sulfide as an electron donor.

In the natural habitat, growth of phototrophic sulfur bacteria is limited mainly by light and sulfide. Sulfide often becomes the growth-limiting factor at the top of the phototrophic sulfur bacterial layers where light intensities are highest, while sulfide has to diffuse through the remainder of the community. The affinity for sulfide during photolithotrophic growth varies between the different groups of anoxygenic phototrophs (including cyanobacteria growing with sulfide) and has been shown to be of selective value during competition experiments. Green sulfur bacteria and *Ectothiorhodospiraceae* exhibit five to seven times higher affinities for sulfide than *Chromatiaceae* (van Gemerden and Mas 1995). On the contrary, affinities for polysulfides are comparable between green sulfur bacteria and *Chromatiaceae*.

Efficiency of Growth and Maintenance Energy Requirements

For any photochemical reaction, the quantum yield is defined as the number of molecules converted per light quantum absorbed. The quantum efficiency is the ratio of energy stored in a compound, to the radiant energy absorbed for its formation. The quantum requirement is the reciprocal of the quantum yield. For CO_2 fixation of purple sulfur bacteria, a quantum requirement of 8–10.5 mol quanta·(mol CO_2)$^{-1}$ is theoretically expected (Brune 1989), considering that reverse electron transport is necessary. Experimentally, a quantum requirement of 12 ± 1.5 and 11.7 mol quanta·(mol CO_2)$^{-1}$ was determined, which corresponds to a quantum yield of 0.083 (Wassink et al. 1942 in Brune 1989; Göbel 1978). In contrast, calculated values for the quantum requirements of green sulfur bacteria lie between 3.5 and 4.5 mol quanta·(mol CO_2)$^{-1}$, assuming noncyclic electron transport. However, earlier measurements had yielded much higher values (9–10; Brune 1989).

The quantum yield for CO_2-fixation determined for *Prochlorococcus* isolates incubated in daylight spectrum fluorescent light was between 0.086 and 0.128 mol C·(mol quanta)$^{-1}$ (Moore et al. 1998), thus reaching Emerson's theoretical maximum for O_2 evolution in oxygenic photosynthesis. In cyanobacteria, typically thriving in oxic environments where only oxidized sources of nitrogen and sulfur are available, a large proportion of the reducing power generated in the light reactions must be diverted to assimilatory nitrate or sulfate reduction, or to nitrogen fixation, so that the quantum requirement for CO_2 fixation can be substantially lower than that for oxygen evolution. In *Oscillatoria limnetica*, capable of both oxygenic and anoygenic photosynthesis, the optimal quantum yield using PSI alone (0.059) with sulfide was significantly higher than that attained with both photosystems (0.033) (Oren et al. 1977).

In a careful study of *Rhodobacter capsulatus* and *Rba. acidophilus* grown with lactate as electron donor in a light chemostat, a value for the maintenance light energy requirement of m_q = 0.012 mol quanta·(g dry weight·h)$^{-1}$ was determined (Göbel 1978). The maintenance energy requirements of green sulfur bacteria are significantly lower compared to those of their purple counterparts (van Gemerden and Mas 1995). This may be explained by the fact that protein turnover is highly energy demanding and that the protein content of the green sulfur bacterial antenna is much lower than in purple sulfur bacterial antenna (◉ *Table 12.4*). A recent study of the brown-colored *Chlorobium* sp. BS1 from the Black Sea chemocline yielded the lowest maintenance energy requirement known for any

bacterium to date. BS1 requires as little as 1.6–4.9 · 10^{-15} kJ · $cell^{-1}$ · day^{-1} which figures almost 4 orders of magnitude lower than the value for *Rhodobacter capsulatus* KB1 and still tenfold lower than the lowest maintenance energy requirement reported so far (Marschall et al. 2010).

Response to Changes in Light Intensity and Quality

Phototrophic bacteria acclimate to changes in light intensity and quality by diverse mechanisms. Anoxygenic phototrophic bacteria as well as cyanobacteria respond to a step-down in irradiance by increasing the specific pigment content and vice versa (references compiled in Sánchez et al. 1998). These changes can be accomplished either by varying the number of photosynthetic units per cell, the size of the individual photosynthetic unit, or both (see section ❯ "Long-Term Adaptations to Changes in Light Intensity" in this chapter). Besides long-term biochemical changes in the composition and the amount of light-harvesting complexes, short-term redistribution of antenna capabilities (see section ❯ "State Transitions" in this chapter) occur in oxygenic phototrophs.

Many species use vertical migration, mediated by tactic responses (see section ❯ "Movement" in this chapter) and formation of gas vesicles to regulate their vertical position and exposure to light. Especially in the stably stratified pelagic habitats of phototrophic sulfur bacteria, the difference in buoyant density from the surrounding water would cause a sedimentation of bacterial cells out of the photic zone and toward the lake bottom. The minimum cellular buoyant density determined in phototrophic cells devoid of gas vesicles was 1,010 $kg \cdot m^{-3}$ (Overmann et al. 1991b). Actively growing cells, which contain storage carbohydrate and, in the case of *Chromatiaceae*, elemental sulfur, can easily attain much higher buoyant densities (up to 1,046 $kg \cdot m^{-3}$; Overmann and Pfennig 1992). By comparison, freshwater has a considerably lower density (e.g., 996 $kg \cdot m^{-3}$; Overmann et al. 1999c). As a consequence, sedimentation losses are significant for natural populations of several species of phototrophic sulfur bacteria (Mas et al. 1990).

Phototrophic bacteria have developed two ways to adjust their vertical position along gradients of light intensity and spectral composition. For purple sulfur bacteria, motility in response to changes in irradiance is known to be of ecological significance in both planktonic and benthic situations. In benthic and terrestrial cyanobacteria, vertical locomotion by gliding is common. Planktonic cyanobacteria inhabiting stratified waters perform vertical migrations by changing their cellular gas vesicle content and ballast mass (intracellular carbohydrates and protein) and hence their buoyant density. Planktonic anoxygenic phototrophic bacteria do not seem to perform vertical migrations mediated by changes in gas vesicle content but rather use these cell organelles to maintain their position within the chemocline (Overmann et al. 1991b, 1994; Parkin and Brock 1981).

Adaptations to Low Light Intensities

The capability to adapt to low light intensities represents a competitive advantage for phototrophic organisms. An estimate of the minimum irradiance I_{min} required for survival of phototrophic cells in the environment can be calculated from a few physiological parameters, namely, the pigment content of the cells, P (in mg bacteriochlorophyll·g C^{-1}); the maintenance energy requirement, m_q (in mol quanta·g $C^{-1} \cdot s^{-1}$); the (bacterio)chlorophyll-specific attenuation coefficient, k (in m^2·mg BChl a^{-1}); the cellular dry weight content, D (in g $C \cdot m^{-3}$); and the mean optical pathlength of one cell, d:

$$I_{min} = m_q \cdot D \cdot d/[1 - \exp(-k \cdot D \cdot P \cdot d)]$$

Employing the appropriate values for m_q (see section ❯ "Efficiency of Growth and Maintenance Energy Requirements"), k and P (see section ❯ "Light Energy and the Spectral Distribution of Radiation" in this chapter), D ($1.21 \cdot 10^5$ g $C \cdot m^{-3}$; Watson et al. 1977), and d (0.5 m for the smaller anoxygenic phototrophs), this yields a minimum irradiance (I_{min}) of 2 µmol quanta·$m^{-2} \cdot s^{-1}$. In many natural habitats of anoxygenic phototrophic bacteria, irradiances of this order of magnitude or lower have been measured. *Prochlorococcus* has been found at deep water layers down to 300 m. However, these bacteria do not grow at light intensities below 3.5 mol quanta·$m^{-2} \cdot s^{-1}$ (Moore et al. 1998) and thus appear to be less low-light-adapted than the green sulfur bacterial strain BS1 isolated from the Black Sea which grows at light intensities as low as 0.015 µmol quanta·$m^{-2} \cdot s^{-1}$ (Marschall et al. 2010). Lower irradiances could be used by phototrophic prokaryotes after a decrease of m_q or an increase of P or both. Both adaptations are present in the Black Sea strain (❯ Long-Term Adaptations to Changes in Light Intensity).

Long-Term Adaptations to Changes in Light Intensity

In those photosynthetic bacteria whose entire photosynthetic apparatus is confined to the membrane, light absorption is often increased by formation of intracellular membrane systems (❯ *Fig. 12.1*). In *Rhodobacter capsulatus*, the number of intracellular membrane vesicles increases by a factor of 6.3 when the cells are shifted from high to low light intensities. As a result, the area of intracellular membranes under these conditions is 2.7-fold larger than the area of the whole cytoplasmic membrane. Photosynthetic species of the *Betaproteobacteria* that do not form extensive intracellular membrane systems (*Rhodocyclus purpureus*, *Rhodocyclus tenuis*, *Rubrivivax gelatinosus*) increase the density of photosynthetic units in their cytoplasmic membrane (Drews and Golecki 1995). Intracellular membranes appear to be absent in *Heliobacteriaceae* and *Heliothrix*, where pigments are confined to the cytoplasmic membrane (❯ *Fig. 12.1*). In *Chloroflexus aurantiacus*, the increase in cellular concentrations of bacteriochlorophylls *a* and *c* is mediated by an

increase in the number and volume of chlorosomes, and the percentage of cell membrane surface covered by chlorosomes (Golecki and Oelze 1987). In a similar manner, green sulfur bacteria adapt to low light intensities by increasing the size and the cellular number of chlorosomes.

During induction of the photosynthesis apparatus in *Proteobacteria*, invaginations of the cytoplasmic membrane increases in the number and size of the photosynthetic units, and bacteriochlorophyll synthesis occur simultaneously. Under anoxic conditions, the amount of pigment synthesized by anoxygenic phototrophic bacteria is inversely related to the available light intensity and varies by a factor of up to 6.6 (Göbel 1978). After a shift to low light intensity, the ratio of light-harvesting complex I per reaction center remains constant (at about 30 bacteriochlorophylls per reaction center), whereas the relative amount of the peripheral light-harvesting complex II increases. As a result, the size of the photosynthetic unit changes by a factor of 2–5. Conversely, the specific NADH dehydrogenase activity decreases as does the amount of cytochrome and ubiquinone per reaction center. In *Rba. capsulatus* and *Rba. sphaeroides*, these changes take about 2–3 generations and the growth rate is lowered during adaptation due to energy limitation. In the purple sulfur bacterium *Allochromatium vinosum*, low-light adaptation is also accomplished by increasing the size of the photosynthetic unit (Sánchez et al. 1998). Species like *Rhodospirillum rubrum* and *Blastochloris viridis*, which harbor only one type of light-harvesting complex, increase the number of photosynthetic units (Drews and Golecki 1995).

In cells of the brown-colored Black Sea *Chlorobium* grown under light limitation, the specific pigment content of MN1 is doubled (Overmann et al. 1991a). Low-light-adapted *Chlorobaculum tepidum* cells contain three times more bacteriochlorophyll than those grown under light saturation. In this green sulfur bacterium, the methylation of BChl c at C-8^2 and C-12^1 is essential for low-light adaptation (Gomez Maqueo Chew et al. 2007). Within the chlorosomes of brown-colored *Chlorobiaceae*, the content of highly alkylated pigment molecules is increased which is thought to improve the efficiency of energy transfer toward the reaction center (Borrego and Garcia-Gil 1995). Furthermore, low-light adaptation of the brown-colored *Chlorobaculum limnaeum* 1549 involves a change in the composition of the carotenoids with higher intracellular concentrations of isorenieratene/β-isorenieratene and less β-zeacarotene, β-carotene, and 7,8-dihydro-β-carotene (Hirabayashi et al. 2004).

Similar to what has been observed in anoxygenic phototrophic bacteria, changes in both the number and the size of the photosynthetic unit have also been described for cyanobacteria. In marine *Synechococcus* strains, the cellular content of the light-harvesting phycoerythrin can vary by a factor of 20 and decreases with increasing light intensity. In marine benthic *Microcoleus chthonoplastes* strains, an increase in the content of total phycobilines and a change in the ratio of PEC to PC occurs with decreasing light intensity. The latter increase the ratio of phycocyanin to chlorophyll *a* during low-light adaptation (Foy and Gibson 1982; Post et al. 1985). Acclimation to very low light intensities usually involves an increase in the size of the photosynthetic unit, such as in metalimnetic *Oscillatoria* (*Leptolyngbya*) *redekei* and *Oscillatoria aghardii*. Changes in both the number and the size of the photosynthetic units seem to occur in *Microcystis* (Zevenboom and Mur 1984). Low-light-adapted genotypes of *Prochlorococcus* contain multiple (i.e., up to eight) copies of *pcb* genes that encode light-harvesting antenna proteins, each binding about 13 chlorophylls, and are constitutively expressed. The Pcb proteins associate with both photosystems in the low-light-adapted forms but only with photosystem II (containing only 30 Chl molecules instead of the 100 Chl molecules present in photosystem I) in high-light-adapted genotypes (Bibby et al. 2003).

Adaptations to High Light Intensities and Ultraviolet Wavelengths

For oxygenic phototrophs, special adaptations to oxygen-dependent photoinhibition of photosynthesis are of particular relevance. The protein D1 of PSII, coded by the *psbA* gene, has been identified as the central target of photoinhibition at high light intensities. In *Synechococcus* PCC 7942, *psbA* contains actually a multigene family coding for three different forms of the protein D1, which are differentially expressed according to the light conditions. Analysis of mutants showed that the isoforms expressed under high light conditions allow for optimal performance of PSII under photoinhibitory conditions (Golden 1994). In addition, carotenoids probably play a central role in preventing oxygen-mediated photosensitized bleaching of photosynthetic pigments and photooxidation of fatty acids under high light conditions. They function as antioxidant quenchers of excited molecules (such as triplet state chlorins before the formation of singlet-excited states of oxygen radicals) in many organisms and perhaps also as inhibitors of free-radical reactions (Britton 1995). The photoprotective xanthophyll cycle typical of green algae and higher plants is not present in cyanobacteria, but judging from its increased specific content at high light intensity, zeaxanthin seems to play an important photoprotective role in some strains (Kana et al. 1988; Masamoto and Furukawa 1997; Millie et al. 1990). Glycosylated myxoxanthophylls seem to attain the same role in others (Nonnengießer et al. 1996; Garcia-Pichel et al. 1998; Ehling-Schulz et al. 1997). Because there is a considerable photooxidation of carotenoids themselves at high light intensities, the maintenance of high carotenoid contents requires an increased expression of their biosynthetic genes.

Exposure to high light intensities in cyanobacterial habitats typically brings about exposure to increased levels of ultraviolet radiation. UV-A radiation (320–400 nm) is not particularly harmful but in the presence of oxygen, when a variety of photosensitized oxidative reactions become of importance. UVA is thus a major environmental stressor for oxygenic phototrophs. UV-B (280–320 nm) is much more efficient in causing oxygen-independent cellular damage, mostly through its direct effects on DNA and proteins (Castenholz and Garcia-Pichel 2000).

A variety of responses is know from cyanobacteria to avoid or counteract UV photodamage, which range from DNA repair, increased target protein turnover, to behavioral motility responses specifically triggered by UV exposure. Perhaps the most unique aspect of these adaptations is the synthesis and accumulation of microbial sunscreen compounds: scytonemin- and mycosporine-like amino acids. Research in the last decade has brought about much progress in the knowledge of the genetics and biochemistry of these compounds (Gao and Garcia-Pichel 2011).

Chromatic Adaptation

Several species of cyanobacteria are capable of changing the amount of peripheral phycoerythrin in response to changes in the spectral composition of light. During growth in white or green light, red-pigmented PE hexamers are added to the peripheral rods whereas additional blue-pigmented PC is added under red light (Sidler 1994). This complementary chromatic adaptation is found only in strains capable of forming PE, but not in those forming PEC. The complementary change in antenna pigment composition optimizes the light-harvesting capabilities of populations of *Oscillatoria* spp., which thrive in deeper layers of stratified lakes where light is predominantly in the blue-green to green wavelength range (Utkilen et al. 1985; *Fig. 12.4*). Bacteriophytochrome is involved in the chromatic adaptation of *Fremyella diplosiphon* (*Calothrix* PCC 7601) (Grossmann et al. 2001).

Genetic Regulation in Response to Light

The synthesis of the photosystem is especially energy consuming because of the high amount of light-harvesting and reaction center protein present in phototrophically grown cells of phototrophic *Proteobacteria* (20 % in purple nonsulfur bacteria). The maintenance energy requirements seem to be increased in low-light-adapted cells (Sánchez et al. 1998). An effective regulation of photosynthesis gene expression therefore would prevent futile synthesis of cellular proteins. The synthesis of the photosystem in anoxygenic phototrophic bacteria is under the control of a complex regulatory network (Bauer and Bird 1996).

The expression of light-harvesting complex I and reaction center genes is controlled (1) by the linkage of genes in superoperons, (2) at the level of transcription initiation, and (3) posttranscriptionally by the decay rate of mRNA (Bauer 1995).

In *Rhodobacter capsulatus*, the genes coding the structural, biosynthetic, and regulatory proteins for light-harvesting I and reaction center complexes are found assembled in a 46-kb-long photosynthetic gene cluster (Alberti et al. 1995). The arrangement of the genes within the cluster seems to be conserved among different phototrophic species of the *Alphaproteobacteria*, like *Rhodobacter sphaeroides*, *Rhodocista centenaria*, and *Rhodospirillum rubrum* (Bauer et al. 1993). Only the *pucBA* operon which codes for structural α- and β-polypeptides of light-harvesting complex II is found in a distant location on the bacterial chromosome (about 18 kb of the *puhA* in *Rhodobacter capsulatus*; Suwanto and Kaplan 1989).

In anoxygenic phototrophic bacteria, transcription of the photosynthesis genes occurs only under anoxic conditions. Different photosynthesis genes exhibit varying levels of expression and degrees of regulation (Bauer and Bird 1996). The *pufA,B,L,M* genes (coding for the α- and β-polypeptide of the light-harvesting complex I and the reaction center L and M structural polypeptides) as well as *puhA* (coding for the structural polypeptide subunit H) are tightly coregulated, transcribed at a high rate under anoxic conditions and strongly regulated (15- to 30-fold). An inverted repeat sequence located between *pufA* and *pufL* affects the longevity of the respective mRNA primary transcript. In *Rhodobacter capsulatus* and *Rba. sphaeroides*, the redox-sensitive repressors CrtJ and PpsR, respectively, bind under oxic conditions to conserved palindrome sequences in promoters of bacteriochlorophyll, carotenoid, light-harvesting complex II genes, and respiratory genes (see sections "Chemotrophic Growth with O_2" and "Genetic Regulation by O_2"). The flavin-containing antirepressor AppA is capable of breaking the disulfide-bond in oxidized PpsR and of forming a stable AppA-$PpsR_2$ complex in *Rba. sphaeroides* (Masuda and Bauer 2002). Upon blue-light excitation, AppA becomes incapable of forming the antirepressor complex with PpsR, which results in blue light repression of photosystem gene expression. In contrast, *Rhodobacter capsulatus* does not exhibit a strong regulation of photosynthesis gene expression by high light intensities and CrtJ is not involved in this regulation. Furthermore, A reduction of light leads to an activation of *puf* and *puh* gene expression by the *hvrA* gene product, which probably directly interacts with the two promoter regions. Light of 450 nm exhibits the most severe repressing effect, indicating that a flavin-binding protein (possibly HvrA itself) is the photoreceptor. Notably in aerobic phototrophic bacteria, a blue-light-sensitive system seems to regulate biosynthesis of bacteriochlorophyll *a* (Shimada 1995).

The intracellular bacteriochlorophyll concentrations appear to affect *puf* and *puc* gene expression not only at the transcriptional but also the posttranscriptional level in *Rhodobacter capsulatus* (Rödig et al. 1999). The polycistronic organization allows the coordinate expression of the structural polypeptides of light-harvesting complex I and the two integral membrane-proteins of the reaction center. Since, however, many light-harvesting I complexes are required per reaction center in *Proteobacteria*, additional regulatory mechanisms must exist. Differential degradation of various portions of the polycistronic mRNA are one means to regulate the stoichiometry of different components of the photosynthetic apparatus. The synthesis of different amounts of gene products is achieved by posttranscriptional regulation (Rödig et al. 1999). Because of a highly stable secondary terminator structure at its 3′-end and the absence of specific recognition sites for endonucleolytic cleavage, the mRNA coding the two light-harvesting polypeptides has much higher stability than that of the entire *puf* gene transcript. The degradation of the downstream *pufLM* section of the mRNA is

mediated by an endonuclease. A similar regulation mechanism may exist for the polycistronic mRNA of bacteriochloro-phyll synthesis genes (*bchFNBHLM-F1696*) and the *puhA*, and operate in regulation of light-harvesting complex II expression.

A shift to low light intensities results in an increase especially of light-harvesting complex II. The corresponding *pucBA* operon is highly expressed but only moderately regulated (fourfold). In the purple nonsulfur bacterium *Rhodobacter capsulatus*, fourfold less *puc* mRNA but at the same time four times as many light-harvesting II complexes were detected after a shift from high to low-light conditions (Zucconi and Beatty 1988). Therefore regulation by light most likely involves posttranscriptional regulation. A posttranscriptional regulation appears to occur (Bauer 1995).

Bacteriochlorophyll and carotenoid biosynthesis genes are only weakly expressed and moderately (two- to fourfold) regulated. Light intensity may control the rate of bacteriochlorophyll degradation (by oxidative degradation of bacteriochlorophyll; Biel 1986) rather than the rate of synthesis (Biel 1995). This is another distinct difference from the regulation by oxygen, where inhibition of δ-aminolevulinate synthase by molecular oxygen appears to occur (see section "Chemotrophic Growth with O_2" in this chapter). Bacteriochlorophyll may be stabilized by insertion in pigment-protein complexes, however. The promoter of the bacteriochlorophyll synthesis gene *bchC* is of the sigma-70 type and leads to one large superoperon (Yurkov and Beatty 1998). In contrast, an alternative sigma factor appears to recognize the strongly regulated structural *puf* and *puh* genes (Bauer 1995). These differences explain the independent and different levels of regulation observed for the two classes of genes.

In *Rhodospeudomonas palustris* and the closely related *Bradyrhizobium* symbiont of the plant *Aeschynomene*, the synthesis of the photosynthetic apparatus is under the control of bacteriophytochrome. Bacteriophytochromes harbor a linear tetrapyrrole as light sensing cofactor and reversibly switch between an inactive red-light-absorbing and an active far-red-light-absorbing form. The chromophore binding site differs between cyanobacteria (cystein as binding site with one exception; Hübschmann et al. 2001) and purple nonsulfur bacteria (histidine) (Giraud et al. 2002). Bacteriophytochromes have also been detected non-phototrophic bacteria such as *Pseudomonas aeruginosa* and *Deinococcus radiodurans*. In the latter, they protect cells from visible light through regulation of the cellular carotenoid content (Davis et al. 1999). Different from other systems that typically function as light-regulated histidine kinase, the regulation of photosynthesis genes in *Rps. palustris* and *Bradyrhizobium* seems not to involve a phosphorelay cascade but direct protein-protein interactions via the transcriptional factor PpsR. PpsR represses the expression of bacteriochlorophyll, carotenoids, and light-harvesting complex II at high light intensity or high oxygen tension. The bacteriophytochrome in *Rps. palustris* and *Bradyrhizobium* acts as an antagonist of the repressor; hence pigment synthesis in these two bacteria depends on light with a wavelength of 740 nm (Giraud et al. 2002). This type of regulation is absent in *Rhodobacter sphaeroides*, *Rba. capsulatus*, *Rhodocista centenaria*, and *Rubrivivax gelatinosus*.

Recent investigations of gene regulation using genomic microarrays and high-throughput transcriptomic analyses have revealed much about gene regulation in general, and about regulation by light in cyanobacterial model strains such as *Synechocystis* (Los et al. 2008). In general, a sudden shift-up in light intensity brings about the fast downregulation of most genes responsible for the synthesis of light harvesting and photochemical reactions in photosynthesis. The same shift causes the upregulation of NADP dehydrogenase and those responsible for the carbon concentration mechanisms, as well as subsets of genes known to be involved in adaptation to heat and photooxidative stresses. Light-driven gene regulation in *Synechocystis* PCC6803 and a few other model organisms examined is likely modulated through phytochrome-like histidine kinases similar to those known in plants (Los et al. 2008).

State Transitions

In cyanobacteria, state transitions involve redirecting the pathways of excitation energy transfer from light-harvesting complexes to both photosystems, and can be recognized by fluorescence analysis. Cyanobacteria can reach two energetically different states, in which one of the photosystems is preferentially excited. This is achieved with fast changes in the coupling between the light-harvesting complexes and the reaction center (van Thor et al. 1999), in a mechanism similar to that in green chloroplasts but that includes independent mechanisms for redistribution of phycobilin-absorbed and chlorophyll-absorbed excitation (McConnell et al. 2002) In Chl *b*-containing *Prochlorothrix* (Matthijs et al. 1994), polypeptides of the PSII antenna (LHCII) are rapidly phosphorylated during overexcitation of this photosystem, and as a consequence, detach from PSII and migrate to the stromal thylakoids. This mechanism ensures a balanced energy distribution between PSII and PSI. The net result of state transitions is the balanced function of both photosystems and an optimization of the quantum yield for photosynthesis during short-term changes, such as those that planktonic cells might experience during vertical transport by water currents.

Movement

Phototrophic *Proteobacteria* swim by means of flagella, whereas one species of the green sulfur bacteria (*Chloroherpeton thalassium*), members of *Chloroflexus* subgroup and cyanobacteria move by gliding. Of the *Alphaproteobacteria*, most phototrophic species are motile. Peritrichous or lateral flagella are only found in *Rhodomicrobium vannielii* and the swarming phase of *Rhodocista centenaria*. About two thirds of the known *Chromatiaceae* species are motile. Larger forms (*Chromatium okenii*, *Chr. weissei*, *Allochromatium warmingii*, *Isochromatium buderi*, *Thiospirillum jenense*) are motile by

means of bipolar multitrichous tufts of flagella. *Thiospirillum jenense* is bipolarly flagellated. Forms with smaller cells are monotrichously flagellated (small *Allochromatium* species, *Lamprocystis*, *Thiocystis*, *Thiorhodococcus*, *Thiorhodovibrio*). All *Ectothiorhodospiraceae* are flagellated.

Cyanobacteria lack flagella, but many are motile by gliding. The mechanisms behind gliding motility are unknown in any detail, and it is thought that the propulsion is based on the excretion and hydration of polysaccharides (Jeon and Dobryinin 2005). A new mode of swimming motility has been described for a unicellular marine *Synechococcus*, which moves in a similar fashion to flagellated bacteria but lacks a flagellum (Waterbury et al. 1985). The motor is likely based on PMF-driven wave-like deformations of the S-layer at the cell surface (Ehlers and Oster 2012).

True phototaxis is the ability to move toward or away from the direction of light. Cyanobacteria are the only prokaryotes displaying true phototaxis (Garcia-Pichel and Castenholz 1999). Phototaxis may not be of competitive value for microorganisms adapted to live at low light intensities in the subsurface of sediments, soils, and mats because the light fields may be close to diffuse deep below the surface. However, directed movements can still be of much use in microorganisms dwelling at or close to the sediment surface, where the light fields contain a significant downward directionality. Photophobic responses are changes in the direction of movement in reaction to abrupt changes in light intensity (Castenholz 1982; Häder 1987). In the step-up photophobic response, organisms will reverse direction when sensing an increase in light intensity, which results in a net accumulation of organisms at lower light intensities. In a step-down photophobic (or scotophobic) response, the organisms will tend to accumulate in the region of higher light intensity. Photophobic responses are the basis of photomovement in all flagellated bacteria (Armitage 1997), and in most gliding cyanobacteria (Castenholz 1982).

In swimming cells of phototrophic *Proteobacteria*, a decrease in light intensity triggers a reversal of flagellar rotation (*Rhodospirillum rubrum*, *Allochromatium* spp.) or an increase in stopping frequency (*Rhodobacter sphaeroides*). Owing to a memory effect, cells of the latter species retain a higher stopping frequency for up to 2 min, which prevents the cells from being trapped in the dark but instead permits reorientation of the cells and a return to higher light intensities (Armitage et al. 1995). As a result of this scotophobic response, the cells accumulate in the light and at wavelengths corresponding to the absorption maxima of photosynthetic pigments. A change in light intensity of as little as 2 % can be sensed (Armitage et al. 1995). Active electron transport is required for the scotophobic response.

The formation of flagella in *Chromatium* and *Allochromatium* species is induced by low sulfide concentrations and low light intensities. These two environmental variables are mutually dependent: the lower the light intensity, the higher the sulfide concentration at which a given strain can persist in its motile stage (Pfennig and Trüper 1989). In the natural environment of purple sulfur bacteria, gradients of light and sulfide are opposed to each other. The control of motility by the two interdependent environmental variables (instead of only one) enables cells to return either from low-sulfide/high-light environment above the chemocline or from the high-sulfide/low-light environment below the chemocline back to their habitat.

In its pelagic habitat, *Chromatium okenii* may display diurnal migrations with a vertical amplitude of about 2 m (Sorokin 1970). In other lakes, vertical migrations of *Thiocystis minor* extended over a distance of 30–35 cm (Lindholm et al. 1985; Pedrós-Alió and Sala 1990). Vertical migration of nonthermophilic *Allochromatium*, and of *Thermochromatium tepidum* also has been observed in ponds and in intertidal or hot spring microbial mats (Castenholz and Pierson 1995; Jørgensen 1982; Pfennig 1978). In the latter environments, *Allochromatium* or *Chromatium* cells migrate upward to the surface of the mat and enter the overlaying water as a result of positive aerotaxis during the night. The cells contain high amounts of intracellular sulfur globules, which are formed during incomplete sulfide oxidation by anoxygenic photosynthesis during daytime. It is assumed that migration into microoxic layers enables the cells to grow chemoautotrophically by oxidation of sulfide or intracellular sulfur with molecular oxygen (Jørgensen 1982; Castenholz and Pierson 1995).

If phototrophic sulfur bacteria would solely follow the light gradient, their scotophobic response would ultimately lead them into oxic water layers. Both the scotophobic behavior and aerotaxis respond to the rate of intracellular electron flow (presumably sensed as changes in the redox state of an intermediate). Because the two tactic responses interact through a common signal, a combination of light and molecular oxygen elicits a differential response. *Rhodobacter sphaeroides* exhibits pronounced aerotaxis when precultivated aerobically, but negative aerotaxis when grown anaerobically in the light. Conversely, cells only swim toward higher light intensities in anoxic medium. A pulse of oxygen in the light causes a transient fall in the membrane potential which probably represents the primary tactic signal. As a result, the bacteria move toward environments where electron transport rate is increased (Armitage et al. 1995).

Rhodocista centenaria exhibits a characteristic swarming behavior. In liquid media, cells move with a single polar flagellum. Upon contact with solid agar media, formation of a large number of lateral flagella is induced. Lateral flagella allow whole colonies to swarm toward or away from the light (Ragatz et al. 1994). The supposedly true phototaxis of these swarming colonies (Ragatz et al. 1995) has later been proven to actually be aerotaxis following microgradients within the colonies (Sackett et al. 1997). The light sensing system in this species appears to be more complex, since infrared light leads to positive and visible light to negative phototaxis. In microbial mats, infrared light penetrates to much greater depths than light of the visible wavelength range (see section "Competition for Light" in this chapter). It has been suggested that the ratio of visible to infrared light may be used to maintain an optimum position in such environments (Armitage et al. 1995; Ragatz et al. 1995). Cells of *Roseospirillum parvum* accumulate scotophobically in two distinct bands at wavelengths of 800–810 and 900–910 nm

corresponding to the position of the long-wavelength absorption maxima of the unusual BChl *a* containing light-harvesting complexes in this species (Glaeser and Overmann 1999).

Cyanobacteria are the only prokaryotes displaying true phototaxis (Garcia-Pichel and Castenholz 1999). Surface-dwelling cyanobacteria such as *Lyngbya* spp. from hot spring mats and intertidal sediments and the motile phases (hormogonia) of terrestrial *Nostoc* spp. from desert soils exhibit this type of movement. The bundle-forming *Microcoleus chthonoplastes* also is able to display a "populational phototaxis" in that bundles of trichomes of this cyanobacterium are able to steer in the direction of the incoming light, whereas single trichomes are apparently not able to do so (Prufert-Bebout and Garcia-Pichel 1994). True phototaxis is a mechanism for the orientation of cells at or close to the sediment surface, where the light field contains a significant downward directionality. In contrast, phototaxis does not provide a selective advantage for bacteria thriving in the subsurface of sediments, soils, and mats because of the diffuse light field. In natural microbial mats, photophobic responses to changes in light intensity are probably involved in the migrations of gliding bacteria (Nelson and Castenholz 1982; Pentecost 1984). In microbial mats, some strains of cyanobacteria are able to migrate vertically following their optimal light intensity over the diel cycle (Garcia-Pichel et al. 1996). The upward migrations of cyanobacteria in mats is preferentially prevented by short wavelengths, especially by UV radiation (Garcia-Pichel and Castenholz 1994; Bebout and Garcia-Pichel 1995; Krutschel and Castenholz 1998) and not by red or green light.

Photoreceptors that are involved in the response to light are either bacteriophytochromes or xanthopsins. Whereas the former contain linear tetrapyrrols as the chromophore, the latter harbor S-coumaryl esters (a 4-OH-cinnamyl-residue) (Hellingwerf 2002). In *Synechocystis* PCC6803, the bilin-binding protein Cph1 appears to mediate phototaxis. Photoactive yellow protein is a xanthopsin that is employed for the photophobic response of *Halorhodospira halophila*. It mediates the response toward high-intensity blue light (Sprenger et al. 1993).

Phototrophic consortia are structural associations between a colorless central bacterium and several surrounding cells of pigmented epibionts (see section "Interactions Between Phototrophic Sulfur Bacteria and Chemotrophic Bacteria" in this chapter; *Fig. 12.4*). Intact consortia of the type "Chlorochromatium aggregatum" exhibit a scotophobic response and accumulate in a spot of white light. In phototrophic consortia, only the central colorless bacterium carries a flagellum (Glaeser and Overmann 2003). The action spectrum of scotophobic accumulation corresponds to the absorption spectrum of the green sulfur bacterial epibionts, however. It was concluded that a rapid signal transfer exists between the light-sensing but immotile epibionts and the colorless motile rod (Fröstl and Overmann 1998). The recent analysis of the genome of the central bacterium revealed the presence of a bacteriophytochrome (Don Bryant, personal communication), offering an alternative explanation of the scotophobic response of intact consortia.

Gas Vesicles

Buoyancy-conferring gas vesicles are common in green sulfur bacteria, *Chromatiaceae*, and cyanobacteria. Gas vesicles are cylindrical structures with conical ends; their length and width are variable and species specific. The sheath of gas vesicles is composed of proteins (Walsby 1994). The gas mixture within the gas vesicles is the same as in the surrounding medium and is at the same partial pressures. Gas vesicles occur in a third of the species of *Chromatiaceae* (belonging to the genera *Amoebobacter*, *Lamprobacter*, *Lamprocystis*, *Thiodictyon*, *Thiopedia*, *Thiolamprovum*) and some green sulfur bacteria (genera *Ancalochloris*, *Pelodictyon*, *Chloroherpeton*). Of the *Ectothiorhodospiraceae*, only *Ectothiorhodospira vacuolata* forms gas vesicles during the stationary phase. This reflects the distribution of both families of purple sulfur bacteria in nature, where *Chromatiaceae* typically colonize low-light stratified aquatic environments, whereas *Ectothiorhodospiraceae* typically inhabit more shallow saline ponds and sediments. Gas vesicles also are present in *Prochlorothrix hollandica*. In planktonic habitats, cells of cyanobacteria and phototrophic sulfur bacteria often contain gas vesicles, which indicates a selective advantage of this cellular property.

Gas vesicle formation in the green sulfur bacterium *Chlorobium clathratiforme* BU1 is induced exclusively at light intensities <5 mol quanta·m^{-2}·s^{-1} (Overmann et al. 1991b). This appears to be the reason for the rare observation of gas vesicles in pure cultures of green sulfur bacteria which routinely are incubated at much higher light intensities. A transfer of *Amoebobacter purpureus* strain ML1 to the dark results in an increase of the gas vesicle volume by a factor of 9 (Overmann and Pfennig 1992). Ambient temperature controls gas vesicle formation in *Thiocapsa pendens* (Eichler and Pfennig 1986).

The buoyancy of many species of *Cyanobacteria* is regulated by the formation of gas vesicles. Highly buoyant cells may float toward the surface of stagnant water bodies. When the turgor pressure within the surrounding cytoplasm rises, such as by accumulation of low-molecular-weight photosynthates during periods of intense photosynthesis, the critical pressure may be exceeded and the gas vesicles collapse. New vesicles are formed by de novo synthesis rather than by reinflation of collapsed vesicles. Short-term regulation of cell buoyant density occurs in cyanobacterial species thriving in stratified lakes, like *Aphanizomenon flos-aquae*, *Anabaena flos-aquae*, and green-colored *Oscillatoria* spp. (Konopka et al. 1978; Oliver and Walsby 1984; Utkilen et al. 1985). In these species, the proteinaceous gas vesicle sheaths are weak enough to permit a collapse at high intracellular turgor pressures as they are reached during periods of intense photosynthesis. By this mechanism, cells lose buoyancy within 30 min and thus can sink out of surface layers of stratified lakes. After de novo synthesis of gas vesicles in lower water layers, utilization of photosynthates, and a decrease of turgor pressure, cells rise back to the surface during the night. Rapid, turgor-mediated reduction of buoyancy together with gas vesicle formation thus represents an adaptation to the pronounced diurnal variations in light intensity and the limitation

of growth by inorganic nutrients as they occur during summer stratification in the surface layer of eutrophic lakes. In some instances (e.g., *Microcystis aeruginosa*), diurnal migrations are mediated by an increase of carbohydrate ballast alone and gas vesicles do not collapse even at maximum turgor pressure (Kromkamp and Mur 1984; Thomas and Walsby 1985). In contrast, gas vesicles of red-colored *Oscillatoria aghardii* and of phototrophic sulfur bacteria are mechanically stronger and do not collapse even at maximum cell turgor pressure. A decrease in the cellular gas vesicle content is therefore the result of their dilution during growth and division of the cells, and thus proceeds rather slowly (Overmann et al. 1991b; Overmann and Pfennig 1992). Bacteria of this category mostly colonize the low-light environments shortly above or within the chemocline of stratified lakes where photosynthetic rates typically are strongly limited by light and hydrostatic pressure is high. Gas vesicles in green sulfur bacteria are rigid enough to persist at hydrostatic pressures down to depths of 38 m (Overmann et al. 1991b). The cyanobacterium *Trichodesmium* contains extremely stable gas vesicles (with mean critical collapse pressures up to 3.7 MPa, corresponding to a depth of 370 m; Walsby 1978). The differences in strength of gas vesicles formed by different species are related to their shape (especially the diameter) and the primary structure of the GvpC protein of their sheath (Walsby 1994).

In addition to the formation of gas vesicles, a new type of buoyant density regulation was detected in *Pelodictyon phaeoclathratiforme*. Cells of this species form large extracellular slime layers during the stationary phase which leads to an increase of the cellular volume by a factor of three (Overmann et al. 1991b).

Advantages of the Vertical Movement

Theoretically, motility based on flagella and vertical migration by means of buoyancy has different advantages under natural conditions. Movement by flagella requires a permanent, (albeit sometimes low) fraction of metabolic energy (proton-motive force), whereas gas vesicle synthesis represents an initial one-time investment of a higher amount of metabolic energy. Once formed, gas vesicles keep bacterial cells in their habitat without any further demand for energy. The purple sulfur bacterium *Lamprobacter modestohalophilus* is capable of both flagella and gas vesicle formation. Motile cells are usually devoid of gas vacuoles and initially dominate during growth in fresh media. Later, cells become immotile and form gas vesicles and slime capsules (Gorlenko et al. 1979). In a very similar manner, cells of *Ectothiorhodospira vacuolata* are flagellated at low sulfide concentrations and light intensities, and become immotile and form gas vesicles in stationary phase (Imhoff et al. 1981). This supports the view that flagellar movement of purple sulfur and purple nonsulfur bacteria is favored under conditions of continuous energy supply, while gas vesicle formation represents an adaptation to conditions of starvation. Within one lake ecosystem, vertical migration of a flagellated species (*Thiocystis minor*) was observed while the gas-vacuolated *Amoebobacter* did not change its vertical position (Pedrós-Alió and Sala 1990).

A minimum quantum requirement of flagellar motility can be estimated from data in the literature. A vertical migration over a distance of 2 m (the maximum amplitude of vertical migration observed in nature) during 6 h corresponds to a swimming speed of 93 $\mu m \cdot s^{-1}$. At a similar speed of 100 $\mu m \cdot s^{-1}$, the frequency of flagellar rotation is $<100\ s^{-1}$ in *Rhodobacter sphaeroides* and requires between 200 and 1,000 H^+ per rotation (Armitage et al. 1995). This yields a proton translocation rate of $\sim 6 \times 10^4\ H^+ \cdot s^{-1}$ at a swimming velocity of 100 $m \cdot s^{-1}$. Based on an absorbing cross-sectional area of the cell of 1 m^2, an absorption of 36 % of the incident light (see section ❯ "Efficiency of Light Harvesting" in this chapter), a ratio of protons translocated to electrons transferred (H^+/e^- ratio) of 2 (see section ❯ "Conversion of Light into Chemical Energy" in this chapter), and assuming that each photon absorbed leads to transport of an electron, the proton translocation rate of $6 \times 10^4\ H^+ \cdot s^{-1}$ would be reached at an underwater irradiance of 0.2 mol quanta $\cdot m^{-2} \cdot s^{-1}$. However, all available quanta would be required just for motility at this irradiance and no vertical migration would be possible during the night. Therefore, motility by flagella will be of competitive advantage only at significantly higher irradiances. In many lakes, underwater irradiances in layers of phototrophic sulfur bacteria are ≤ 1 mol quanta $\cdot m^{-2} \cdot s^{-1}$ (Overmann and Tilzer 1989; Overmann et al. 1999a). Under these conditions, purple sulfur bacteria harboring gas vesicles dominate over flagellated forms in the chemocline community (❯ *Fig. 12.4*). At least in some lakes, gas vesicles appear to be of selective advantage also at higher underwater irradiances (Overmann et al. 1991b; Overmann and Pfennig 1992).

Interestingly, the extremely low-light-adapted *Chlorobium phaeobacteroides* strain MN1 isolated from the chemocline of the Black Sea was not capable of gas vesicle formation. The green sulfur bacterial layer is located at an 80-m depth and with respect to light intensity represents the lower limit for growth of a phototrophic organism. The isolated strain exhibits an extremely low maintenance energy requirement. It therefore appears that gas vesicle formation is too energy demanding at the very low light intensities available at an 80-m depth in the Black Sea.

Carbon Metabolism of Phototrophic Prokaryotes

In the natural environment, the principal carbon source of phototrophic bacteria in many instances is CO_2 (Madigan et al. 1989; Sinninghe Damsté et al. 1993; Takahashi et al. 1990). In *Cyanobacteria*, *Chromatiaceae*, *Ectothiorhodospiraceae*, and purple nonsulfur bacteria, CO_2 is assimilated by the reductive pentose phosphate or Calvin cycle. Employing this cycle, the formation of one molecule of glyceraldehyde-3-phosphate requires 6 NAD(P)H + H^+ and 9 ATP. By comparison, the reductive tricarboxylic acid cycle used for CO_2-assimilation by green sulfur bacteria requires 4 NADH + H^+, 2 reduced ferredoxins, and only 5 ATP. As two of the reactions of the reductive tricarboxylic acid cycle (the α-oxoglutarate synthase and

pyruvate synthase reactions) require reduced ferredoxin as electron donor, this pathway of CO_2 fixation can only proceed under strongly reducing conditions. Furthermore, reduced ferredoxin is a primary product of the light reaction only in FeS-type reaction centers. Ultimately, the lower demand for ATP is possible because of the adaptation of green sulfur bacteria to the strongly reducing conditions of their natural environment. CO_2-fixation by the hydroxypropionate cycle in *Chloroflexus aurantiacus* requires 8 ATP per glyceraldehyde-3-phosphate and therefore is energetically less favorable than in green sulfur bacteria.

Organic carbon as it is present in canonical microbial biomass (<$C_4H_8O_2N$>; Harder and van Dijken 1976) is considerably more reduced than CO_2. Given the high energy demand of autotrophic growth, the capability for assimilation of organic carbon compounds is of selective advantage especially if natural populations are limited by light or by low concentrations of electron-donating substrates, as is typically the case for phototrophic sulfur bacteria. At limiting concentrations of sulfide or thiosulfate, the cell yield of green sulfur bacteria is increased three times if acetate is available as an additional carbon source (Overmann and Pfennig 1989). Acetate represents one of the most important intermediates of anaerobic degradation of organic matter (Wu et al. 1997). That almost all anoxygenic phototrophic bacteria (with the exception of *Rhodopila globiformis*; Imhoff and Trüper 1989) are capable of acetate assimilation is therefore not surprising. In most phototrophic *Proteobacteria*, acetate is assimilated by acetyl-CoA synthetase and the enzymes of the glyoxylate cycle. In green sulfur bacteria, the ferredoxin-dependent pyruvate synthetase, PEP synthetase, and reactions of the reductive tricarboxylic acid cycle serve this purpose. The capacity for organotrophic growth seems to correlate with the presence of α-oxoglutarate dehydrogenase. The latter is a key enzyme for the complete oxidation of the carbon substrates in the tricarboxylic acid cycle (Kondratieva 1979), whereas a complete cycle is not needed for the photoassimilation during the presence of inorganic electron donors. The range of carbon substrates utilized and the capacity for photoorganotrophy or chemoorganotrophy vary considerably among the different groups of phototrophic prokaryotes (Pfennig and Trüper 1989).

Organic carbon compounds not only are assimilated but also can serve as photosynthetic electron donors in purple nonsulfur bacteria, some *Chromatiaceae* and *Ectothiorhodospiraceae*, all *Heliobacteriaceae*, and members of the *Chloroflexus* subdivision.

Green sulfur bacteria are the least versatile of all phototrophic prokaryotes. All known species are obligately photolithotrophic and assimilate only very few simple organic carbon compounds (acetate, propionate, pyruvate). Few strains have been shown to assimilate fructose or glutamate. Whereas green sulfur bacteria have a higher growth affinity for sulfide than purple sulfur bacteria, acetate seems to be used by purple sulfur bacteria at an affinity 30 times higher than in green sulfur bacteria (Veldhuis and van Gemerden 1986). In addition, uptake of acetate in *Chlorobium phaeobacteroides* is inhibited by light (Hofman et al. 1985).

Based on their metabolic flexibility, two groups can be distinguished among the *Chromatiaceae*. Several species (*Chromatium okenii*, *Chr. weissii*, *Allochromatium warmingii*, *Isochromatium buderi*, *Thermochromatium tepidum*, *Thiospirillum jenense*, *Lamprocystis roseopersicina*, *Thiodictyon elegans*, *Thiodictyon bacillosum*, *Thiococcus pfennigii*, *Thiopedia rosea*) are obligately phototrophic and strictly anaerobic and photoassimilate acetate and pyruvate only in the presence of CO_2 and sulfide. Assimilatory sulfate reduction is absent in these species (Pfennig and Trüper 1989). However, particularly those species with limited metabolic flexibility form dense blooms under natural conditions (see sections "Interactions with Other Microorganisms" and "Coexistence of Phototrophic Bacteria" in this chapter). The second physiological group within the *Chromatiaceae* comprises the small-celled *Marichromatium gracile*, *Thiocystis minor*, *Allochromatium minutissimum*, as well as *Allochromatium vinosum*, *Lamprobacter modestohalophilus*, *Thiocystis* spp., and *Thiocapsa*. Most of these species use thiosulfate as electron donor and a wide range of organic carbon compounds including glucose, fructose, glycerol, fumarate, malate, succinate, formate, propionate, and butyrate for photoassimilation, and often are capable of assimilatory sulfate reduction. In some species (especially *Allochromatium vinosum*), these organic carbon substrates also serve as electron-donor for phototrophic or chemotrophic growth.

Most *Ectothiorhodospiraceae* species are capable of photoorganotrophic growth, with *Halorhodospira halophila* and *Halorhodospira halochloris* being the exceptions. The spectrum of electron-donating carbon substrates for photoorganotrophic growth resembles that found in the versatile *Chromatium* species (Pfennig and Trüper 1989). Assimilation of acetate and propionate proceeds by carboxylation and therefore depends on the presence of CO_2.

Chloroflexus aurantiacus grows preferably by photoorganoheterotrophy (Pierson and Castenholz 1995). The carbon substrates utilized comprise acetate, pyruvate, lactate, butyrate, C_4-dicarboxylic acids, some alcohols, sugars, and amino acids (glutamate, aspartate). This versatility has been seen as the major cause for the profuse growth of *Chloroflexus* in microbial mats where accompanying microorganisms, especially cyanobacteria, may provide the required carbon substrates (Sirevåg 1995). However, high rates of formation of low-molecular-weight organic carbon substrates by the anaerobic food chain have also been observed in other stratified systems, where the dominating anoxygenic phototrophs could utilize only a narrow range of carbon substrates (Overmann 1997; Overmann et al. 1996). Therefore, the presence of low-molecular-weight organic carbon substrates is not necessarily the most selective factor in the natural environment.

Slow photolithoautotrophic growth with H_2S or H_2 as electron-donating substrates has been shown in laboratory cultures of *Chloroflexus aurantiacus* and in hot spring populations (Pierson and Castenholz 1995). Carbon fixation proceeds by carboxylation of acetyl-CoA and via hydroxypropionyl-CoA as an intermediate and yields glyoxylate as the net product (hydroxypropionate cycle; Holo 1989; Strauß and Fuchs 1993;

Eisenreich et al. 1993). So far this cycle has not been found in any other member of the Bacteria. Glyoxylate is further assimilated into cell material with tartronate semialdehyde and 3-phosphoglycerate as intermediates (Menendez et al. 1999).

The highest metabolic versatility is found in phototrophic *Alpha-* and *Betaproteobacteria* (purple nonsulfur bacteria). All representatives grow photoorganoheterotrophically and (with the exception of *Blastochloris viridis*) photolithoautotrophically with H_2 in the light. In addition to the substrates used by versatile purple sulfur bacteria, the spectrum of substrates that can serve as electron donors comprise long-chain fatty acids (like pelargonate), amino acids (aspartate, arginine, glutamate), sugar alcohols (sorbitol, mannitol), or aromatic compounds (benzoate; Imhoff and Trüper 1989). With the exception of *Rubrivivax gelatinosus*, none of the purple nonsulfur bacteria is capable of degradation of polymers and therefore depends on the anaerobic food chain for the supply of electron-donating substrates required for growth. This dependence and the competition with chemotrophs for the carbon substrates might be the major reason why dense blooms of purple nonsulfur bacteria do not occur under natural conditions (see section "Habitats of Phototrophic Prokaryotes" in this chapter). Some species are capable of also using reduced sulfur compounds as electron donors. However, most species oxidize sulfide to elemental sulfur only (Hansen and van Gemerden 1972).

In *Heliobacteriaceae*, only a limited number of carbon substrates can serve as photosynthetic electron donor including pyruvate, ethanol, lactate, acetate, propionate, and butyrate. Heliobacteriaceae are not capable of autotrophic growth, which for *Heliobacterium modesticaldum* has been attributed to the lack of ATP citrate lyase of the reductive tricarboxylic acid cycle. However, non-autotrophic assimilation of CO_2 mostly via pyruvate:ferredoxin oxidoreductase enhanced the cell yield of *H. modesticaldum* (Tang et al. 2010). Glucose and fructose have been reported as growth substrates of *Heliobacterium gestii*. Using a defined growth medium, growth of *Heliobacterium modesticaldum* on D-ribose, D-fructose, and D-glucose could be demonstrated (Tang et al. 2010). This suggests that the substrate spectrum of other *Heliobacteriaceae* could be broader than assumed to date. *Heliorestis acidaminivorans* isolated from an Egyptian soda lake is capable of photoassimilating the amino acids alanine, arginine, glutamate, glutamine, histidine, lysine, and serine (Asao et al. 2012). High levels of sulfide are inhibitory (Madigan 1992; Madigan and Ormerod 1995).

While Cyanobacteria are obligate photoautotrophs, small-molecular-weight organic compounds such as acetate, sugars, and amino acids are assimilated. In the case of amino acids, the presence of various efficient uptake systems has been interpreted as a means of recovery of leaked organic nitrogen, rather than a true chemotrophic capability (Montesinos et al. 1997). While it was previously assumed that the obligate photolithoautotrophy of cyanobacteria stems from an incomplete tricarboxylic acid cycle, recent analyses have revealed that cyanobacterial genomes (except those of *Synechococcus* and *Prochlorococcus* spp.) encode 2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase which functionally can complete the cycle (Zhang and Bryant 2011). Certain strains of cyanobacteria can grow facultatively as chemoheterotrophs in the dark (Rippka et al. 1979), but even under these conditions, all of the photosynthetic machinery is synthesized. This lack of regulation implies that chemotrophy has played no significant evolutionary role in these organisms.

Chemotrophic Growth with O_2

Ecophysiology of Chemotrophic Growth

In lakes, purple sulfur and green sulfur bacteria are confined to environments where light reaches sulfide-containing water layers. The physiological properties restrict the distribution of these bacteria in the pelagic habitat (Pfennig 1978). Dense accumulations of anoxygenic phototrophic bacteria, which apparently are growing chemotrophically, are only known for *Chloroflexus* (see section "Habitats of Phototrophic Prokaryotes" in this chapter). Although populations of other anoxygenic phototrophic bacteria do not seem to grow permanently by chemotrophy, the ability of many strains to shift to an aerobic chemotrophic mode of growth is of selective advantage in environments like intertidal sediments.

Green sulfur bacteria and *Heliobacteriaceae* are obligate anaerobes. Under oxic conditions, the reaction of reduced ferredoxin of the type I reaction center with molecular oxygen would create superoxide and other activated oxygen species. *Heliobacteriaceae* are rapidly damaged by exposure to molecular oxygen. This has been attributed not only to the formation of toxic oxygen radicals but also the destruction of the unsaturated fatty acids present in the cell membrane by activated oxygen species (Madigan and Ormerod 1995). In green sulfur bacteria, it has been observed that the energy transfer from light-harvesting bacteriochlorophylls *c*/*d*/*e* to bacteriochlorophyll *a* drops by a factor of 10 after an increase in redox potential due to the quenching by chlorobium quinone. This mechanism may protect the cells during brief anoxic/oxic transitions (Frigaard et al. 1997).

The newly discovered "*Candidatus* Chloracidobacterium thermophilum" is an aerobic phototrophic bacterium that employs a photosynthetic apparatus very similar to that of green sulfur bacteria including a type I homodimeric reaction center, FMO-proteins and chlorosomes (see section "Taxonomy of Phototrophic Prokaryotes"). While the principle molecular architecture of the FMO protein in Cab. thermophilum is similar to that of the Chlorobiaceae, the amino acid sequence was only distantly related and exciton coupling was found to be weaker than in the Chlorobiaceae FMO which has been interpreted as an adaptation to an efficient light-harvesting under oxic conditions (Tsukatani et al. 2010). As other possible mechanisms of enhanced protection against reactive oxygen species that prevail in its natural habitat, the chlorosomes of Cab. thermophilum contain the xanthophylls echinenone and canthaxanthin and dihydrogenated menaquinone-8. While the ketocarotenoids may be involved in photoprotection and

protection against oxygen radicals, the latter may act as a quencher of energy transfer under oxic conditions. Finally, the major esterifying alcohol of bacteriochlorophylls in Cab. thermophilum is the straight-chain saturated octadecanol which might represent an adaptation to avoid peroxidation of the double bonds in the isoprenoid alcohol farnesol that is found as the major esterifying alcohol of bacteriochlorophylls in green sulfur bacteria (Garcia Costas et al. 2012b).

All other groups of phototrophic prokaryotes comprise species that not only generate metabolic energy by photosynthesis but are also capable of chemosynthesis with O_2.

Chloroflexus aurantiacus is capable of growth as an aerobic heterotroph. During phototrophic growth, β-carotene, γ-carotene, and hydroxy-γ-carotene-glucoside are the major carotenoids, whereas echinenone and myxobactone predominate in aerobically grown cells (Pierson and Castenholz 1995). Unlike in purple nonsulfur or purple sulfur bacteria, synthesis of some carotenoids by *C. aurantiacus* is greatly enhanced under aerobic conditions (Pierson and Castenholz 1974). The expression of the chlorosome CsmA protein is transcriptionally or posttranscriptionally regulated by oxygen (Theroux et al. 1990).

Almost all known species of phototrophic *Alpha-* and *Betaproteobacteria* (purple nonsulfur bacteria) are capable of microaerophilic or aerobic chemoorganoheterotrophic growth with oxygen as terminal electron acceptor. Of the purple sulfur bacteria, *Ectothiorhodospira* species, and eight small-celled *Chromatiaceae* (*Thiocapsa rosea*; *Marichromatium gracile*; *Thiocystis minor*; *Allochromatium vinosum*; *Thiocystis violascens*; *Thiocapsa roseopersicina*; *Thiocystis violacea*; *Thiorhodovibrio winogradskyi*) can grow by chemolithotrophy, oxidizing sulfide or thiosulfate with molecular oxygen (De Wit and van Gemerden 1987b; Kømpf and Pfennig 1980; Overmann and Pfennig 1992). Only few species grow also chemoorganotrophically with organic carbon substrates as electron donor of respiration. The group of facultatively chemotrophic *Chromatiaceae* includes typical inhabitants of benthic microbial mats like *Thiocapsa roseopersicina* and *Thiorhodovibrio winogradskyi*. This is not surprising considering the pronounced oxic/anoxic fluctuations in this type of habitat. The cells of purple sulfur bacteria in benthic systems are often immotile and form aggregates together with sand grains, apparently as an adaptation to the hydrodynamic instability of the habitat (van den Ende et al. 1996). At the same time, however, immotile cells are exposed to strong diurnal variations in oxygen concentrations. The growth affinities for sulfide are lower for chemotrophically growing *Thiocapsa roseopersicina* than for colorless sulfur bacteria, which may explain that no natural populations of purple sulfur bacteria are known that grow permanently by chemotrophy (see section ❯ "Interactions Between Phototrophic Sulfur Bacteria and Chemotrophic Bacteria" in this chapter).

When grown anaerobically in the light, facultatively chemotrophic species of the purple nonsulfur and purple sulfur bacteria contain a potentially active respiratory system and exhibit ≥50 % of the respiratory activity of chemotrophically growing cells (De Wit and van Gemerden 1987a; Kømpf and Pfennig 1980; Overmann and Pfennig 1992; Pfennig 1978). In cells that still contain bacteriochlorophyll, respiration is inhibited by light. This indicates that respiration and photosynthesis are coupled (e.g., by the membrane potential or common redox carriers; Richaud et al. 1986). An example is the soluble cytochrome c_2 which has a dual function in *Rhodobacter sphaeroides* where it is needed for electron transfer from the cytochrome bc_1 complex to the reaction center during photosynthesis, and to the cytochrome c oxidase during respiration with molecular oxygen. During photosynthetic growth, expression of cytochrome c_2 is increased. At limiting concentrations of the electron-donating substrate, photosynthesis is preferred over respiration as long as the intracellular bacteriochlorophyll content is maintained at a sufficiently high level (4–7 g bacteriochlorophyll a·mg protein$_{-1}$ in *Thiocapsa roseopersicina* at light saturation; De Wit and van Gemerden 1990a).

Growth continues after a shift to microoxic or aerobic conditions. Under oxic conditions, the synthesis of pigments and of pigment-binding proteins of the photosynthetic apparatus ceases. The number of intracellular membrane vesicles is reduced dramatically and the composition of membrane lipids is altered. The pigment content in purple sulfur bacteria is inversely related to the ambient oxygen concentration (Kämpf and Pfennig 1986). At 25 % air saturation (52 M) of oxygen, pigment synthesis in *Thiocapsa roseopersicina* is completely repressed and cells become colorless (De Wit and van Gemerden 1987b). In continuous cultures of purple sulfur bacteria, active degradation has not been observed and intracellular bacteriochlorophyll concentrations follow the washout curve. Thus bacteriochlorophyll does not seem to be actively degraded but is diluted out by cell division (De Wit and van Gemerden 1987b). Concomitantly, the activities of respiratory enzymes (NADH dehydrogenase, cytochrome c oxidases) are increased in chemotrophically grown cells. When the cells of *Thiocapsa roseopersicina* become colorless, they use only one third of the electron donor for reduction of CO_2. The remaining two thirds are used for energy generation and respired. Correspondingly, the protein yield reaches one third of that of phototrophically grown cells (De Wit and van Gemerden 1987b, 1990b).

In aerobic phototrophic bacteria, aerobic growth is stimulated by light that is absorbed by bacteriochlorophyll *a*. This stimulation is only transient, however, since bacteriochlorophyll synthesis is repressed even by low light intensities (Yurkov and van Gemerden 1993), thus leading to a loss of the photosynthetic apparatus under continuous illumination.

Respiration in *Cyanobacteria* involves a full respiratory chain including a cytochrome aa_3 terminal oxidase. Monomeric sugars are degraded using the oxidative pentose phosphate cycle. They apparently make use of a non-canonical tricarboxylic acid cycle (Zhang and Bryant 2011). The NADPH formed in sugar catabolism is fed to the membrane-bound electron transport chain at the level of plastoquinone. This is in contrast to green chloroplasts, in which plastoquinol is autoxidized (Peltier and Schmidt 1991). The respiratory electron transport chain of cyanobacteria is located in both the plasma and the thylakoidal membrane, and it shares many functional components with

photosynthetic electron transport. The role of exogenous respiration of organic substrates is probably minor under natural conditions. Under anoxia, the known electron acceptor alternatives to oxygen for cyanobacterial chemoorganotrophy are some organic compounds and elemental sulfur. Fermentation seems to be a relatively widespread ability in benthic and bloom-forming cyanobacteria, but it is not universal (Moezelaar and Stal 1994).

Genetic Regulation by O_2

A shift from anoxic to oxic growth conditions requires the expression of new proteins and cofactors. On the genetic level, the formation of the photosynthetic apparatus and the intracytoplasmic membrane system is regulated by two main environmental variables, light intensity (see section "Response to Changes in Light Intensity and Quality" in this chapter) and molecular oxygen. The two factors act independently of one another and are involved in different mechanisms of regulation of bacteriochlorophyll synthesis (Arnheim and Oelze 1983). Compared to light, molecular oxygen acts as a stronger repressor, however. Although oxygen is a major factor controlling the formation of the photosynthetic apparatus in most of the facultatively phototrophic *Proteobacteria*, *Rhodovulum sulfidophilum* and *Rhodocista centenaria* are exceptional in that these species form the photosynthetic apparatus under both aerobic and anaerobic conditions (Hansen and Veldkamp 1973; Nickens et al. 1996). Photopigment synthesis is not repressed by O_2 in *Rhodocista centenaria*.

The regulation of bacteriochlorophyll synthesis in purple nonsulfur bacteria is complex. The cells synthesize very little bacteriochlorophyll, probably because of the inhibition of bacteriochlorophyll biosynthesis enzymes (the δ–aminolevulinic acid synthesis and enzymes for the conversion of coproporphyrin; Oelze 1992) by O_2. Oxygen does not seem to exert an effective transcriptional control. Under oxic conditions, the transcription of bacteriochlorophyll synthesis genes decreases twofold, while that of light-harvesting I and reaction-center genes decreases by a factor of 30–100 (Bauer 1995). The tetrapyrrole synthesis pathway has four different branches (leading to heme, bacteriochlorophyll, siroheme and vitamin B_{12}). While the bacteriochlorophyll content is drastically reduced in the presence of oxygen (Arnheim and Oelze 1983), heme synthesis remains unaffected (Lascelles 1978). The intracellular activity of δ-aminolevulinic acid synthase, the key enzyme of tetrapyrrol synthesis in *Alphaproteobacteria*, is reduced in the presence of oxygen. Regulation by oxygen may occur also during some later steps of tetrapyrrole synthesis. It appears that oxygen inhibits magnesium chelatase, thereby increasing the protoporphyrin IX pool, which in turn leads to increased formation of heme. Feedback inhibition of δ-aminolevulinate synthase by heme would then slow down the synthesis of intermediates but still guarantee the amount needed for heme biosynthesis (Beale 1995; Biel 1995; Rebeiz and Lascelles 1982).

After return to anoxic conditions, the synthesis of the photosynthetic apparatus and intracellular membranes occurs in a light-independent manner. Anoxygenic photosynthetic bacteria contain a distinct light-independent protochlorophyllide reductase, composed of probably three subunits (BchN, BchB, and BchL). In angio-sperms, the reduction of the fourth ring of the Mg-tetrapyrrole intermediate by NADPH-protochlorophyllide oxidoreductase is a light-dependent step in the chlorophyll biosynthetic pathway. This protein represents one of the only two enzymatic transformations known to require light (Suzuki and Bauer 1995). Cyanobacteria, green algae, and gymnosperms contain both the light-dependent and light-independent protochlorophyllide reductase. The capacity to synthesize (bacterio)chlorophyll in the dark is of significance for the competitive success of *Chromatiaceae* in intertidal microbial mats. During anoxic conditions in the dark, *Thiocapsa roseopersicina* can synthesize bacteriochlorophyll *a* at maximum rate. Under the fluctuating conditions as they are observed in benthic microbial mats (oxic light, anoxic dark phase), purple sulfur bacteria therefore can maintain a photosynthetic mode of growth as long as bacteriochlorophyll synthesis during the night compensates for the wash out of pigments during the day (De Wit and van Gemerden 1990b).

A multicomponent regulatory cascade controls the coordinate expression of the light-harvesting and reaction center *puf*, *puh*, and *puc* genes and involves various transcription factors (Bauer 1995; Bauer and Bird 1996). In *Rhodobacter capsulatus* and *Rba. sphaeroides*, the redox-sensitive repressors CrtJ and PpsR, respectively, bind under oxic conditions to conserved palindrome sequences in promoters of bacteriochlorophyll, carotenoid, light-harvesting complex II genes, and respiratory genes. Under oxic conditions, CrtJ or PpsR bind tightly and as tetramers to the promoters. This redox sensing is mediated by an intramolecular disulfide bond that is formed under oxic conditions (Masuda and Bauer 2002). A second system for the regulation of the *puf*, *puh*, and *puc* operons probably consists of three components, a membrane-spanning sensor kinase (RegB), a soluble response regulator (RegA), and a hypothetical activator of the nonspecific alternative sigma factor σ^P (RegX). A decrease in oxygen tension causes autophosphorylation of the membrane-spanning sensor kinase RegB, which then phosphorylates the cytoplasmic response regulator RegA. The latter acts as an intermediate and probably transfers its phosphate to a putative third DNA-binding component that activates gene expression. The RegA-RegB system also is involved in the regulation of the expression of cytochrome c_2 and the Calvin cycle CO_2 fixation genes and therefore is of general significance for the regulation of cellular metabolism.

The transcripts of the photosynthetic gene cluster exceed 10 kb and extend from pigment biosynthesis genes across promoter regions and into the genes for light-harvesting complex I and reaction center proteins. In *Rhodobacter capsulatus*, transcription of the genes coding structural polypeptides of the reaction center and light-harvesting complex I are not the only peptides initiated at their respective promoters. The transcripts of the bacteriochlorophyll biosynthesis *bchCA* operon extends

through the promoter and coding sequences of the downstream *puf BALM* operon, and the transcript of the carotenoid biosynthesis *crtEF* operon extends through both (Wellington et al. 1992). Similarly, the *bchFBKHLM-F1696* and *puhA* operons are transcriptionally linked. The linkage of operons of different components of the photosynthetic apparatus in such superoperons also has been detected in other species of purple nonsulfur bacteria and may play a significant role in the adaptation of cells to changes in environmental oxygen tension. According to a model (Wellington et al. 1992), the presence of superoperons ensures a rapid physiological response to a decrease in oxygen tension. In the presence of oxygen, a basal level of light-harvesting I and reaction center polypeptides is constantly formed and incorporated into the membrane, but these polypeptides disappear again in the absence of bacteriochlorophyll (Dierstein 1984; Drews and Golecki 1995) due to degradation. After a shift from oxic to anoxic conditions, the presence of a basal level of structural polypeptides considerably shortens the lag time for the change from aerobic respiratory to anaerobic photosynthetic growth. During this lag phase, the cellular amount of structural polypeptides of the photosynthetic apparatus is further increased by increasing the transcription rate of the *puf* and *puh* genes.

Oxygen does not only regulate the transcription of photosynthesis genes but also later steps in gene expression. Posttranscriptional regulation involves mRNA processing (mRNA degradation) and possibly some later steps (Rödig et al. 1999).

In most bacteria, the formation of multiple sigma factors is a prerequisite for the coordination of the regulation of a large number of genes in response to changes in environmental conditions. Sigma factors are dissociable subunits that confer promoter specificity on eubacterial core RNA polymerase and are required for transcription initiation. In phototrophic bacteria, the diversity of sigma factors of the σ^{70} family as they are present in the different phylogenetic groups appears to be correlated with their metabolic flexibility. In the unicellular cyanobacteria *Synechococcus* sp. and *Synechocystis* sp., nine different sigma factors (one member of group 1, four members of group 2, and four members of group 3) have been found, whereas one group 1 and three group 2 sigma factors have been found in *Chloroflexus* spp. In contrast to most other bacteria, the green sulfur bacterium *Chlorobium tepidum* contains only one group 1, but no alternative group 2 sigma factor (Gruber and Bryant 1998). In *Chloroflexus*, one group 2 σ^{70} factor (SigB) is transcribed at fourfold higher levels during aerobic growth and therefore appears to be involved in the shift in metabolism. It has been proposed that SigB is involved in the regulation of pigment synthesis (Gruber and Bryant 1998).

Significance of Anoxygenic Photosynthesis for the Pelagic Carbon and Sulfur Cycles

The carbon fixation of phototrophic sulfur bacteria has been determined in a wide range of habitats, mostly inland lakes (Overmann 1997; van Gemerden and Mas 1995). The theoretical maximum of primary production by phototrophic sulfur bacteria has been estimated to be 10,000 mg $C \cdot m^{-2} \cdot d_{-1}$. Purple and green sulfur bacteria can contribute up to 83 % of total primary productivity in these environments. This high number notwithstanding, anoxygenic primary production only represents a net input of organic carbon to the food web if (1) the anaerobic food chain is fueled by additional allochthonous carbon from outside and (2) aerobic grazers have access to the biomass of phototrophic sulfur bacteria. Based on recent experimental evidence, these conditions are met at least in some aquatic ecosystems (Overmann 1997).

With the exception of geothermal springs, the sulfide required by phototrophic sulfur bacteria for CO_2-assimilation originates from sulfate or sulfur reduction during the terminal degradation of organic matter. This organic matter cannot be provided solely by anoxygenic phototrophic bacteria, since growth (hence accumulation of reduced carbon) constantly diverts electrons from their cycling between anoxygenic phototrophic bacteria and sulfate-reducing bacteria. At least part of the sulfide formation is therefore fueled by carbon that has already been fixed by oxygenic photosynthetic organisms within or outside the ecosystem. Consequently, anoxygenic photosynthesis represents not new but secondary primary production. A complete degradation of the carbon fixed by phototrophic sulfur bacteria in the anaerobic food chain (and thus an efficient recycling of electrons) in an anoxygenic primary production has been estimated to exceed oxygenic photosynthesis by as much as ten times (Overmann 1997). In reality, anoxygenic photosynthesis surpasses that of phytoplankton mostly in oligotrophic lakes. In many oligotrophic lakes, the input of allochthonous carbon derived from terrestrial sources in the watershed is significant (Rau 1980; Sorokin 1970). In an oligotrophic saline meromictic lake (Mahoney Lake, B.C., Canada), purple sulfur bacteria together with the anaerobic food chain efficiently converted allochthonous organic carbon into easily degradable bacterial biomass (Overmann 1997). It appears likely that phototrophic sulfur bacteria have this ecological function also in other aquatic ecosystems.

The presence of hydrogen sulfide in layers of phototrophic sulfur bacteria may prevent their biomass from entering the grazing food chain. This has been substantiated by stable carbon and sulfur isotope data, which indicated that phototrophic sulfur bacteria are not consumed to a significant extent by higher organisms (Fry 1986). In addition, a quantitative analysis of loss processes conducted in a few lakes indicates that predation must be of minor significance (Mas et al. 1990; van Gemerden and Mas 1995). In contrast, recent investigations have revealed that at least in one lake ecosystem, a major fraction of purple sulfur bacterial biomass enters the aerobic food chain via rotifers and calanoid copepods (Overmann et al. 1999b, c). The key environmental factors that caused this efficient link between anoxic and oxic water layers were the autumnal upwelling of phototrophic bacteria into oxic water layers by mixing currents, and the formation of gas vesicles and large cell aggregates by the dominant species, *Amoebobacter purpureus*.

Sulfide formation by sulfate- and sulfur-reducing bacteria and sulfide oxidation back to sulfur and sulfate occur at comparable rates in several lakes (Overmann et al. 1996; Parkin and Brock 1981). This leads to a closed sulfur cycle and a detoxification of sulfide without concomitant depletion of oxygen (Pfennig 1978).

The significance of phototrophic sulfur bacteria for the oxidation of sulfide in stratified environments is critically dependent on their cell density rather than the absolute biomass per surface area of the ecosystem (Jørgensen 1982). Dense populations in laminated microbial mats can account for 100 % of the total sulfide oxidation in those systems, whereas some dilute pelagic populations oxidize only very small amounts (e.g., 4 % in the Black Sea) of the sulfide diffusing from below into the chemocline (Overmann et al. 1991a, 1996).

No information on the ecological significance of aerobic phototrophic bacteria is available to date.

Interactions with Other Microorganisms

Competition for Light

Blue light prevails in very clear open oceans (*Fig. 12.5*) where marine *Synechococcus* cells thrive under conditions of low photon flux (~10 mol quanta·m^{-2}·s^{-1}; Carr and Mann 1994). Two ecotypes of the marine *Synechococcus* exist which differ in the intracellular ratio of phycourobilin to phycoerythrobilin (Waterbury et al. 1986). Two subpopulations are distinguished according to the predominant chromophore associated with the phycoerythrin. Phycourobilin-rich strains are characteristic of the open oceans whereas strains with a lower PUB content predominate in shelf waters (Olson et al. 1990a). Compared to PEB-containing antennae (absorption maximum, ~550 nm), incorporation of PUB (absorption maximum, ~495 nm) increases the efficiency of light absorption significantly in deeper water layers of oligotrophic oceans.

Similarly, coexisting and phylogenetically closely related but genetically distinct populations of *Prochlorococcus* are adapted for growth at different light intensities, which results in their broad depth distribution (Moore et al. 1998). The low-light-adapted ecotype has a higher intracellular content of chlorophylls a and b, a higher chlorophyll b/a ratio, and exhibits a higher maximum quantum yield reaching the theoretical maximum of 0.125 mol C·(mol quanta)$^{-1}$. Its properties enable this ecotype to colonize very low water layers. It has been suggested that the distribution of different ecotypes in the same water column would result in greater integrated production than could be achieved by a single ecotype (Moore et al. 1998).

Based on the specific physiological properties of oxygenic and anoxygenic phototrophic bacteria, multilayered microbial communities frequently develop in stratified pelagic and in benthic (*Fig. 12.4a, b*) habitats. Cyanobacteria, eukaryotic algae, and even plants (*Lemna* spp.) form the topmost layers overlying populations of *Chromatiaceae* and green sulfur bacteria (Dubinina and Gorlenko 1975; Caldwell and Tiedje 1975; Pfennig 1978; Camacho et al. 1996; Pierson et al. 1990).

Phototrophic sulfur bacteria require the simultaneous presence of light and sulfide, which usually restricts their occurrence to layers well below the surface of lakes and sediments. As a consequence of the absorption of light in the overlying water, the light energy available to phototrophic sulfur bacteria in most pelagic environments is rather low (0.02–10 % of surface light intensity; van Gemerden and Mas 1995; Parkin and Brock 1980b; Camacho et al. 1996). Similar values have been determined for purple layers in benthic microbial mats (Kühl and Jørgensen 1992; Pierson et al. 1990; Garcia-Pichel et al. 1994). A tight correlation between anoxygenic photosynthesis and the amount of light reaching phototrophic sulfur bacteria strongly suggests that light is the main environmental variable controlling the anoxygenic photosynthesis (van Gemerden and Mas 1995). Therefore, a selective pressure for efficient light harvesting and maximum quantum yield exists in anoxygenic phototrophs. The same holds true for a few niche-specialized, deep-dwelling cyanobacteria.

The ecological niches of green sulfur bacteria and *Chromatiaceae* show considerable overlap because both groups grow preferably or exclusively by photolithotrophic metabolism, using ambient sulfide as electron-donating substrate. Different species of the same group should be even more competitive. Besides differences in maintenance energy demand, in adaptation to low light intensities and metabolic flexibility, another important factor determining the species composition of phototrophic sulfur bacteria in their natural habitats is the spectral composition of underwater light. In the overlying layers, light is absorbed by water itself, dissolved yellow substance (gilvin), phytoplankton, and inanimate particulates. The limited wavelength range available at great depth selects for species of anoxygenic phototrophic bacteria with complementary absorption spectra. In many lacustrine habitats, light absorption by phytoplankton exceeds that of gilvin or water itself (Kirk 1983), and light of the blue-green to green wavelength range reaches layers of phototrophic sulfur bacteria. Those *Chromatiaceae* which contain the carotenoid okenone (*Fig. 12.7*) dominated in 63 % of the natural communities studied (van Gemerden and Mas 1995). It was proposed that energy transfer from carotenoid antenna pigments to the reaction center is more efficient in okenone-forming strains than in other purple sulfur bacteria (Guerrero et al. 1986). In addition, the capability of gas vesicle formation and the different kinetics of sulfide oxidation (see section "Interactions with Other Microorganisms" in this chapter) appear to be of selective value for the colonization of pelagic habitats. Below accumulations of purple sulfur bacteria, the green-colored forms of the green sulfur bacteria dominate because of their superior capability to harvest the light reaching them, which has its spectrum shifted to a maximum intensity at 420–450 nm (*Table 12.3*) (Montesinos et al. 1997). In contrast, the brown-colored forms of the green sulfur bacteria dominate in lakes where the chemocline is located at depths greater than 9 m and in eutrophic lakes with a pronounced light absorption in the oxic zone.

A similar niche separation occurs in the phototrophic consortia, which encompass green-colored or brown-colored epibionts (Overmann et al. 1999b). The ecological niche of the brown-colored green sulfur bacteria may be attributed to their use of significantly lower light intensities than purple sulfur bacteria for phototrophic growth and to their lower maintenance energy requirements (see section "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter). An extremely low-light-adapted strain of the green sulfur bacterium *Chlorobium phaeobacteroides* has been isolated from the chemocline of the Black Sea located at an 80-m depth (Overmann et al. 1991a). This isolate (strain BS1) could grow at light intensities as low as 0.015 µmol quanta·m^{-2}·s^{-1} (Marschall et al. 2010).

In sedimentary environments with their particular optical properties (*Fig. 12.5*), the irradiance reaching anoxygenic phototrophic bacteria may be reduced to <1 % of the surface value for light in the visible region, while >10 % of the near-infrared light is still available (Kühl and Jørgensen 1992; see section "Light Energy and the Spectral Distribution of Radiation"). As a consequence, the long-wavelength Q_y bands of bacteriochlorophylls are significant for light harvesting in sediments, whereas light absorption of anoxygenic phototrophic bacteria in lakes is mediated by carotenoids and the Soret bands of bacteriochlorophylls. In microbial mats, the spectral quality of the scalar irradiance is strongly modified as it penetrates. The presence of populations of phototrophic microorganisms imposes strong absorption signatures on the spectrum of the scalar irradiance (Jørgensen and Des Marais 1988; Pierson et al. 1987). As a result of vertical niche separation, benthic microbial mats can consist of up to five distinctly colored layers that are formed (from the top) by diatoms and cyanobacteria, cyanobacteria alone, purple sulfur bacteria with bacteriochlorophyll *a*, purple sulfur bacteria with bacteriochlorophyll *b*, and green sulfur bacteria (Nicholson et al. 1987). In this vertical sequence, different wavelength bands of red and infrared light (compare *Table 12.3*, *Fig. 12.6*) are successively absorbed by the different microbial layers (Pierson et al. 1990). Distinct blooms of bacteriochlorophyll *b*–containing anoxygenic phototrophic bacteria have been observed only in benthic habitats. Employing this pigment, the phototrophic Proteobacteria *Blastochloris viridis*, *Blastochloris sulfoviridis*, *Thiococcus pfennigii*, *Halorhodospira halochloris*, and *Halorhodospira abdelmalekii* harvest light of a wavelength range (1,020–1,035 nm) which cannot be exploited by any other photosynthetic organism.

Until recently, no strain of anoxygenic photosynthetic bacteria was known that could absorb light in the wavelength range between 900 and 1,020 nm. Because of the prevalence of infrared radiation in the anoxic layers of microbial mats and the strong competition for this wavelength region, bacteria containing other types of photosynthetic antenna complexes would have a high selective advantage. Recently, the α-Proteobacterium *Rhodospira trueperi* was isolated, which contains bacteriochlorophyll *b* in a light-harvesting complex with a maximum absorption at 986 nm (Pfennig et al. 1997). Employing a selective enrichment strategy, the *Alphaproteobacterium Roseospirillum parvum* could be isolated which harbors another new type of photosynthetic antenna complex. Here, bacteriochlorophyll *a* is the light-harvesting pigment, and in vivo exhibits an absorption maximum at 911 nm (Glaeser and Overmann 1999, *Fig. 12.6*). Furthermore, A $BChl_a$-containing purple sulfur bacterium with the most extreme band-shift recognized so far (Q_y at 963 nm) has been isolated (Permentier et al. 2001; Rücker et al. 2011). These three isolates originate from benthic microbial mats, indicating that the diversity of pigment-protein complexes in *Proteobacteria* is higher than previously assumed. The variation in the in vivo absorption spectra of the same pigment must be the result of differences in binding to light-harvesting proteins. In contrast, changes in the absorption spectra of the light-harvesting complex of green sulfur bacteria are the result of chemical alterations (e.g., methylation) of the pigment molecules (Bobe et al. 1990) because pigment-pigment interactions dominate in the chlorosomes (see section "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter).

Because methanogenesis is the predominant pathway of terminal degradation in rice fields, *Heliobacteriaceae* probably compete with the photoheterotrophic purple nonsulfur bacteria in their natural environment (Madigan and Ormerod 1995). Owing to the presence of bacteriochlorophyll *g*, *Heliobacteriaceae* take advantage of a wavelength region of the electromagnetic spectrum, which is not absorbed by other phototrophic bacteria. As a result of the small and fixed size of the photosynthetic antenna (see section "Light Absorption and Excitation Transfer in Prokaryotes" in this chapter), these bacteria are adapted to higher light intensities than other anoxygenic phototrophic bacteria (≈1,000 mol quanta·m^{-2}·s^{-1}).

In addition to the capacity of absorbing light in the long wavelength range, metabolic flexibility is of highly selective value for the colonization of benthic habitats with their high fluctuations in oxygen and sulfide concentrations (see section "Chemotrophic Growth with O_2").

However, the composition of communities of phototrophic sulfur bacteria is not solely determined by competition. The simultaneous presence of green sulfur bacteria and *Chromatiaceae* possibly is also based on syntrophic interactions (see section "Coexistence of Phototrophic Bacteria" in this chapter).

Coexistence of Phototrophic Bacteria

Within the *Chromatiaceae*, many small-celled species exhibit a considerably greater metabolic flexibility than the large-celled *Chromatium* species (see sections "Carbon Metabolism of Phototrophic Prokaryotes" and "Chemotrophic Growth with O_2" in this chapter). In addition, small-celled species like *Allochromatium vinosum* have a higher growth affinity for sulfide. Based on these pure culture data, it is therefore unexpected that large-celled forms in fact dominate in natural ecosystems. The large-celled *Chromatium weissei* oxidizes sulfide twice as fast as the small-celled *Allochromatium vinosum*. Whereas the former preferentially oxidizes sulfide to zero-valent sulfur, the latter oxidizes a larger fraction directly to sulfate. Under fluctuating

conditions, as they occur in the chemocline of lakes, *Chromatium weissei* is capable of rapidly oxidizing sulfide at the onset of illumination, thereby accumulating zero-valent sulfur. During the remaining light period and because of its higher affinity for sulfide, *Allochromatium vinosum* utilizes most of the sulfide. Continuous cocultures of both species have thus been established by illumination in light-dark cycles (van Gemerden 1974).

Furthermore, stable coexistence of two organisms is feasible in the presence of two substrates for which the two competitors have complementary affinities. Stable syntrophic interactions can be established in laboratory cocultures of purple sulfur (*Allochromatium vinosum*) and green sulfur bacteria (*Chlorobium limicola* f.sp. thiosulfatophilum; van Gemerden and Mas 1995). Because of its higher affinity, the green sulfur bacterium oxidizes sulfide to zero-valent sulfur. The extracellular sulfur is mobilized as polysulfide, which can be used instantaneously as an electron donor of the purple sulfur bacterium. The presence of sulfide inhibits the green sulfur bacterium from using polysulfide. Sulfide and polysulfide thus are the mutual substrates for the two different phototrophic sulfur bacteria.

Purple and green sulfur bacteria also have complementary affinities for sulfide and acetate (see section ❯ "Carbon Metabolism of Phototrophic Prokaryotes"). Accordingly, stable continuous cocultures of *Chlorobium phaeobacteroides* and *Thiocapsa roseopersicina* can be established (Veldhuis and van Gemerden 1986).

Recent culture-independent determination of the metabolic activity of different anoxygenic phototrophic sulfur bacteria in a stratified alpine lake revealed that *Chromatium okenii* contributed 70 % of total carbon uptake and 40 % of total ammonium uptake despite a relative cell abundance of only 0.4 %, enabling a doubling time of 5 days, whereas the numerically dominant *Chlorobium clathratiforme* reached only a doubling time of 36 days (Musat et al. 2008). Obviously, the competitiveness of anoxygenic phototrophs cannot be deduced from their abundance in the natural environment.

Interactions Between Phototrophic Sulfur Bacteria and Chemotrophic Bacteria

A considerable number of strains of *Chromatiacae* are capable of switching to a chemolithotrophic growth mode after prolonged incubation in the presence of molecular oxygen (see section ❯ "Chemotrophic Growth with O_2" in this chapter). Under these conditions, purple sulfur bacteria compete with colorless sulfur bacteria like the *Thiobacillus* spp. Compared to thiobacilli, the purple sulfur bacterium *Thiocapsa roseopersicina* attains a higher growth yield under chemolithotrophic conditions (De Wit and van Gemerden 1987a). However, the growth affinity for sulfide of the colorless sulfur bacteria is up to 47 times higher than that of *Chromatiacae* (De Wit and van Gemerden 1987b; van Gemerden and Mas 1995). Therefore *Chromatiacae* growing exclusively by chemolithotrophy would be rapidly outcompeted by colorless sulfur bacteria.

Culture experiments indicate that *Thiocapsa roseopersicina*, a typical inhabitant of laminated microbial mats in temperate environments, can replenish its photosynthetic pigments during anoxic periods in the dark, thereby maintaining a phototrophic growth mode also during the subsequent oxic light period (De Wit and van Gemerden 1990b). Based on microelectrode measurements, purple sulfur bacteria in marine microbial mats of the North Sea barrier islands are exposed to oxygen during most of the day, whereas anoxic conditions prevail during the night (De Wit et al. 1989). Thus, the anoxygenic phototrophs cannot grow during the night and face competition for sulfide by colorless sulfur bacteria during the day. Because of their higher affinity for sulfide, the latter would be expected to outcompete phototrophically growing purple sulfur bacteria. In cocultures of *Thiocapsa roseopersicina* and *Thiobacillus thioparus*, sulfide is indeed entirely used by the colorless sulfur bacterium in the presence of oxygen. If oxygen concentrations are limiting, however, sulfide is oxidized incompletely by the chemolithotroph and soluble zero-valent sulfur formed (either as polysulfide or polythionates) that in turn is used by the purple sulfur bacterium for phototrophic growth (van den Ende et al. 1996). Both diurnal fluctuations between oxic light and anoxic dark periods and syntrophism based on sulfur compounds may permit a stable coexistence of these groups and explain their simultaneous presence in natural microbial mats.

Stable associations can be established between green sulfur bacteria and sulfur- or sulfate-reducing bacteria. These associations are based on a cycling of sulfur compounds but not carbon (see section ❯ "Significance of Anoxygenic Photosynthesis for the Pelagic Carbon and Sulfur Cycles" in this chapter). The simultaneous growth of both types of bacteria is fueled by the oxidation of organic carbon substrates and light. In a similar manner, cocultures of *Chromatiaceae* with sulfate-reducing bacteria have been established in the laboratory (van Gemerden 1967).

The most spectacular type of association involving phototrophic bacteria is represented by the phototrophic consortia. These consortia consist of green sulfur bacterial epibionts that are arranged in a regular fashion around a central chemotrophic betaproteobacterium (Pfannes et al. 2007). To date, 19 different types of phototrophic consortia have been detected worldwide using 16S rRNA gene sequence–based methods (Glaeser and Overmann 2004). Whereas the successful isolation of the epibiont of "*Chlorochromatium aggregatum*" (*Chlorobium chlorochromatii*; Vogl et al. 2006) demonstrates that the association in phototrophic consortia is not an obligatory one at least for the green sulfur bacterial partner, both types of bacteria still exhibit highly specific adaptations to the life in association. Epibiont cells in the symbiotic state show a conspicuous and previously unknown type of intracellular sorting of their chlorosomes which are entirely absent at the site of attachment to the central bacterium (Vogl et al. 2006; Wanner et al. 2008). Genome analysis of *Chl. chlorochromatii* identified candidate symbiosis genes that are unique among the *Chlorobiaceae* and, unexpectedly, resemble known virulence factors of *Proteobacteria* (Vogl et al. 2008). It has been hypothesized

that these genes were acquired by the epibiont through lateral gene transfer. While only a comparatively small number of unique genes could be identified in *Chl. chlorochromatii*, a much larger number of at least 352 genes are differentially described in the symbiotic state, most of them encoding components of the central metabolic pathways which suggested a considerable preadaptation of the ancestor of *Chl. chlorochromatii* to a life in symbiosis (Wenter et al. 2010). A rapid signal transfer exists between the two partners and permits phototrophic consortia to scotophobotactically accumulate at preferred light intensities and wavelengths. In this association, the immotile green sulfur bacteria attain motility like purple sulfur bacteria. The high numbers of phototrophic consortia found in many lakes indicate that this strategy must be of high competitive value under certain environmental conditions.

A commensal relationship may exist between coccoid epibiotic bacteria and the purple sulfur bacterium *Chromatium weissei* (Clarke et al. 1993). This unidentified epibionts attaches to healthy cells but does not form lytic plaques on lawns of host cells like the morphologically similar parasite *Vampirococcus* (see section "Significance of Bacteriophages and Parasitic Bacteria" in this chapter). Possibly, the epibiont grows chemotrophically on carbon compounds excreted by the purple sulfur bacterium.

A syntrophic interaction between cyanobacteria and sulfate-reducing bacteria appears to exist in microbial mats where both types of microorganisms occur in close spatial proximity, if not intermixed with each other. In these ecosystems, the excretion of organic carbon substrates by cyanobacteria may provide the electron-donating substrates for sulfate-reducing bacteria (Jorgensen and Cohen 1977; Skyring and Bauld 1990; Fründ and Cohen 1992). The glycolate produced by photorespiration (Fründ and Cohen 1992) as well as the formate, acetate, and ethanol produced by glycogen fermentation (Moezelaar and Stal 1994) most likely are the substrates excreted by cyanobacteria.

Despite a pronounced limitation of sulfate reduction by carbon substrates (Overmann et al. 1996; Overmann 1997), no close syntrophic relationship was found between purple sulfur and sulfate-reducing bacteria in a meromictic lake. In this specific environment, degradation of biomass by the entire anaerobic food chain rather than excretion of small carbon molecules and their direct utilization by sulfate-reducing bacteria provides the electron-donating substrates for sulfate-reducing and sulfur-reducing bacteria.

Symbioses Between Phototrophic Bacteria and Eukaryotes

Only one example is known for an intracellular symbiosis of anoxygenic phototrophic bacteria with a eukaryotic organism. The ciliate *Strombidium purpureum* inhabits the photic zone of sulfide-containing marine sands and harbors 200–700 purple endosymbionts. Symbionts are arranged along the periphery of the host cell and contain intracellular tubular or vesicular membranes, bacteriochlorophyll *a*, and spirilloxanthin (Fenchel and Bernard 1993a; Fenchel and Bernard 1993b). The ciliate shows a photosensory behavior, accumulating at wavelength that corresponds to the absorption maxima of the endosymbionts. It has been suggested that the intracytoplasmic purple bacteria increase the efficiency of the fermentative host by using its end products for anoxygenic photosynthesis. Furthermore, respiration of the bacteria may protect the host against oxygen toxicity.

In the course of evolution, *Cyanobacteria* have entered into symbiotic associations with a multitude of organisms (Schenk 1992). Besides all eukaryotic phototrophs, from microalgae to *Sequoia sempervirens*, which have intracellular cyanobacterial symbioses, the most common extracellular symbioses of nonheterocystous cyanobacteria are in the form of cyanolichens and involve the unicellular genera *Chroococcidiopsis*, *Gloeocapsa*, "*Chroococcus*," and *Gloeothece*, as well as members of the genera *Nostoc*, *Calothrix*, *Scytonema*, *Stigonema*, and *Fischerella* as photobionts. Heterocystous cyanobacteria in the genus *Nostoc* form extracellular symbioses with liverworts and higher plants (Cycads, duckweed). *Anabaena* enters in symbiosis with water ferns of the genus *Azolla*. *Prochloron* strains, large-celled *Synechocystis*, and small-celled *Acaryochloris marina* are known from extracellular symbioses with ascidians in tropical or subtropical marine waters; *Prochloron* is found as an ectosymbiont on the marine didemnid ascidian *Lissoclinum patella* (Lewin and Withers 1975). Extracellular symbioses of the *Pseudanabaena*-like "*Konvophoron*" occur in Mediterranean invertebrates. Finally, intracellular symbioses of nonheterocystous cyanobacteria are known with tropical sponges ("*Aphanocapsa*," *Oscillatoria*, *Synechocystis*, *Prochloron*), with green algae (*Phormidium*), and dinoflagellates (unidentified). Heterocystous cyanobacteria occur intracellularly in oceanic diatoms of the genera *Hemiaulus* and *Rhizosolenia* (and the cyanobacterium *Richelia intracellularis*). The cyanobacterial symbiont consists of a short cell filament with a terminal heterocyst (Mague 1977). The numbers of filaments varies with host species. *Nostoc* thrives intracellularly in *Trifolium* (clover) and also in the terrestrial non-lichenic fungus *Geosiphon pyriforme*. With the notable exception of lichenic photobionts, many symbiotic cyanobacteria have resisted cultivation in spite of continued efforts.

Significance of Bacteriophages and Parasitic Bacteria

In addition to grazing and light and nutrient limitation, cyanophage infection of cyanobacteria may be a significant factor limiting primary productivity in the marine environment. However, because of inactivation by solar radiation and resistance of the host cells, the role of cyanophages has remained unclear (Bergh et al. 1989; Proctor and Fuhrman 1990; Suttle et al. 1990, 1993; Waterbury and Valois 1993). The so-called gene transfer agents (GTAs) represent an unusual class of virus-like entities that have been detected first in *Rhodobacter capsulatus*

but occur widespread in other phototrophic and nonphototrophic *Alphaproteobacteria* as well as some other bacteria (Lang and Beatty 2006). Based on phylogenetic analyses, a GTA may have been present in the ancestor of *Alphaproteobacteria* and then be transmitted predominantly in a vertical fashion. In contrast to the canonical viruses, GTAs appear to function in transfer of comparatively small (<14 kb) and random pieces of genomic DNA between cells but do not bear any negative effect on their host. In *Rba. capsulatus*, the GTA resembles a small-tailed phage, but is under the control of the cellular regulatory system and does not seem to cause cell lysis during release from the cell. In *Rba. capsulatus* cultures, GTA-dependent transduction frequencies increase in later exponential and stationary phases of growth due to transcriptional regulation and are also under control of quorum sensing (Lang and Beatty 2006). This regulation might favor genetic recombination at higher cell densities of the bacterial population.

Several bacteria have been discovered that attack phototrophic bacteria (Guerrero et al. 1986; Nogales et al. 1997). *Vampirococcus* attaches to the cell surface of *Chromatium* spp. where it divides, forming chains of up to three cells. Concomitantly, the cytoplasm of the host cell appears to be degraded. *Daptobacter* penetrates the cell envelope and divides intracellularly by binary fission. In contrast to *Vampirococcus*, *Daptobacter* has been cultivated in the absence of the host and grows by fermentative metabolism. *Bdellovibrio* has a broad host range, and under laboratory conditions attacks also purple sulfur bacteria. *Bdellovibrio* forms daughter cells by multiple divisions in the periplasmic space of the host cell. The Gram-negative chemotrophic bacterium *Stenotrophomonas maltophilia* is a non-obligatory parasite of green sulfur bacteria, which causes cell lysis and ghost formation (Nogales et al. 1997). Its host range is not limited to green sulfur bacteria. The presence of parasitic bacteria in water samples becomes evident by the formation of lytic plaques on lawns of host bacteria (Esteve et al. 1992; Nogales et al. 1997). Up to 94 % of the cells of phototrophic sulfur bacteria may be infected by parasitic bacteria in natural samples. Since infection is largely limited to nongrowing cells, the impact of parasitism on populations of phototrophic sulfur bacteria appears to be limited (van Gemerden and Mas 1995).

Evolutionary Considerations

Porphyrins are found in all organisms from archaebacteria through plants to animals, and are indispensable as prosthetic groups for energy conservation. In contrast, the partially reduced derivates of porphyrins, the (bacterio)chlorophylls, are only known to be synthesized by members of six bacterial phyla (*Table 12.1*). This indicates that the capability for synthesis of porphyrins is a very ancient trait, whereas only a few prokaryotes acquired the capability to form photosynthetic pigments. Photosynthesis requires the presence of various complex protein structures and cofactors, and thus the expression of a large number of different genes. Previously, it had therefore appeared justified to consider all phototrophic prokaryotes as a monophyletic group only distantly related to nonphototrophic bacteria (Pfennig and Trüper 1974; Trüper and Pfennig 1978). Two lines of evidence have been used to reconstruct the evolution of photosynthesis.

Fossil Evidence

The oldest fossils of microorganisms have been dated back to the early Archaean (3.8 billion years ago) and may represent remains of cyanobacteria (Awramik 1992). They consist of chemical fossils and stromatolites that have been detected especially in sedimentary rocks of the Pilbara region, Western Australia, and the Barberton Mountain Land, South Africa. Stromatolites are laminated convex domes and columns of centimeter to decimeter size and have been found in 3.5–0.8-billion-year-old rocks. Although scarce in biosynthetic molecular skeletons, the insoluble, high-molecular-weight organic matter (kerogen) contains isotopic evidence for autotrophic carbon fixation. The ratio of stable carbon isotopes ($\delta^{13}C$ values) are in the range of −35.4 ‰ to −30.8 ‰, which is typical for CO_2-carbon fixed by the ribulose-1,5-bisphosphate cycle (Hayes et al. 1983). In fact, the earliest evidence for biological carbon fixation originates from 3.8 Ga old rocks (Mojzsis et al. 1996).

As deduced from geochemical proxies, like the distribution of redox-sensitive metals, the Earth's atmosphere has been oxygenated since the "Great Oxidation Event" about 2.4 Ga ago, with possibly several transient oxygen pulses preceding this event (Buick 2008). Derivatives of carotenoids that today represent specific biomarkers of *Chlorobiaceae* (chlorobactene, isorenieratene) and *Chromatiaceae* (okenone) have been discovered in up to 1.64-Ga-old rocks deposited in a stratified marine environment (Brocks et al. 2005). The Archaean microfossil recorded is only limited and does not provide strong morphological evidence for oxygen-producing cyanobacteria. The oldest microfossils that are generally accepted as cyanobacteria date back to the Paleoproterozoic, as far as some 2.5 billion years old and contain C-isotopic signatures which suggest photoautotrophy. Additionally, chemofossil lipid biomarkers attributed to cyanobacteria ($>C_{31}$ 2α-methylhopanols) and present in fluid-inclusion oils are almost as old as the microfossils (2.7 Ga; Summons et al. 2006). Yet, 2-methylhopanoids are also produced in significant amounts in anoxygenic phototrophs such as *Rhodopseudomonas palustris* (Rashby et al. 2007). Older Archaean time microfossils do exists but their taxonomic identity remains controversial (Buick 2008; Knoll 2008). Ancient sediments as old as 3.5 billion years contain laminated domes and columns of centimeter to decimeter size, which in analogy to extant stromatolites have been interpreted as organosedimentary structures produced by the trapping, binding, and precipitation activity of filamentous microorganisms, possibly cyanobacteria. However, uncertainties about the biotic origin of these structures have arisen (Homann-Marriott and Blankenship 2011).

Alternatively, it has been proposed that anoxygenic photosynthetic bacteria and not the oxygenic cyanobacteria formed

the oldest stromatolites. The presence of sulfate (as barite) and reduced sulfur compounds (as pyrite) in the above-mentioned older stromatolites may indicate a sulphuretum ecosystem driven by anoxygenic photosynthesis (Buick 2008). Based on the phylogenetic analysis of the 16S rRNA gene sequence (Oyaizu et al. 1987) and the ecophysiology (Ward et al. 1989) of the filamentous green photosynthetic bacterium *Chloroflexus aurantiacus*, similar anoxygenic phototrophic bacteria may be the more likely candidate microorganisms that built the most ancient stromatolites. However, according to analyses of the nucleotide sequences of its reaction center polypeptides and primary sigma factor (see section "Molecular Evidence" in this chapter), *Chloroflexus aurantiacus* does not represent a deep branch of bacterial evolution. Gypsum layers within the supposed stromatolites have been interpreted as indicators of sulfide oxidation by either anoxygenic phototrophs or colorless sulfur-oxidizing bacteria (Awramik 1992). However, similar structures have been discovered in lacustrine, and thus sulfur-depleted, settings with little input of allochthonous organic carbon (Buick 1992). Therefore, at least some 2.7-billion-year-old stromatolites are more likely to have harbored oxygenic cyanobacteria. Taken together with the fossil evidence, this would indicate that diversification of the major groups of phototrophic microorganisms did occur during the early Archaean (Awramik 1992).

Geochemical analyses of 3.4-Ga-old South African cherts that contain some of the oldest alleged microbial mats suggest that photosynthesis of these microorganisms was based on utilization of H_2 as the electron donor and hence anoxygenic (Tice and Lowe 2006).

Molecular Evidence

Given the complexity of the photosynthetic apparatus, it is unlikely that photosynthesis has evolved more than once during the evolution of the domain Bacteria (Woese 1987). Chlorophyll-based photosystems are only found in the Bacteria and chloroplasts, suggesting that this type of energy conversion originated in the bacterial lineage after the divergence of Archaea and Eukarya. At present, six of the known bacterial phyla comprise phototrophic species (*Table 12.1*, see section "Taxonomy of Phototrophic Prokaryotes" in this chapter). Based on 16S rRNA sequences, extant phototrophic species of different lineages are only very distantly related to each other. So far, photosynthetic species have not been discovered in the very early lineages of the bacterial radiation (e.g., the thermophilic oxygen reducers and *Thermotogales*). Because most species of these lineages are chemolithotrophic, it has been proposed that chemolithoautotrophy preceded phototrophy during the evolution of the Bacteria (Pace 1997). This conclusion is supported by the fact that in phylogenetic trees based on protein sequences of elongation factor EF-Tu and the β-subunit of ATP synthase, only the *Aquificales* and *Thermotogales* branch deeper than the majority of the bacterial divisions, while the *Chloroflexus* subdivision does not (Stackebrandt et al. 1996), thus indicating that *Chloroflexus* does not represent the descendant of a more ancient ancestor than other phototrophic bacteria as originally concluded from the limited 16S rRNA gene sequence data set available (Blankenship 1992).

Nevertheless, the phylogenetic analysis indicates that either an early ancestor of most known bacteria had acquired the capacity for photosynthetic growth (Stackebrandt et al. 1988) or, alternatively, that the genes coding the photosynthetic apparatus were transferred laterally between phylogenetically distant bacteria. Photosynthesis involves genes encoding reaction center and antenna polypeptide structural genes, pigment biosynthesis genes, genes for electron transfer components and carbon fixation machinery. While there is general agreement that these genes have not had the same evolutionary history (as shown, e.g., by the whole genome analysis of Raymond et al. 2002), very different scenarios of the evolution of bacterial photosynthesis are discussed in the literature.

It had been proposed (Oparin 1938; Gest and Schopf 1983) that anaerobic, heterotrophic prokaryotes capable of fermenting hexose sugars were among the earliest life forms and that electron transport and photosynthesis evolved as a response to the depletion of organic nutrients from the primordial soup. One model assumes that reaction centers formed consecutively but already in the prebiotic phase, whereas another pictures their evolution from cytochrome *b* in bacteria (Olson and Blankenship 2004).

Based on one hypothesis (the Granick hypothesis; Granick 1965), the biosynthetic pathway of photosynthesis pigment molecules may be taken as a recapitulation of evolution such that compounds with shorter biosynthetic pathways reflect the more ancestral state. The synthesis of bacteriochlorophyll requires one additional enzymatic reduction than that of chlorophyll. Because chlorophyll precedes bacteriochlorophyll in the biosynthetic pathway, the former should have existed earlier in nature. According to the selective loss model (Pierson and Olson 1989) a non-oxygenic photosynthetic ancestor containing chlorophyll *a* and the two types of reaction centers evolved prior to the major radiation event of the Bacteria. During the subsequent radiation, oxygen evolution appeared in one line of descent whereas either the quinone or the FeS-type photosystem was lost in other lineages, concomitant with the emergence of the different bacteriochlorophylls. Besides avoiding an a priori lateral gene transfer of the complete photosynthetic gene cluster, this Pierson-Olson hypothesis takes into account the ecological conditions of the early biosphere in which the absence of oxygen and ozone caused a predominance of radiation in the blue and UV wavelength range, which in turn would render the red-shifted absorption maxima of bacteriochlorophylls of little selective advantage (Boxer 1992). Analysis of the photosynthesis genes in the core genome of 15 cyanobacteria led to the conclusion that cyanobacteria indeed represent the most ancestral phototrophs (Mulkidjanian et al. 2006) From these "procyanobacteria" that are thought to have been anoxygenic phototrophs employing type I reaction centers, photosynthesis genes could have spread to other bacterial groups by lateral gene transfer. However, this model has been questioned because it was derived

without using accepted rooted phylogenies of photosynthesis genes (Xiong 2006).

As an argument against the Granick and Pierson-Olson hypotheses, several types of phototrophic bacteria that would be expected are apparently missing in nature. As an example, anoxygenic chlorophyll-containing forms have never been found, although it has been argued that the 8-hydroxychlorophyll-containing *Heliobacteriaceae* represents this type inasmuch as bacteriochlorophyll *g* is easily converted to chlorophyll *a* by oxidation. Bacteriochlorophylls occur in two types of reaction centers, the pheophytin type (*Proteobacteria*, *Chloroflexus*) and the FeS type. This could indicate that the presence of bacteriochlorophyll represents a primitive trait. The chlorophyll-first hypothesis postulates that bacteriochlorophyll has replaced chlorophyll independently in at least three different bacterial lineages. Chlorophyll, however, is presently only found in oxygen-evolving organisms of the phylum *Cyanobacteria*. *Cyanobacteria* that contain two different photosystems and thus have the most complex photosynthetic apparatus. In addition, the much higher complexity of the oxygen-evolving PSII of oxygenic phototrophic organisms may imply that it appeared later than the other photosystems during evolution. Molecular phylogenetic studies of the enzymes involved in the (bacterio)chlorophyll biosynthetic pathways (encoded by the *bch* genes), rooting their phylogeny using homologous enzyme families, indicate that anoxygenic Proteobacteria were the most divergent and hence probably represent the earliest group of phototrophs (Xiong et al. 2000). A scenario was put forward in which pigment biosynthesis genes were laterally transferred to *Chlorobiaceae*, then to *Chloroflexi* and *Cyanobacteria*, and from the latter to *Heliobacteriaceae* (Xiong 2006; Xiong and Bauer 2002). This would imply that, contrary to the Granick hypothesis, the evolution at least of pigment biosynthesis proceeded from bacteriochlorophyll to chlorophyll and hence must have involved gene loss rather than stepwise acquisition of genes. As an example, the discrepancy between the presence of chlorophyll exclusively in the most highly evolved bacteria and its shorter biosynthetic pathway may be explained by the finding that the chlorin reductase, which catalyzes the additional step of the biosynthetic pathway for bacteriochlorophyll, is phylogenetically older than the enzyme (protochlorophyllide reductase) that catalyzes the preceding step. This enzyme is present in both the chlorophyll- and bacteriochlorophyll-containing bacteria (Burke et al. 1993). An ancient reductase may have been able to perform the reduction of both protochlorophyllide and chlorin, such that bacteriochlorophyll was the photochemically active pigment in the last common ancestor of all extant phototrophic bacteria.

As another argument against the Pierson-Olson hypothesis, chlorophyll itself should have been of little selective advantage in the Earth's early biosphere, and it has been proposed that quinone-iron complexes represented the first photosynthetic unit (Boxer 1992). In contrast to the complex porphyrin pigments, quinones can form spontaneously from acetyl thioesters (Hartman 1992).

The fusion model postulates that the reaction center types I and II evolved in two different evolutionary lineages. Reaction center type I would have given rise to reaction centers in *Heliobacteriaceae* and *Chlorobiaceae*, whereas the type II led to reaction centers in *Proteobaceria* and *Chloroflexi*. The photosynthetic apparatus of the *Cyanobacteria* then resulted from the fusion of the two reaction center types (Olson and Blankenship 2004). An analysis of the distribution of the different types of reaction centers among the different bacterial phyla and the amino acid sequences of reaction center proteins (Blankenship 1992) suggests different ways for the evolution of these structural genes. Both the pheophytin/quinone and the FeS-type reaction centers are found in phylogenetically distant groups (e.g., a pheophytin/quinone reaction center in *Chloroflexus* and phototrophic members of the Alphaproteobacteria). Even more significantly, a phylogenetic analysis of the amino acid sequences of pheophytin-type reaction center polypeptides from the three different bacterial lineages *Chloroflexaceae*, *cyanobacteria*, and Alphaproteobacteria indicated that the reaction center of *Chloroflexus aurantiacus* is more closely related to that of phototrophic members of the Alphaproteobacteria than to the PSII reaction center of cyanobacteria (Blankenship 1992). Thus the reaction center of *Chloroflexus* must have evolved after (and not prior to) the divergence of the D1/D2 branch from the L/M line of descent. Similarly, additional Baysian analysis suggested that the purple bacterial L-subunit represents the more ancestral than *Chloroflexi* and cyanobacterial type II reaction centers and that the heliobacterial reaction center is the ancestor of type I reaction center proteins (Xiong and Bauer 2002). Another essential component of the photosynthetic apparatus of *Chloroflexus* and green sulfur bacteria are the light-harvesting chlorosomes. Based on amino acid sequence comparison of protein constituents, chlorosomes of both groups have a common evolutionary origin (Wagner-Huber et al. 1988). Similarly, a comparison of the amino acid sequences of the group 1 σ^{70} primary sigma factor also has demonstrated a close relationship to the green sulfur bacteria with respect to this component of the central housekeeping function (Gruber and Bryant 1998). Other features of *Chloroflexus aurantiacus* appear to be unique (like the lipid and carotenoid composition) or ancient (like the hydroxypropionate pathway of CO_2-fixation), such that *Chloroflexus aurantiacus* seems to represent a "chimeric" organism.

Based on the most parsimonious assumption that homodimeric reaction centers are ancestral to heterodimeric ones, the reaction centers of green sulfur bacteria and *Heliobacteriaceae* would resemble most the reaction center of the ancestor of all extant bacteria. It has been hypothesized (Gruber and Bryant 1998) that the reaction center of *Chloroflexus aurantiacus* was acquired by a recent lateral gene transfer event that may have replaced a type I reaction center with a type II reaction center, whereas other features like primary sigma factor or chlorosomes still reflect the common descent of *Chloroflexus* and the green sulfur bacteria. Alternatively, it has been suggested that transfer of the genetic information of the relatively simple chlorosomes occurred after the evolution of the two classes of reaction centers and that the green sulfur bacteria represent a relatively modern evolutionary invention (Stackebrandt et al. 1996).

The presence of two homologous polypeptides in all known reaction centers would suggest a single gene duplication event in an early ancestor of all phototrophic bacteria. As an additional result of the phylogenetic analysis of the amino acid sequences of pheophytin-type reaction center polypeptides from the three different bacterial lineages (*Chloroflexaceae*, *cyanobacteria*, and Alphaproteobacteria; Blankenship 1992), the most likely occurrence of two independent gene duplications is suggested: one leading to the reaction center of PSII in cyanobacteria and green plants (polypeptides D1 and D2) and another to the reaction center of *Chloroflexus* and purple nonsulfur bacteria (polypeptides L and M). Another, third, independent gene duplication has to be assumed during the evolution of the FeS-type reaction center after the cyanobacterial branch split from the *Chlorobiaceae*, *Chloracidobacterium* and *Heliobacteriaceae* that retained the homodimeric form (Homann-Marriott and Blankenship 2011). The reason for the paraphyletic development of the three lineages may be a functional advantage of dimeric reaction centers over monomeric ones. It has been argued that the common ancestor of the type II reaction center most likely encompassed 5 instead of 11 transmembrane helices and that this type represents also the ancestor of the type I (FeS) reaction center based on the different evolutionary origin of some of the FeS clusters in the latter (Homann-Marriott and Blankenship 2011). In fact, independent support for the lateral gene transfer of reaction center genes stems from the discovery of genes encoding photosystem II core protein D1 (Mann et al. 2003) and photosystems I subunits in marine cyanobacterial phages (Sharon et al. 2009).

Yet another evolutionary scenario for photosynthetic reaction centers (Vermaas 1994) has been based on the finding that the sixth membrane-spanning region of the heliobacterial (FeS- or PSI-type) reaction center shows a great similarity to the sixth membrane-spanning region of the CP47 antenna polypeptide of (the quinone-type) PSII, and the preceding N-terminal five hydrophobic regions still show significantly greater similarity to CP47 (and to another PSII antenna protein, CP43) than to the respective portion of PSI. According to this model, an ancestral homodimeric antenna/reaction center complex comprised 11 putative transmembrane regions and contained two quinones and an F_x-type Fe_4S_4 iron-sulfur center. Relatively few modifications may have led to the homodimeric complex of green sulfur bacteria and *Heliobacteriaceae*, whereas a gene duplication event and divergent evolution led to the heterodimeric PSI. As a parallel line of descent, splitting of the ancestral reaction center complex into a reaction center and a separate antenna protein may have occurred. Operon duplication, loss of the FeS, and divergent evolution are assumed to have resulted in two separate lineages. By association with an additional water-splitting enzyme system, PSII was formed. In contrast, the separate antenna polypeptide was lost and replaced by a modified antenna complex (light-harvesting I) during evolution of the reaction center of Proteobacteria and *Chloroflexus*. Significantly, however, this theory does not explain the occurrence of the quinone-type reaction center in these latter two groups, which are phylogenetically very distant. In addition, the combination of a reaction center typical for *Proteobacteria* with an antenna structure characteristic for green sulfur bacteria would still need to be explained by lateral gene transfer of either of the two components.

Based on the obvious discrepancy between the phylogeny of ribosomal RNA and reaction center proteins, the hypothesis of lateral transfer of photosynthesis genes between distantly related groups of bacteria has been put forward. Lateral gene transfer as yet seems to provide the simplest explanation for the distribution pattern of photosynthesis genes within the bacterial radiation (Blankenship 1992; Nagashima et al. 1993, 1997). This holds true particularly if the core of photosynthesis genes is analyzed across the genomes of different phototrophic lineages (Raymond et al. 2002). Such a lateral gene transfer would encompass reaction center structural genes, genes coding for other electron transfer proteins, and genes needed for the biosynthesis of pigments and cofactors. In purple nonsulfur bacteria, the majority of these genes indeed form a single cluster of 46 kb (which does not encompass the genes for the light-harvesting II complex, however; Bauer and Bird 1996; Wellington et al. 1992; Yildiz et al. 1992). The genetic organization may be taken as evidence for lateral gene transfer as the cluster represents only ~1.3 % of the total genome size. It should be mentioned, however, that clustering of most photosynthesis genes may also be due to structural or regulatory constraints. Supporting the latter argument (Yildiz et al. 1992), photosynthesis genes in Alphaproteobacteria are transcriptionally coupled in superoperons involving overlapping transcripts. The particular genetic organization is the prerequisite for adaptation of the cells to changing light intensity (see section ❯ "Genetic Regulation in Response to Light" in this chapter) and oxygen tension (see section ❯ "Genetic Regulation by O_2" in this chapter). Therefore a selective pressure may exist to retain the linkage order and would make the genetic organization of the photosynthesis genes less suitable for phylogenetic inference. Furthermore, the high correlation between the phylogenetic trees for 16S rRNA and cytochrome c in phototrophic members of the Alphaproteobacteria has been taken as evidence that a lateral transfer of photosynthesis genes did not occur at least within this phylogenetic group (Woese et al. 1980). Thus, the presence of reaction centers in aerobic bacteriochlorophyll-containing Alphaproteobacteria may represent an atavistic trait, and the genes coding the reaction center might have been lost frequently during the evolution of aerobic representatives in this group (Stackebrandt et al. 1996). Comparative analyses of whole genomes of photosynthetic bacteria revealed that photosynthesis-specific and photosynthesis-related genes yield highly incongruent phylogenetic trees (Raymond et al. 2002). This lends further support to the hypothesis that the evolution of the photosynthetic apparatus involves rampant lateral gene transfer. Also, the reaction center *pufLM* genes are located on linear plasmids in the aerobic anoxygenic phototrophs *Roseobacter litoralis* and *Sulfitobacter guttiformis*, which would facilitate horizontal transfer of these genes (Pradella et al. 2004)

Early theories that green plant plastids were derived from Chl *b* containing cyanobacteria (known at the time as

"prochlorophytes"; van Valen and Maiorana 1980; Lewin 1981) have found no support in phylogenetics studies. Cyanobacteria containing chlorins other than Chl *a* are clearly not monophyletic, and the monophyletic clade of eukaryotic plastids represents clearly an early evolutionary offshoot of the cyanobacterial clade. A combined phylogenetic and paleontological study indicated that filamentous cyanobacteria capable of cell differentiation (production of heterocysts and akinetes) are monophyletic and may have diverged between 2,450 and 2,100 million years ago (Tomitani et al. 2006).

The present phylogenetic distribution of rhodopsins may be explained by lateral gene transfer among different bacterial lineages (Sharma et al. 2006) and between Archaea and Bacteria (Frigaard et al. 2006).

References

Achtman M, Wagner M (2008) Microbial diversity and the genetic nature of microbial species. Nat Rev Microbiol 6:431–440

Alberti M, Burke DH, Hearst JE (1995) Structure and sequence of the photosynthesis gene cluster. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht/Boston/London, pp 1083–1106

Amesz J (1995) The antenna-reaction center complex of Heliobacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 687–697

Anagnostidis K, Komárek J (1988) Modern approach to the classification system of the cyanophytes. 3. Oscillatoriales. Arch Hydrobiol/Algol Stud 53(Suppl 80):327–472

Angerhofer A, Cogdell RJ, Hipkins MF (1986) A spectral characterization of the light-harvesting pigment-protein complexes from *Rhodopseudomonas acidophila*. Biochim Biophys Acta 848:333–341

Armitage JP (1997) Behavioral responses of bacteria to light and oxygen. Arch Microbiol 168:249–261

Armitage JP, Kelly DJ, Sockett RE (1995) Flagellate motility, behavioral responses and active transport in purple non-sulfur bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 1005–1028

Arnheim K, Oelze J (1983) Differences in the control of bacteriochlorophyll formation by light and oxygen. Arch Microbiol 135:299–304

Asao M, Madigan MT (2010) Taxonomy, phylogeny, and ecology of the helicobacteria. Photosynth Res 104:103–111

Asao M, Takaichi S, Madigan MT (2012) Amino acid-assimilating phototrophic heliobacteria from soda lake environments: *Heliorestis acidaminivorans* sp. nov. and '*Candidatus* Heliomonas lunata'. Extremophiles 16:585–595

Awramik SM (1992) The oldest records of photosynthesis. Photosynth Res 33:75–89

Barry BA, Boerner RJ, de Paula JC (1994) The use of cyanobacteria in the study of the structure and function of photosystem II. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht/Boston/London, pp 217–257

Bauer CE (1995) Regulation of photosynthesis gene expression. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 1221–1234

Bauer CE, Bird TH (1996) Regulatory circuits controlling photosynthesis gene expression. Cell 85:5–8

Bauer CE, Bollivar DW, Suzuki JY (1993) Genetic analyses of photopigment biosynthesis in eubacteria: a guiding light for algae and plants. J Bacteriol 175:3919–3925

Beale SI (1995) Biosynthesis and structures of porphyrins and hemes. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 153–177

Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, VanDover CL, Martinson TA, Plumley GF (2005) An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. Proc Natl Acad Sci 102:9306–9310

Bebout BM, Garcia-Pichel F (1995) UVB-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environm Microbiol 61:4215–4222

Beck H, Hegeman GD, White D (1990) Fatty acid and lipopolysaccharide analyses of three *Heliobacterium* spp. FEMS Microbiol Lett 69:229–232

Beer-Romero P, Favinger JL, Gest H (1988) Distinctive properties of bacilliform photosynthetic heliobacteria. FEMS Microbiol Lett 49:451–454

Béjà O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB, Gates CM, Feldman RA, Spudich JL, Spudich EN, DeLong EF (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science 289:1902–1906

Béjà O, Spudich EN, Spudich JL, Leclerc M, DeLong EF (2001) Proteorhodopsin phototrophy in the ocean. Nature 411:786–789

Béjà O, Suzuki MT, Heidelberg JF, Nelson WC, Preston CM, Hamada T, Eisen JA, Fraser CM, DeLong ED (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. Nature 415:630–633

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

Bibby TS, Mary I, Nield J, Partensky F, Barber J (2003) Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature 424:1051–1054

Biebl H, Drews G (1969) Das in vivo Spektrum als taxonomisches Merkmal bei Untersuchungen zur Verbreitung der Athiorhodaceae. Zbl Bakt Abt II 123:425–452

Biel AJ (1986) Control of bacteriochlorophyll accumulation by light in *Rhodobacter capsulatus*. J Bacteriol 168:655–659

Biel AJ (1995) Genetic analysis and regulation of bacteriochlorophyll biosynthesis. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 1125–1134

Blankenship RE (1992) Origin and early evolution of photosynthesis. Photosynth Res 33:91–111

Blankenship RE, Olson JM, Miller M (1995) Antenna complexes from green photosynthetic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 399–435

Bobe FW, Pfennig N, Swanson KL, Smith KM (1990) Red shift of absorption maxima in Chlorobiaceae through enzymic methylation of their antenna bacteriochlorophylls. Biochemistry 29:4340–4348

Boomer SM, Lodge DP, Dutton BE, Pierson B (2002) Molecular characterization of novel red green nonsulfur bacteria from five distinct hot spring communities in Yellowstone National Park. Appl Environ Microbiol 68:346–355

Borrego CM, Garcia-Gil LJ (1995) Rearrangement of light harvesting bacteriochlorophyll homologues as a response of green sulfur bacteria to low light intensities. Photosynth Res 45:21–30

Bowyer JR, Hunter CN, Ohnishi T, Niederman RA (1985) Photosynthetic membrane development in *Rhodopseudomonas sphaeroides*. J Biol Chem 260:3295–3304

Boxer SG (1992) Some speculations concerning the evolution of photosynthetic function. Photosynth Res 33:113–119

Brinkhoff T, Giebel H-A, Simon M (2008) Diversity, ecology, and genomics of the *Roseobacter clade*: a short overview. Arch Microbiol 189:531–539

Brocks JJ, Love GD, Summons RE, Knoll AH, Logan GA, Bowden SA (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. Nature 437:866–870

Brune DC (1989) Sulfur oxidation by phototrophic bacteria. Biochim Biophys Acta 975:189–221

Bryant DA (1982) Phycoerythrocyanin and phycoerythrin: properties and occurrence in cyanobacteria. J Gen Microbiol 128:835–844

Bryant DA, Garcia Costas AM, Maresca JA, Gomez Maqueo Chew A, Klatt CG, Bateson MM, Tallon LJ, Hostetler J, Nelson WC, Heidelberg JF, Ward DM (2007) *Candidatus* Chloracidobacterium thermophilum: an aerobic phototrophic Acidobacterium. Science 317:523–526

Büdel B, Karsten U, Garcia-Pichel F (1997) Ultraviolet-absorbing scytonemin and mycosporine-like amino acids in exposed. rock-inhabiting cyanobacterial lichens. Oecologia 112:165–172

Buick R (1992) The antiquity of oxygenic photosynthesis: evidence from stromatolites in sulphate-deficient Archaean lakes. Science 255:74–77

Buick R (2008) When did oxygenic photosynthesis evolve? Phil TRans R Soc B 363:2731–2743
Burke DH, Hearst JE, Sidow A (1993) Early evolution of photosynthesis: clues from nitrogenase and chlorophyll iron proteins. Proc Natl Acad Sci USA 90:7134–7138
Caldwell DE, Tiedje JM (1975) The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. Can J Microbiol 21:377–385
Camacho A, Garcia-Pichel F, Vicente E, Castenholz RW (1996) Adaptation to sulfide and to the underwater light field in three cyanobacterial isolates from lake Arcas (Spain). FEMS Microbiol Ecol 21:293–301
Campbell L, Vaulot D (1993) Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Res 40:2043–2060
Campbell L, Nolla HA, Vaulot D (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnol Oceanogr 39:954–961
Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ (1977) Trichodesmium, a globally significant cyanobacterium. Science 276:1221–1229
Carr NG, Mann NH (1994) The oceanic cyanobacterial picoplankton. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht/Boston/London, pp 27–48
Castenholz RW (1982) Motility and taxes. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific, Oxford, pp 413–440
Castenholz RW (2001a) Phylum BX. Oxygenic photosynthetic bacteria. In: Garrity GM (chief ed) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 473–599
Castenholz RW (2001b) Class I. "Chloroflexi". In: Garrity GM (chief ed) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, pp 427–446
Castenholz RW, Garcia-Pichel F (1999) Cyanobacterial responses to UV-radiation. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer, Dordrecht, p 704
Castenholz RW, Garcia-Pichel F (2000) Cyanobacterial responses to UV-radiation. In: Potts M, Whitton BA (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 591–611
Castenholz RW, Pierson BK (1995) Ecology of thermophilic anoxygenic phototrophs. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 87–103
Castenholz RW, Utkilen HC (1984) Physiology of sulfide tolerance in a thermophilic *Oscillatoria*. Arch Microbiol 138:299–305
Castenholz RW, Bauld J, Jørgensen BB (1990) Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium* sp. FEMS Microbio Ecol 74:325–336
Chen M, Schliep M, Willows RD, Cai Z-L, Neilan BA, Scheer H (2010) A red-shifted chlorophyll. Science 329:1318–1319
Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334:340–343
Clarke KJ, Finlay BJ, Vicente E, Lloréns H, Miracle MR (1993) The complex life-cycle of a polymorphic prokaryote epibiont of the photosynthetic bacterium Chromatium weissei. Arch Microbiol 159:498–505
Cohen Y, Jørgensen BB, Padan E, Shilo M (1975) Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Nature 257:489–492
Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407
Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2009) The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucl Acids Res 37(Suppl 1):D141–D145
Coolen MJL, Overmann J (1998) Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. Appl Environ Microbiol 64:4513–4521
Cotrell MT, Mannino A, Kirchman DL (2006) Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic bight and the North Pacific Gyre. Appl Environ Microbiol 72:557–564
Crowe SA, Jones CA, Katsev S, Magen C, O'Neill AH, Sturm A, Canfield DE, Haffner GD, Mucci A, Sundby B, Fowle DA (2008) Photoferrotrophs thrive in an Archean ocean analogue. Proc Natl Acad Sci USA 105:15938–15943
Csotonyi JT, Swiderski J, Stackebrandt E, Yurkov VV (2008) Novel halophilic aerobic anoxygenic phototrophs from a Canadian hypersaline spring system. Extremophiles 12:529–539
Davies JH, Davies DR (2010) Earth's Surface heat flux. Solid Earth 1:5–24
Davis SJ, Vener AV, Vierstra RD (1999) Bacteriophytochromes: phytochrome-like photoreceptors from nonphotosynthetic eubacteria. Science 286:2517–2520
De Ruyter, YS, Fromme P (2008) Molecular structure of the photosynthetic Apparatus. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Horizon Scientific Press, London, pp 217–270
De Wit R, van Gemerden H (1987a) Chemolithotrophic growth of the phototrophic sulfur bacterium Thiocapsa roseopersicina. FEMS Microbiol Ecol 45:117–126
De Wit R, van Gemerden H (1987b) Oxidation of sulfide to thiosulfate by Microcholeus chtonoplastes. FEMS Microbiol Ecol 45:7–13
De Wit R, van Gemerden H (1990a) Growth and metabolism of the purple sulfur bacterium Thiocapsa roseopersicina under combined light/dark and oxic/anoxic regimens. Arch Microbiol 154:459–464
De Wit R, van Gemerden H (1990b) Growth of the phototrophic purple sulfur bacterium Thiocapsa roseopersicina under oxic/anoxic regimens in the light. FEMS Microbiol Ecol 73:69–76
De Wit R, van Boekel WHM, van Gemerden H (1988) Growth of the cyanobacterium *Microcoleus chtonoplastes* on sulfide. FEMS Microbiol Ecol 53:203–209
De Wit R, Jonkers HM, van den Ende FP, van Gemerden H (1989) In situ fluctuations of oxygen and sulphide in marine microbial sediment ecosystems. Neth J Sea Res 23:271–281
Dierstein R (1984) Synthesis of pigment-binding protein in toluene-treated *Rhodopseudomonas capsulata* and in cell-free systems. Eur J Biochem 138:509–518
Dietrich G, Kalle K, Krauss W, Siedler G (1975) Allgemeine Meereskunde. Gebrüder Bornträger, Berlin/Stuttgart, p 593
Drews G, Golecki JR (1995) Structure, molecular organization, and biosynthesis of membranes of purple bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 231–257
DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen (2012) http://www.dsmz.de/bacterial-diversity/bacterial-nomenclature-up-to-date.html
Dubinina GA, Gorlenko VM (1975) New filamentous photosynthetic green bacteria containing gas vacuoles. Mikrobiologiya 44:511–517
Ehlers K, Oster G (2012) On the mysterious propulsion of *Synechococcus*. PLoS One 7:e36081
Ehling-Schulz M, Bilger W, Scherer S (1997) UVB induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium Nostoc commune. J Bacteriol 179:1940–1945
Eichler B, Pfennig N (1986) Characterization of a new platelet-forming purple sulfur bacterium *Amoebobacter pedioformis* sp. nov. Arch Microbiol 146:295–300
Eisen JA, Nelson KE, Paulsen IT, Heidelberg JF, Wu M, Dodson RJ, Deboy R et al (2002) The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. Proc Natl Acad Sci USA 99:9509–9514
Eisenreich W, Strauß G, Werz U, Fuchs G, Bacher A (1993) Retrobiosynthetic analysis of carbon fixation in the phototrophic eubacterium Chloroflexus aurantiacus. Eur J Biochem 215:619–632
Essen L-O, Oesterhelt D (1998) A cold break for photoreceptors. Nature 392:131–133
Esteve I, Gaju N, Mir J, Guerrero R (1992) Comparison of techniques to determine the abundance of predatory bacteria attacking Chromatiaceae. FEMS Microbiol Ecol 86:205–211
Euzéby JP (2012) LPSN, List of Prokaryotic names with Standing in Nomenclature. http://www.bacterio.cict.fr/
Evans WR, Fleischman DE, Calvert HE, Pyati PV, Alter GM, Rao NSS (1990) Bacteriochlorophyll and photosynthetic reaction centers in *Rhizobium* strain BTA:1. Appl Environ Microbiol 56:3445–3449
Fenchel T, Bernard C (1993a) A purple protist. Nature 362:300
Fenchel T, Bernard C (1993b) Endosymbiotic purple non-sulphur bacteria in an anaerobic ciliated protozoon. FEMS Microbiol Lett 110:21–25

Ferris MJ, Ruff-Roberts AL, Kopczynski ED, Bateson MM, Ward DM (1996) Enrichment culture and microscopy conceal diverse thermophilic Synechococcus populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 62:1045–1050

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240

Fleischman DE, Evans WR, Miller IM (1995) Bacteriochlorophyll-containing *Rhizobium* species. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 123–136

Foy RH, Gibson CE (1982) Photosynthetic characteristics of planktonic blue-green algae: changes in photosynthetic capacity and pigmentation of *Oscillatoria redekei* Van Goor under high and low light. Br Phycol 17:183–193

Friedmann EI (1982) Endolithic microorganisms in the Antactc Cold Desert. Science 215:1045–1053

Frigaard N-U, Takaishi S, Hirota M, Shimada K, Matsuura K (1997) Quinones in chlorosomes of green sulfur bacteria and their role in the redox-dependent fluorescence studied in chlorosome-like bacteriochlorophyll aggregates. Arch Microbiol 167:343–349

Frigaard N-U, Gomez Maqueo Chew A, Li H, Maresca JA, Bryant DA (2003) *Chlorobium tepidum*: insights into the structure, physiology, and metabolism of a green sulfur bacterium derived from the complete genome sequence. Photosynth Res 78:93–117

Frigaard N-U, Maresca JA, Yunker CE, Jones AD, Bryant DA (2004) Genetic manipulation of carotenoid biosynthesis in the green sulfur bacterium *Chlorobium tepidum*. J Bacteriol 186:5210–5220

Frigaard N-U, Martinez A, Mincer TJ, DeLong EF (2006) Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. Nature 439:847–850

Fröstl JM, Overmann J (1998) Physiology and tactic response of the phototrophic consortium "*Chlorochromatium aggregatum*". Arch Microbiol 169:129–135

Fründ C, Cohen Y (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl Environ Microbiol 58:70–77

Fry B (1986) Sources of carbon and sulfur nutrition for consumers in three meromictic lakes of New York state. Limnol Oceanogr 31:79–88

Ganapathy S, Oostergetel GT, Wawrzyniak PK, Reus M, Gomez Maqueo Chew A, Buda F, Boekema EJ, Bryant DA, Holzwarth AR, de Groot HJM (2009) Alternating *syn-anti* bacteriochlorophylls form concentric helical nanotubes in chlorosomes. Proc Natl Acad Sci USA 106:8525–8530

Gao Q, Garcia-Pichel F (2011) Microbial Ultraviolet Sunscreens. Nature Reviews Microbiol 9:791–802

Garcia Costas AM, Liu Z, Tomsho LP, Schuster SC, Ward DM, Bryant DA (2012a) Complete genome of *Candidatus* Chloracidobacterium thermophilum, a chlorophyll-based photoheterotroph belonging to the phylum Acidobacteria. Environ Microbiol 14:177–190

Garcia Costas AM, Tsukatani Y, Rijpstra IC, Schouten S, Welander PV, Summons RE, Bryant DA (2012b) Identification of the bacteriochlorophylls, carotenoids, quinones, lipids, and hopanoids of "*Candidatus* Chloracidobacterium thermophilum". J Bacteriol 194:1158–1168

Garcia-Pichel F (1994) A model for internal self-shading in planktonic microorganisms and its implications for the usefulness of sunscreens. Limnol Ocean 39:1704–1717

Garcia-Pichel F (1995) A scalar irradiance fiber-optic microprobe for the measurement of ultraviolet radiation at high spatial resolution. Photochem Photobiol 61:248–254

Garcia-Pichel F (1999) Cyanobacteria. In: Lederberg J (ed) Encyclopedia of microbiology. Academic, San Diego

Garcia-Pichel F (2008) Molecular ecology and environmental genomics. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Horizon Scientific Press, London, pp 59–88

Garcia-Pichel F, Bebout B (1996) The penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. Mar Ecol Prog Ser 131:257–262

Garcia-Pichel F, Belnap J (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. J Phycol 32:774–782

Garcia-Pichel F, Castenholz RW (1990) Comparative anoxygenic photosynthetic capacity in 7 strains of a thermophilic cyanobacterium. Arch Microbiol 153:344–355

Garcia-Pichel F, Castenholz RW (1994) On the significance of solar ultraviolet radiation for the ecology of microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 77–84

Garcia-Pichel F, Castenholz RW (1999) Photomovements of microorganisms in sediments and soils. In: Häder D-P (ed) Photomovements. Elsevier, Amsterdam

Garcia-Pichel F, Wojciechowski MF (2009) The Evolution of a Capacity to Build Supra-Cellular Ropes Enabled Filamentous Cyanobacteria to Colonize Highly Erodible Substrates. PLoS One 4:e7801

Garcia-Pichel F, Mechling M, Castenholz RW (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl Environ Microbiol 60:1500–1511

Garcia-Pichel F, Prufert-Bebout L, Muyzer G (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl Environ Microbiol 62:3284–3291

Garcia-Pichel F, Nübel U, Muyzer G (1998) The phylogeny of unicellular, extremely halotolerant cyanobacteria. Arch Microbiol 169:469–482

Garcia-Pichel F, López-Cortés A, Nübel U (2001) Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Appl Environ Microbiol 67:1902–1910

Garcia-Pichel F, Belnap J, Neuer S, Schanz F (2003) Estimates of global cyanobacterial biomass and its distribution. Arch Hydrobiol Suppl 148:213–227

Gates DM (1962) Energy exchange in the biosphere. Harper & Row, New York, p 151

Geitler L (1932) Cyanophyceae. Akademische Verlagsgesellschaft, Leipzig, p 1035

Gest H (1993) Photosynthetic and quasi-photosynthetic bacteria. FEMS Microbiol Lett 112:1–6

Gest H, Favinger JL (1983) *Heliobacterium chlorum*: an anoxygenic brownish-green photosynthetic bacterium containing a new form of bacteriochlorophyll. Arch Microbiol 136:11–16

Gest H, Schopf JW (1983) Biochemical evolution of anaerobic energy conversion: the transition from fermentation to anoxygenic photosynthesis. In: Schopf JW (ed) Earth's earliest biosphere. Princeton University Press, Princeton, pp 135–148

Gich F, Garcia-Gil J, Overmann J (2001) Unknown and phylogenetically diverse members of the Green Nonsulfur Bacteria are indigenous to freshwater lakes. Arch Microbiol 177:1–10

Giovanonni SJ, Turner S, Olsen GJ, Barns S, Lane DJ, Pace NR (1988) Evolutionary relationships among cyanobacteria and green chloroplasts. J Bacteriol 170:3548–3592

Glaeser J, Overmann J (1999) Selective enrichment and characterization of *Roseospirillum parvum*, gen. nov. and sp. nov., a new purple nonsulfur bacterium with unusual light absorption properties. Arch Microbiol 171:405–416

Glaeser J, Overmann J (2003) The significance of organic carbon compounds for in situ metabolism and chemotaxis of phototrophic consortia. Environ Microbiol 5:1053–1063

Glaeser J, Overmann J (2004) Biogeography, evolution and diversity of the epibionts in phototrophic consortia. Appl Environ Microbiol 70:4821–4830

Glaeser J, Baneras L, Rütters H, Overmann J (2002) Novel bacteriochlorophyll *e* structures and species-specific variability of pigment composition in green sulfur bacteria. Arch Microbiol 177:475–485

Göbel F (1978) Quantum efficiencies of growth. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 907–925

Goericke R, Welschmeyer NA (1993) The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. Deep-Sea Res 40:2283–2294

Golbeck JH (1994) Photosystem I in cyanobacteria. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht/Boston/London, pp 319–360

Golden SS (1994) Light responsive gene expression and the biochemistry of photosystem II reaction center. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht, pp 693–714

Golecki JR, Oelze J (1987) Quantitative relationship between bacteriochlorophyll content, cytoplasmic membrane structure and chlorosome size in Chloroflexus aurantiacus. Arch Microbiol 148:236–241

Gomez Maqueo Chew A, Frigaard NU, Bryant DA (2007) Bacteriochlorophyllide c C-8^2 and C-12^1 methyltransferases are essential for adaptation to low light in *Chlorobaculum tepidum*. J Bacteriol 189:6176–6184

Gomez-Consarnau L, Gonzalez JM, Coll-Llado M, Gourdon P, Pascher T, Neutze R, Pedros-Alio C, Pinhassi J (2007) Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. Nature 445:210–213

Gorlenko VM, Krasilánikova EN, Kikina OG, Tatarinova NY (1979) The new motile purple sulfur bacterium *Lamprobacter modestohalophilus* nov. gen., nov. sp. with gas vacuoles. Izv Akad Nauk S S S R Ser Biol 5:755–767

Gottschalk G (1986) Bacterial metabolism. Springer, New York, p 359

Granick S (1965) Evolution of heme and chlorophyll. In: Bryson V, Vogel HJ (eds) Evolving genes and proteins. Academic, New York, pp 67–88

Gregersen LH, Bryant DA, Frigaard N-U (2011) Mechanisms and evolution of oxidative sulfur metabolism in green sulfur bacteria. Front Microbiol 2:116

Griffin BM, Schott J, Schink B (2007) Nitrite, an electron donor for anoxygenic photosynthesis. Science 316:870

Grossmann AR, Bhaya D, He Q (2001) Tracking the light environment by cyanobacteria and the dynamic nature of light harvesting. J Biol Chem 286:1149–11452

Grote M, O'Malley MA (2011) Enlightening the life sciences: the history of halobacterial and microbial rhodopsin research. FEMS Microbiol Rev 35:1082–1099

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

Guyoneaud R, Matheron R, Baulaigue R, Podeur K, Hirschler A, Caumette P (1996) Anoxygenic phototrophic bacteria in eutrophic coastal lagoons of the French Mediterranean and Atlantic Coasts. Hydrobiologia 329:33–43

Haberl H, Erb KH, Krausmann F, Gaube V, Bondeau A, Plutzar C, Gingrich S, Lucht W, Fischer-Kowalski M (2007) Quantifying and mapping the human appropriation of net primary production in earth's terrestrial ecosystems. Proc Natl Acad Sci USA 104:12942–12945

Häder D-P (1987) Photosensory behavior in prokaryotes. Microbiol Rev 51:1–21

Hanada S, Takaishi S, Matsuura K, Nakamura K (2002) *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. Int J Syst Evol Microbiol 52:187–193

Hansen TA, van Gemerden H (1972) Sulfide utilization by purple nonsulfur bacteria. Arch Mikrobiol 86:49–56

Hansen TA, Veldkamp H (1973) *Rhodopseudomonas sulfidophila* nov. sp., a new species of the purple nonsulfur bacteria. Arch Microbiol 92:45–58

Harashima K, Shiba T, Totsuka T, Simidu U, Taga N (1978) Occurrence of bacteriochlorophyll a in a strain of an aerobic heterotrophic bacterium. Agricult Biol Chem Tokyo 42:1627–1628

Harder W, van Dijken JP (1976) Theoretical considerations on the relation between energy production and growth of methane-utilizing bacteria. In: Schlegel HG, Gottschalk G, Pfennig N (eds) Symposium on microbial production and utilization of gases (H_2, CH4, CO). E Goltze KG, Göttingen, pp 403–418

Hartman H (1992) Conjectures and reveries. Photosynth Res 33:171–176

Hartmann R, Sickinger H-D, Oesterhelt D (1980) Anaerobic growth of halobacteria. Proc Natl Acad Sci USA 77:3821–3825

Hayes JM, Kaplan IR, Wedeking KW (1983) Precambrian organic geochemistry, preservation of the record. In: Schopf JW (ed) Earth's earliest biosphere. Princeton University Press, Princeton, pp 93–134

Heising S, Richter L, Ludwig W, Schink B (1999) *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a "Geospirillum" sp. strain. Arch Microbiol 172:116–124

Hellingwerf KJ (2002) The molecular basis of sensing and responding to light in microorganisms. Ant van Leeuwenhoek 81:51–59

Hess WR (2008) Comparative genomics of marine cyanobacteria and their phages. In: Herrero A, Flores E (eds) The cyanobacteria. Molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 89–116

Hirabayashi H, Ishii T, Takaishi S, Inoue K, Uehara K (2004) The role of carotenoids in the photoadaptation of the brown-colored sulfur bacterium *Chlorobium phaeobacteroides*. Photochem Photobiol 79:280–285

Hofman PAS, Veldhuis MJW, van Gemerden H (1985) Ecological significance of acetate assimilation by *Chlorobium phaeobacteroides*. FEMS Microbiol Ecol 31:271–278

Holo H (1989) Chloroflexus aurantiacus secretes 3-hydroxypropionate, a possible intermediate in the assimilation of CO_2 and acetate. Arch Microbiol 151:252–256

Homann-Marriott MF, Blankenship RE (2011) Evolution of photosynthesis. Annu Rev Plant Biol 62:515–548

Hoppe W, Lohmann W, Markl H, Ziegler H (1983) Biophysik. Springer, Berlin/Heidelberg/New York

Hudnell HK (2008) Cyanobacterial harmful algal blooms: state of the science and research needs. Springer, New York, pp 950

Hübschmann T, Jorissen HJMM, Börner T, Gärtner W, Tandeau de Marsac N (2001) Phosphorylation of proteins in the light-dependent signalling pathway of a filamentous cyanobacterium. Eur J Biochem 268:3383–3389

Iino T, Mori K, Uchino Y, Naka-gawa T, Harayama S, Suzuki K (2010) *Ignavibacterium album* gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of *Ignavibacteria classis* nov., for a novel lineage at the periphery of green sulfur bacteria. Int J Syst Evol Microbiol 60:1376–1382

Imhoff JF (2003) Phylogenetic taxonomy of the family Chlorobiaceae on the basis of 16S rRNA and fmo (Fenna–Matthews–Olson protein) gene sequences. Int J Syst Evol Microbiol 53:941–951

Imhoff JF, Caumette P (2004) Recommended standards for the description of new species of anoxygenic phototrophic bacteria. Int J Syst Evol Microbiol 54:1415–1421

Imhoff JF, Trüper HG (1989) Purple nonsulfur bacteria. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, pp 1658–1682

Imhoff JF, Tindall BJ, Grant WD, Trüper HG (1981) *Ectothiorhodospira vacuolata* sp. nov., a new phototrophic bacterium from soda lakes. Arch Microbiol 130:238–242

International Committee on Systematics of Prokaryotes (2007) Subcommittee on the taxonomy of phototrophic bacteria. Int J Syst Evol Microbiol 57:1169–1171

Jannasch HW (1989) Chemosynthetically sustained ecosystems in the deep sea. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer, New York, pp 147–166

Jeon J, Dobryinin AV (2005) Polymer confinement and bacterial gliding motility. Eur Phys J E 17:361–372

Jørgensen BB (1982) Ecology of the bacteria of the sulphur cycle with special reference to anoxic-oxic interface environments. Phil Trans Royal Soc Lond B 298:543–561

Jorgensen BB, Cohen Y (1977) Solar Lake (Sinai). 5. The sulfur cycle of the benthic microbial mats. Limnol Oceanogr 22:657–666

Jørgensen BB, Des Marais DJ (1986) A simple fiber-optic microprobe for high resolution light measurements: applications in marine sediments. Limnol Oceanogr 31:1374–1383

Jørgensen BB, Des Marais DJ (1988) Optical properties of benthic photosynthetic communities: fiber-optic studies of cyanobacterial mats. Limnol Oceanogr 33:99–113

Jørgensen BB, Kuenen JG, Cohen Y (1979) Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol Oceanogr 24:799–822

Kämpf C, Pfennig N (1986) Chemoautotrophic growth of Thiocystis violacea, Chromatium gracile and C. vinosum in the dark at various O_2-concentrations. J Basic Microbiol 26:517–531

Kana T, Gilbert PM, Goericke R, Welschmeyer NA (1988) Zeaxanthin and beta-carotene in Synechococcus WH7803 respond differently to irradiation. Limnol Oceanogr 33:1623–1627

Karr EA, Sattley WM, Jung DO, Madigan MT, Achenbach LA (2003) Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. Appl Environ Microbiol 69:4910–4914

Keppen OI, Baulina OI, Kondratieva EN (1994) Oscillochloris trichoides neotype strain DG-6. Photosynthesis Res 41:29–33

Keppen OI, Tourova TP, Kuznetsov BB, Ivanovsky RN, Gorlenko VM (2000) Proposal of *Oscillochloridaceae* fam. nov. on the basis of a phylogenetic analysis of the filamentous anoxygenic phototrophic bacteria, and emended

description of *Oscillochloris* and *Oscillochloris trichoides* in comparison with further new isolates. Int J Syst Evol Microbiol 50:1529–1537

Kimble LK, Mandelco L, Woese CR, Madigan MT (1995) *Heliobacterium modesticaldum*, sp. nov., a thermophilic heliobacterium of hot springs and volcanic soils. Arch Microbiol 163:259–267

Kirk JTO (1983) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, p 401

Klappenbach JA, Pierson BK (2004) Phylogenetic and physiological characterization of a filamentous anoxygenic photoautotrophic bacterium 'Candidatus Chlorothrix halophila' gen. nov., sp. nov., recovered from hypersaline microbial mats. Arch Microbiol 181:17–25

Knoll A (2008) Cyanobacteria and Earth's history. In: The cyanobacteria: molecular biology, genomics and evolution, A. Herrero & E. Flores (Eds), Horizon Sci.Press, London. pp.1–20

Kok B (1973) Photosynthesis. In: Gibbs M, Hollaender A, Kok B, Krampitz LO, San Pietro A (eds) Proceedings of the workshop on bio-solar conversion. National Science Foundation, Bethesda

Komárek J, Anagnostidis K (1989) Modern approach to the classification system of cyanophytes. 4. Nostocales. Arch Hydrobiol/Algol Stud 56(Suppl 823):247–345

Kømpf C, Pfennig N (1980) Capacity of Chromatiaceae for chemotrophic growth. Specific respiration rates of Thiocystis violacea and Chromatium vinosum. Arch Microbiol 127:125–135

Kondratieva EN (1979) Interrelation between modes of carbon assimilation and energy production in phototrophic purple and green bacteria. In: Quale JR (ed) Microbial biochemistry. University Park Press, Baltimore, pp 117–175

Konopka A, Brock TD, Walsby AE (1978) Buoyancy regulation by planktonic blue-green algae in Lake Mendota. Wisconsin Arch Hydrobiol 83:524–537

Kopp G, Lean J (2011) A new, lower value of total solar irradiance: evidence and climate significance. Geophys Res Lett 38:L01706

Kromkamp JC, Mur LR (1984) Buoyant density changes in the cyanobacterium Microcystis aeruginosa due to changes in the cellular carbohydrate content. FEMS Microbiol Lett 25:105–109

Krutschel C, Castenholz RW (1998) The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypesaline waters. FEMS Microbiol Ecol 27:53–72

Kühl M, Jørgensen BB (1992) Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. Limnol Oceanogr 37:1813–1823

Lancaster CRD, Michel H (1996) Three-dimensional structures of photosynthetic reaction centers. Photosynth Res 48:65–74

Lang AS, Beatty JT (2006) Importance of widespread gene transfer agent genes in α-proteobacteria. Trends Microbiol 15:54–62

Lascelles J (1978) Regulation of pyrrole synthesis. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 795–808

Lassen C, Plough H, Jørgensen BB (1992) A fiber-optic scalar irradinace microsensor: application for spectral light measurements in sediments. FEMS Microbiol Ecol 86:247–254

Lewin RA (1981) Prochloron and the theory of symbiogenesis. Ann N Y Acad Sci 361:325–329

Lewin RA, Withers NW (1975) Extraordinary pigment composition of a prokaryotic alga. Nature 256:735–737

Ley RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72:3685–3695

Lindholm T, Weppling K, Jensen HS (1985) Stratification and primary production in a small brackish lake studied by close-interval siphon sampling. Verh Internat Verein Limnol 22:2190–2194

Liu H, Nolla HA, Campbell L (1997) *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. Aquat Microb Ecol 12:39–47

Liu Z, Frigaard N-F, Vogl K, Iino T, Kosako Y, Overmann J, Bryant DA (2012a) Complete genome of *Ignavibacterium album*, a metabolically versatile, flagellated, facultative anaerobe from the phylum Chlorobi. Frontiers Evol Genomic Microbiol 3:185

Liu Z, Klatt C, Ludwig M, Rusch DB, Jensen SI, Kühl M, Ward DM, Bryant DA (2012b) 'Candidatus Thermochlorobacter aerophilum:' an aerobic chlorophotoheterotrophic member of the phylum Chlorobi defined by metagenomics and metatranscriptomics. ISME J. doi:10.1038/ismej.2012.24

Loach PA, Parkes-Loach PS (1995) Structure-function relationships in core light-harvesting complexes (LHI) as determined by characterization of the structural subunit and by reconstitution experiments. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 437–471

Los DA, Suzuki I, Zinchencko V, Murata N (2008) Stress resposes comparative genomics of marine cyanobacteria and their phages. In: Herrero A, Flores E (eds) The Cyanobacteria. Molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 89–116

Madigan MT (1992) The family *Heliobacteriaceae*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer, New York, pp 1982–1992

Madigan MT, Ormerod JG (1995) Taxonomy, physiology and ecology of Heliobacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 17–30

Madigan MT, Takigiku R, Lee RG, Gest H, Hayes JM (1989) Carbon isotope fractionation by thermophilic phototrophic sulfur bacteria: evidence for autotrophic growth in natural populations. Appl Environ Microbiol 55:639–644

Mague TH (1977) Ecological aspects of dinitrogen fixation by blue-green algae. In: Hardy RWF, Gibson AH (eds) A treatise on dinitrogen fixation. Wiley, New York, pp 85–140

Mann NH, Cook A, Millard A, Bialey S, Clokie M (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424:741

Marschall E, Jogler M, Henssge U, Overmann J (2010) Large scale distribution and activity patterns of an extremely low-light adapted population of green sulfur bacteria in the Black Sea. Environ Microbiol 12:1348–1362

Mas J, Pedrós-Alió C, Guerrero R (1990) In situ specific loss and growth rates of purple sulfur bacteria in Lake Cisó. FEMS Microbiol Ecol 73:271–281

Masamoto K, Furukawa KI (1997) Accumulation of zeaxanthin in the cell of the cyanobacterium *Synechococcus* sp. strain PCC7942 grown under high irradiance. J Plant Physiol 151:257–261

Masuda S, Bauer CE (2002) AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in *Rhodobacter sphaeroides*. Cell 110:613–623

Matthijs HCP, van der Staay GWM, Mur LR (1994) Prochlorophytes: the "other" cyanobacteria? In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht, pp 49–64

Mauzerall D (1992) Light, iron, Sam Granick and the origin of life. Photosynth Res 33:163–170

McConnell MD, Koop R, Vasil'ev S, Bruce D (2002) Regulation of the distribution of chlorophyll and phycobilin-absorbed excitation energy in cyanobacteria. A structure-based model for the light state transition. Plant Physiol 103:1201–1212

Menendez C, Bauer Z, Huber H, Gad'on N, Stetter K-O, Fuchs G (1999) Presence of acetyl coenzyme A (CoA) carboxylase and propionyl-CoA carboxylase in autotrophic Crenarchaeota and indication for operation of a 3-hydroxypropionate cycle in autotrophic carbon fixation. J Bacteriol 181:1088–1098

Miller SR, Augustine S, Olson TL, Blankenship RE, Selker J, Wood AM (2005) Discovery of a free-living chlorophyll *d*-producing cyanobacterium with a hybrid proteobacterial/cyanobacterial small-subunit rRNA gene. Proc Natl Acad Sci USA 102:850–855

Millie DF, Ingram DA, Dionigi CP (1990) Pigment and photosynthetic responses of *Oscillatoria aghardii* (Cyanophyta) to photon flux and spectral quality. J Phycol 26:660–666

Miyachi S, Strassdat K, Miyashita H, Senger H (1997) Quantum requirement of photosynthesis in the primarily Chlorophyll d containing prokaryote Acaryochloris marina. Z Naturforsch C-A 52:636–638

Miyashita H, Ikemoto H, Kurano N, Adachi K, Chihara M, Miyachi S (1996) Chlorophyll d as a major pigment. Nature 383:402

Moezelaar R, Stal LJ (1994) Anaerobic dark energy generation in the mat-building cyanobacterium *Microcoleus chthonoplastes*. In: Stal LJ, Caumette P (eds) Microbial mats. Springer, New York, pp 273–278

Mojzsis SJ, Arrhenius G, Mckeegan KD, Harrison TM, Nutman AP, Friend CRL (1996) Evidence for life on Earth before 3800 million years ago. Nature 383:55–59

Montaño GA, Bowen BP, LaBelle JT, Woodbury NW, Pizziconi VB, Blankenship RE (2003) Characterization of *Chlorobium tepidum* chlorosomes: a calculation of bacteriochlorophyll *c* per chlorosome and oligomer modeling. Biophys J 85:2560–2565

Montesinos ML, Herrero A, Flores E (1997) Amino acid transport in taxonomically diverse cyanobacteria and identification of two genes encoding elements of a neutral aminoacid permease putatively involved in recapture of leaked hydrophobic amino acids. J Bacteriol 179:853–862

Moore LR, Rocap G, Chisholm SW (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393:464–467

Mulkidjanian AY, Koonin EV, Makarova KS, Mekhedov SL, Sorokin A, Wolf YI, Dufresne A, Partensky F, Burd H, Kaznadzey D et al (2006) The cyanobacterial genome core and the origin of photosynthesis. Proc Natl Acad Sci USA 103:13126–13131

Munoz R, Yarza P, Ludwig W, Euzéby J, Amann R, Schleifer KH, Glöckner FO, Rosselló-Móra R (2011) Release LTPs104 of the all-species living tree. Syst Appl Microbiol 34:169–170

Musat N, Halm H, Winterholler B, Hoppe P, Peduzzi S, Hillion F, Horreard F, Amann R, Jørgensen BB, Kuypers MMM (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. Proc Natl Acad Sci USA 105:17861–17866

Nagashima KVP, Shimada K, Matsuura K (1993) Phylogenetic analysis of photosynthesis genes of Rhodocyclus gelatinosus: possibility of horizontal gene transfer in purple bacteria. Photosynth Res 36:185–191

Nagashima KVP, Hiraishi A, Shimada K, Matsuura K (1997) Horizontal transfer of genes coding for the photosynthetic reaction centers of purple bacteria. J Mol Evol 45:131–136

Nagashima S, Shimada K, Matsuura K, Nagashima KVP (2002) Transcription of three sets of genes coding for the core light-harvesting proteins in the purple sulfur bacterium, *Allochromatium vinosum*. Photosynth Res 74:269–280

Nelissen B, van de Peer Y, Wilmotte A, de Wachter R (1995) An early origin of plastids within the cyanobacterial divergence is suggested by evolutionary trees based on complete 16S rDNA sequences. Mol Biol Evol 12:1166–1173

Nelson DC, Castenholz RW (1982) Light responses in Beggiatoa. Arch Microbiol 131:146–155

Nicholson JAM, Stolz JF, Pierson BK (1987) Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol Ecol 45:343–364

Nickens D, Fry CJ, Ragatz L, Bauer CE, Gest H (1996) Biotype of the purple nonsulfur photosynthetic bacterium, Rhodospirillum centenum. Arch Microbiol 165:91–96

Nogales B, Guerrero R, Esteve I (1997) A heterotrophic bacterium inhibits growth of several species of the genus *Chlorobium*. Arch Microbiol 167:396–399

Noguchi T, Hayashi H, Shimada K, Takaichi S, Tasumi M (1992) In vivo states and function of carotenoids in an aerobic photosynthetic bacterium, Erythrobacter longus. Photosynth Res 31:21–30

Nonnengießer A, Schuster A, Koenig F (1996) Carotenoids and reaction center II-D1 protein in light regulation of the photosynthetic apparatus in Aphanocapsa. Bot Acta 109:115–124

Nübel U (1999) Diversität und Salinitätsabhängiges Verhalten benthischer, cyanobakterieller Lebensgemeinschaften. PhD thesis, University of Bremen, Bremen

Oelze J (1992) Light and oxygen regulation of the synthesis of bacteriochlorophylls a and c in Chloroflexus aurantiacus. J Bacteriol 174:5021–5026

Oesterhelt D, Krippahl G (1983) Phototropic growth of halobacteria and its use for isolation of photosynthetically-deficient mutants. Ann Microbiol (Inst Pasteur) 134B:137–150

Oesterhelt D, Stoeckenius W (1973) Function of a new photoreceptor membrane. Proc Natl Acad Sci USA 70:2853–2857

Oh-Hama T (1989) Evolutionary consideration of the two pathways of 5-aminolevulinic acid biosynthesis in organelles and prokaryotes. Endocytology IV:589–592

Oh-Hama T, Santander PJ, Stolowich NJ, Scott AI (1991) Bacteriochlorophyll c formation via the C5 pathway of 5-aminolevulinic acid synthesis in Chloroflexus aurantiacus. FEBS Lett 281:173–176

Okamura K, Takamiya K, Nishimura M (1985) Photosynthetic electron transfer system is inoperative in anaerobic cells of Erythrobacter species strain OCh114. Arch Microbiol 142:12–17

Okubo Y, Futamata H, Hiraishi A (2006) Characterization of phototrophic purple nonsulfur bacteria forming colored microbial mats in a swine wastewater ditch. Appl Environ Microbiol 72:6225–6233

Oliver RL, Walsby AE (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of Anabaena flos-aquae. Limnol Oceanogr 29:879–886

Olson JM (1998) Chlorophyll organization and function in green photosynthetic bacteria. Photochem Photobiol 67:61–75

Olson JM, Blankenship RE (2004) Thinking about the evolution of photosynthesis. Photosynth Res 80:373–386

Olson RJ, Chisholm SW, Zettler ER, Armbrust EV (1990a) Pigment, size and distribution of Synechococcus in the North Atlantic and Pacific oceans. Limnol Oceanogr 35:45–58

Olson RJ, Chisholm SW, Zettler ER, Altabet MA, Dusenberry JA (1990b) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep Sea Res 37:1033–1051

Oparin AI (1938) The origin of life. Macmillan, New York

Oren A (2004) A proposal for further integration of the cyanobacteria under the bacteriological code. Int J Syst Evol Microbiol 54:1895–1902

Oren A, Padan E, Avron M (1977) Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Proc Natl Acad Sci USA 74:2152–2156

Overmann J (1997) Mahoney Lake: a case study of the ecological significance of phototrophic sulfur bacteria. In: Jones JG (ed) Advances in microbial ecology, vol 15. Plenum Press, New York, pp 251–288

Overmann J (2008) Green nonsulfur bacteria. In: Encyclopedia of life sciences (ELS). Wiley, Chichester. doi:10.1002/9780470015902.a0000457

Overmann J, Pfennig N (1989) *Pelodictyon phaeoclathratiforme* sp. nov., a new brown-colored member of the Chlorobiaceae forming net-like colonies. Arch Microbiol 152:401–406

Overmann J, Pfennig N (1992) Buoyancy regulation and aggregate formation in *Amoebobacter purpureus* from Mahoney Lake. FEMS Microbiol Ecol 101:67–79

Overmann J, Tilzer MM (1989) Control of primary productivity and the significance of photosynthetic bacteria in a meromictic kettle lake, Mittlerer Buchensee, West-Germany. Aquatic Sci 51:261–278

Overmann J, Beatty JT, Hall KJ, Pfennig N, Northcote TG (1991a) Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake. Limnol Oceanogr 36:846–859

Overmann J, Lehmann S, Pfennig N (1991b) Gas vesicle formation and buoyancy regulation in *Pelodictyon phaeoclathratiforme* (green sulfur bacteria). Arch Microbiol 157:29–37

Overmann J, Beatty JT, Hall KJ (1994) Photosynthetic activity and population dynamics of *Amoebobacter purpureus* in a meromictic saline lake. FEMS Microbiol Ecol 15:309–320

Overmann J, Beatty JT, Krouse HR, Hall KJ (1996) The sulfur cycle in the chemocline of a meromictic salt lake. Limnol Oceanogr 41:147–156

Overmann J, Hall KJ, Northcote TG, Beatty JT (1999a) Grazing of the copepod Diaptomus connexus on purple sulfur bacteria in a meromictic salt lake. Environ Microbiol 1:213–222

Overmann J, Coolen MJL, Tuschak C (1999b) Specific detection of different phylogenetic groups of chemocline bacteria based on PCR and denaturing gradient gel electrophoresis of 16S rRNA gene fragments. Arch Microbiol 172:83–94

Overmann J, Hall KJ, Ebenhöh W, Chapman MA, Beatty JT (1999c) Structure of the aerobic food chain in a meromictic lake dominated by purple sulfur bacteria. Arch Hydrobiol 144:127–156

Oyaizu H, Debrunner-Vossbrinck B, Mandelco L, Studier JA, Woese CR (1987) The green non-sulfur bacteria: a deep branching in the eubacterial line of descent. System Appl Microbiol 9:47–53

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276:734–740

Padan E (1979) Impact of facultative anaerobic phototrophic metabolism and ecology of cyanobacteria. Adv Microb Ecol 3:1–48

Padan E, Cohen Y (1982) Anoxygenic photosynthesis. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Botanical monographs, vol 19. Blackwell Scientific, Oxford, pp 215–235

Papiz MZ, Prince SM, Howard T, Cogdell RJ, Isaacs NW (2003) The structure and thermal motion of the B800-850 LH2 complex from *Rps. acidophila* at 2.0 Å resolution and 100 K: new structural features and functionally relevant motions. J Mol Biol 326:1523–1538

Parkin TB, Brock TD (1980a) The effects of light quality on the growth of phototrophic bacteria in lakes. Arch Microbiol 125:19–27

Parkin TB, Brock TD (1980b) Photosynthetic bacterial production in lakes: the effects of light intensity. Limnol Oceanogr 25:711–718

Parkin TB, Brock TD (1981) The role of phototrophic bacteria in the sulfur cycle of a meromictic lake. Limnol Oceanogr 26:880–890

Pedrós-Alió C, Sala MM (1990) Microdistribution and diel vertical migration of flagellated vs gas-vacuolate purple sulfur bacteria in a stratified water body. Limnol Oceanogr 35:1637–1644

Peltier G, Schmidt GW (1991) Chlororespiration: An adaptation to nitrogen deficiency in Chlamydomonas reinhardtii. Proc Natl Acad Sci USA 88:4791–4795

Pentecost A (1984) Effects of sedimentation and light intensity on mat-forming Oscillatoriaceae with particular reference to *Microcoleus lyngyaceus* Gomont. J Gen Microbiol 130:983–990

Permentier HP, Neerken S, Overmann J, Amesz J (2001) A bacteriochlorophyll *a* antenna complex from purple bacteria absorbing at 963 nm. Biochemistry 40:5573–5578

Pfannes KR, Vogl K, Overmann J (2007) Heterotrophic symbionts of phototrophic consortia: members of a novel diverse cluster of *Betaproteobacteria* characterised by a tandem *rrn* operon structure. Environ Microbiol 9:2782–2794

Pfennig N (1978) General physiology and ecology of photosynthetic bacteria. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 3–18

Pfennig N, Trüper HG (1974) The phototrophic bacteria. In: Buchanan RE, Gibbons NE (eds) Bergey's manual of determinative bacteriology, 8th edn. Williams and Wilkins, Baltimore, pp 24–64

Pfennig N, Trüper HG (1989) Anoxygenic phototrophic bacteria. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, pp 1635–1709

Pfennig N, Lünsdorf H, Süling J, Imhoff JF (1997) *Rhodospira trueperi* gen. nov., spec. nov., a new phototrophic Proteobacterium of the alpha group. Arch Microbiol 168:39–45

Pierson BK, Castenholz RW (1974) Studies of pigments and growth in Chloroflexus aurantiacus, a phototrophic filamentous bacterium. Arch Microbiol 100:283–305

Pierson BK, Castenholz RW (1995) Taxonomy and physiology of filamentous anoxygenic phototrophs. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 31–47

Pierson BK, Olson JM (1989) Evolution of photosynthesis in anoxygenic photosynthetic procaryotes. In: Cogen Y, Rosenberg E (eds) Microbial mats: Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 402–427

Pierson BK, Oesterle A, Murphy GL (1987) Pigments, light penetration and photosynthetic activity in the multi-layered microbial mats of Great Sippewisset Salt Marsh, Massachussets. FEMS Microbiol Ecol 45:365–376

Pierson BK, Sands VM, Frederick JL (1990) Spectral irradinace and distribution of pigments in a highly layered microbial mat. Appl Environ Microbiol 56:2327–2340

Pierson BK, Valdez D, Larsen M, Morgan E, Mack EE (1994) Chloroflexus-like organisms from marine and hypersaline environments: distribution and diversity. Photosynthesis Res 41:35–52

Post AF, de Wit R, Mur LR (1985) Interactions between temperature and light intensity on growth and photosynthesis of the cyanobacterium *Oscillatoria agardhii*. J Plankton Res 7:487–495

Potts M (1994) Desiccation tolerance in prokaryotes. Microbiol Rev 58:755–805

Pradella S, Allgaier M, Hoch C, Päuker O, Stackebrandt E, Wagner-Döbler I (2004) Genome organization and localization of the *pufLM* genes of the photosynthesis reaction center in phylogenetically diverse marine *Alphaproteobacteria*. Appl Environ Microbiol 70:3360–3369

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62

Prufert-Bebout L, Garcia-Pichel F (1994) Field and cultivated *Microcoleus chthonoplastes*: the search for clues to its prevalence in marine microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats: structure, development and environmental significance. Springer, New York/Heidelberg, pp 265–271

Psencik J, Ma Y-Z, Arellano JB, Garica-Gil J, Holzwarth AR, Gillbro T (2002) Excitation energy transfer in chlorosomes of *Chlorobium phaeobacteroides* strain CL1401: the role of carotenoids. Photosynth Res 71:5–18

Psencik J, Ikonen TP, Laurinmäki PA, Merckel MC, Butcher SJ, Serimaa RE, Tuma R (2004) Lamellar organization of pigments in chlorosomes, the light harvesting complexes of green photosynthetic bacteria. Biophys J 87:1165–1172

Ragatz L, Jiang Z-Y, Bauer C, Gest H (1994) Phototactic purple bacteria. Nature 370:104

Ragatz L, Jiang Z-Y, Bauer CE, Gest H (1995) Macroscopic phototactic behaviour of the purple photosynthetic bacterium Rhodospirillum centenum. Arch Microbiol 163:1–6

Rashby SE, Sessions AL, Summons RE, Newman DK (2007) Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. Proc Natl Acad Sci USA 105:15099–15104

Rathgeber C, Beatty JT, Yurkov V (2004) Aerobic phototrophic bacteria: new evidence for the diversity, ecological importance and applied potential of this previously overlooked group. Photosynthesis Res 81:113–128

Rau GH (1980) Carbon-13/Carbon-12 variation in subalpine lake aquatic insects: food source implications. Can J Fish Aquat Sci 37:742–746

Raymond J, Zhaxybayeva O, Gogarten JP, Gerdes SY, Blankenship RE (2002) Whole genome analysis of photosynthetic prokaryotes. Science 298:1616–1620

Rebeiz CA, Lascelles J (1982) Biosynthesis of pigments in plants and bacteria. In: Godvindjee (ed) Energy conversion by plants and bacteria, vol I. Academic, New York, pp 699–780

Reeves RH (1996) 16S ribosomal RNA and the molecular phylogeny of the Cyanobacteria. Nova Hedwiga 112:55–67

Repeta DJ, Simpson DJ, Jørgensen BB, Jannasch HW (1989) Evidence for the existence of anoxygenic photosynthesis from the distribution of bacteriochlorophylls in the Black Sea. Nature 342:69–72

Richaud P, Marrs BL, Vermiglio A (1986) Two modes of interaction between photosynthetic and respiratory electron chains in whole cells of *Rhodopseudomonas capsulata*. Biochim Biophys Acta 850:256–263

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Rödig J, Jock S, Klug G (1999) Coregulation of the synthesis of bacteriochlorophyll and pigment-binding proteins in *Rhodobacter capsulatus*. Arch Microbiol 171:198–204

Roszak AW, Howard TD, Southall J, Gardiner AT, Law CJ, Isaacs NW, Cogdell RJ (2003) Crystal structure of the RC-LH1 core complex from *Rhodopseudomonas palustris*. Science 302:1969–1972

Rücker O, Köhler A, Behammer B, Sichau K, Overmann J (2012) *Puf* operon sequences and inferred structures of light-harvesting complexes of three closely related *Chromatiaceae* exhibiting different absorption characteristics. Arch Microbiol 194:123–134

Ruff-Roberts AL, Kuenen JG, Ward DM (1994) Distribution of cultivated and uncultivated cyanobacteria and Chloroflexus-like bacteria in hot spring microbial mats. Appl Environ Microbiol 60:697–704

Sackett MJ, Armitage JP, Sherwood EE, Pitta TP (1997) Photoresponses of the purple nonsulfur bacteria Rhodospirillum centenum and *Rhodobacter sphaeroides*. J Bacteriol 179:6764–6768

Sánchez O, van Gemerden H, Mas J (1998) Acclimation of the photosynthetic response of Chromatium vinosum to light-limiting conditions. Arch Microbiol 170:405–410

Schenk HEA (1992) Cyanobacterial symbioses. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes. Springer, New York, pp 3819–3854

Schiller H, Senger H, Miyashita H, Miyashi S, Dau H (1997) Light-harvesting in Acaryochloris marina—Spectroscopic characterization of a Chlorophyll d-dominated photosynthetic antenna system. FEBS Lett 410:433–436

Schott J, Griffin BM, Schink B (2010) Anaerobic phototrophic nitrite oxidation by *Thiocapsa* sp. strain KS1 and *Rhodospeudomonas* sp. strain LQ17. Microbiol UK 156:2428–2437

Sharma AK, Spudich JL, Doolittle WF (2006) Microbial rhodopsins: functional versatility and genetic mobility. Trends Microbiol 14:463–469

Sharma AK, Zhaxybayeva O, Papke TR, Doolittle WF (2008) Actinorhodopsins: proteorhodopsin-like sequences found predominantly in non-marine environments. Environ Microbiol 10:1039–1056

Sharon I, Alperovitch A, Rohwer F, Haynes M, Glaser F et al (2009) Photosystem I gene cassettes are present in marine virus genomes. Nature 461: 258–262

Shiba T (1989) Overview of the aerobic photosynthetic bacteria. In: Harashima K, Shiba T, Murata N (eds) Aerobic photosynthetic bacteria. Springer, Berlin, pp 1–8

Shiba T, Harashima K (1986) Aerobic photosynthetic bacteria. Microbiol Sci 3:377–378

Shiba T, Simidu U, Taga N (1979) Distribution of aerobic bacteria which contain bacteriochlorophyll a. Appl Environ Microbiol 38:43–45

Shimada K (1995) Aerobic anoxygenic phototrophs. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 105–122

Sidler WA (1994) Phycobilisome and phycobiliprotein structures. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht/Boston/London, pp 139–216

Sinninghe Damsté JS, Wakeham SG, Kohnen MEL, Hayes JM, de Leeuw JW (1993) A 6, 000-year sedimentary molecular record of chemocline excursions in the Black Sea. Nature 362:827–829

Sirevåg R (1995) Carbon metabolism in green bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 871–883

Skyring GW, Bauld J (1990) Microbial mats in coastal environments. Adv Microb Ecol 11:461–498

Smith RC, Baker KS (1981) Optical properties of the clearest seawaters Appl. Optics 20:177–184

Sorokin YI (1970) Interrelations between sulphur and carbon turnover in meromictic lakes. Arch Hydrobiol 66:391–446

Sprenger WW, Hoff WD, Armitage JP, Hellingwerf KJ (1993) The eubacterium *Ectothiorhodospira halophila* is negatively phototactic, with a wavelength dependence that fits the absorption spectrum of the photoactive yellow protein. J Bacteriol 175:3096–3104

Stackebrandt E, Embley M, Weckesser J (1988) Phylogenetic, evolutionary, and taxonomic aspects of phototrophic bacteria. In: Olson JM, Stackebrandt E, Trüper HG (eds) Green photosynthetic bacteria. Plenum Publishing Cooperation, New York, pp 201–215

Stackebrandt E, Rainey FA, Ward-Rainey N (1996) Anoxygenic phototrophy across the phylogenetic spectrum: current understanding and future perspectives. Arch Microbiol 166:211–223

Stanier RY (1977) The position of cyanobacteria in the world of phototrophs. Carlsberg Res Comm 42:77–98

Steenbergen CLM, Korthals HJ (1982) Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (The Netherlands). Limnol Oceanogr 27:883–895

Steudel R (1989) On the nature of the "elemental sulfur" (S_o) produced by sulfur-oxidizing bacteria-a model for S_o globules. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer, New York, pp 289–303

Steudel R, Holdt G, Visscher PT, van Gemerden H (1990) Search for polythionates in cultures of Chromatium vinosum after sulfide incubation. Arch Microbiol 153:432–437

Strauß G, Fuchs G (1993) Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium Chloroflexus aurantiacus, the hydroxypropionate cycle. Eur J Biochem 215:633–643

Summons RE, Bradley AS, Jahnke LL, Waldbauer JR (2006) Steroids, triterpenoids and molecular oxygen. Phil Trans R Soc B 361:951–968

Suttle CA, Chan AM, Cottrell MT (1990) Infection of phytoplankton by viruses and reduction of primary productivity. Nature 347:467–469

Suttle CA, Chan AM, Feng C, Garza DR (1993) Cyanophages and sunlight: a paradox. In: Guerrero R, Pedrós-Alió C (eds) Trends in microbial ecology. Spanish Society for Microbiology, Barcelona, pp 303–307

Suwanto A, Kaplan S (1989) Physical and genetic mapping of the *Rhodobacter sphaeroides* 2.4.1 genome: genome size, fragment identification, and gene localization. J Bacteriol 171:5840–5849

Suzuki JY, Bauer CE (1995) A prokaryotic origin for light-dependent chlorophyll biosynthesis of plants. Proc Natl Acad Sci USA 92:3749–3753

Swoager WC, Lindstrom ES (1971) Isolation and counting of Athiorhodaceae with membrane filters. Appl Microbiol 22:683–687

Takahashi K, Wada E, Sakamoto M (1990) Carbon isotope discrimination by phytoplankton and photosynthetic bacteria in monomictic Lake Fukamiike. Arch Hydrobiol 120:197–210

Takaishi S, Inoue K, Akaikie M, Kobayashi M, Oh-oka H, Madigan MT (1997) The major carotenoid in all known species of heliobacteria is the C30 carotenoid 4,4′- diaponeurosporene, not neurosporene. Arch Microbiol 168:277–281

Takaishi S, Oh-oka H, Maoka T, Jung DO, Madigan MT (2003) Novel carotenoid glucoside esters from alkaliphilic heliobacteria. Arch Microbiol 179:95–100

Tanada T, Kitadokoro K, Higuchi Y, Inaka K, Yasui A, Deruiter PE, Eker APM, Miki K (1997) Crystal structure of DNA photolyase from Anacystis nidulans Nature Struct. Biol 4:887–891

Tang KH, Yue H, Blankenship RE (2010) Energy metabolism of *Heliobacterium modesticaldum* during phototrophic and chemotrophic growth. BMC Microbiol 10:150

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriolog Rev 41:100–180

Theroux SJ, Redlinger TE, Fuller RC, Robinson SJ (1990) Gene encoding the 5.7-kilodalton chlorosome protein of Chloroflexus aurantiacus: regulated message levels and a predicted carboxy-terminal protein extension. J Bacteriol 172:4497–4504

Thomas RH, Walsby AE (1985) Buoyancy regulation in a strain of Microcystis. J Gen Microbiol 131:799–809

Thorne SW, Newcomb EH, Osmond CB (1977) Identification of chlorophyll b in extracts of prokaryotic algae by fluorescence spectroscopy. Proc Natl Acad Sci 74:575–578

Tice MM, Lowe DR (2006) Hydrogen-based carbon fixation in the earliest known photosynthetic organisms. Geology 34:37–40

Tomitani A, Knoll AH, Cavanaugh CM, Ohno T (2006) The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. Proc Natl Acad Sci USA 103:5442–5447

Tronrud DE, Wen J, Gay L, Blankenship RE (2009) The structural basis for the difference in absorbance spectra for the FMO antenna protein from various green sulfur bacteria. Photosynth Res 100:443–454

Trüper HG, Pfennig N (1978) Taxonomy of the *Rhodospirillales*. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum, New York, pp 19–27

Trüper HG, Pfennig N (1981) Characterization and identification of the anoxygenic phototrophic bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes: a handbook on habitats, isolation and identification of bacteria. Springer, New York, pp 299–312

Tsukatani Y, Wen J, Blankenship RE, Bryant DA (2010) Characterization of the FMO protein from the aerobic chlorophototroph, *Candidatus* Chloracidobacterium thermophilum. Photosynth Res 201:201–209

Turner S (1887) Molecular systematics of oxygenic photosynthetic bacteria. Plant Syst Evol 11:13–52

Tuschak C, Beatty JT, Overmann J (2004) Photosynthesis genes and LH1 proteins of *Roseospirillum parvum* 930I, a purple non-sulfur bacterium with unusual spectral properties. Photosynthesis Res 81:181–199

Tuschak C, Leung MM, Beatty JT, Overmann J (2005) The *puf* operon of the purple sulfur bacterium *Amoebobacter purpureus*: Structure, transcription and phylogenetic analysis. Arch Microbiol 183:431–443

Urakami T, Komagata K (1984) Protomonas, a new genus of facultatively methylotrophic bacteria. Int J Syst Bacteriol 34:188–201

Utkilen HC, Skulberg OM, Walsby AE (1985) Buoyancy regulation and chromatic adaptation in planktonic *Oscillatoria* species: alternative strategies for optimizing light absorption in stratified lakes. Arch Hydrobiol 104:407–417

van den Ende FP, Laverman AM, van Gemerden H (1996) Coexistence of aerobic chemotrophic and anaerobic phototrophic sulfur bacteria under oxygen limitation. FEMS Microbiol Ecol 19:141–151

van Gemerden H (1967) In the bacterial sulfur cycle of inland waters. University of Leiden

van Gemerden H (1974) Coexistence of organisms competing for the same substrate: An example among the purple sulfur bacteria. Microb Ecol 1:104–119

van Gemerden H, Mas J (1995) Ecology of phototrophic sulfur bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 49–85

van Gemerden H, Tughan CS, de Wit R, Herbert RA (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbial Ecol 62:87–102

van Thor JJ, Mullineaux CW, Matthijs HPC, Hellingwert KJ (1998) Light harvesting and state transitions in cyanobacteria. Bot Acta 111:430–443

van Valen LM, Maiorana VC (1980) The archaebacteria and eukaryotic origins. Nature 287:248–250

Vassilieva EV, Stirewalt VL, Jakobs CU, Frigaard N-U, Inoue-Sakamoto K, Baker MA, Sotak A, Bryant DA (2002) Subcellular localization of chlorosome proteins in *Chlorobium tepidum* and characterization of three new chlorosome proteins: CsmF, CsmH, and CsmX. Biochemistry 41:4358–4370

Veldhuis MJW, van Gemerden H (1986) Competition between purple and brown phototrophic bacteria in stratified lakes: sulfide, acetate, and light as limiting factors. FEMS Microbiol Ecol 38:31–38

Vermaas WFJ (1994) Evolution of heliobacteria: implications for photosynthetic reaction center complexes. Photosynth Res 41:285–294

Vogl K, Glaeser J, Pfannes KR, Wanner G, Overmann J (2006) *Chlorobium chlorochromatii* sp. nov., a symbiotic green sulfur bacterium isolated from the phototrophic consortium "*Chlorochromatium aggregatum*". Arch Microbiol 185:363–372

Vogl K, Wenter R, Dreßen M, Schlickenrieder M, Plöscher M, Eichacker L, Overmann J (2008) Identification and analysis of four candidate symbiosis genes from "*Chlorochromatium aggregatum*", a highly developed bacterial symbiosis. Environ Microbiol 10:2842–2856

Wagner-Huber R, Brunisholz R, Frank G, Zuber H (1988) The BChl c/e-binding polypeptides from chlorosomes of green photosynthetic bacteria. FEBS Lett 239:8–12

Wakao N, Yokoi N, Isoyama N, Hiraishi A, Shimada K, Kobayachi M, Kise H, Iwaki M, Itoh S, Takaishi S, Sakurai Y (1996) Discovery of natural photosynthesis using Zn-containing bacteriochlorophyll in an aerobic bacterium Acidiphilium rubrum. Plant Cell Physiol 37:889–896

Wakim B, Oelze J (1980) The unique mode of adjusting the composition of the photosynthetic apparatus to different environmental conditions by Rhodospirillum tenue. FEMS Microbiol Lett 7:221–223

Walsby AE (1978) The properties and buoyancy-providing role of gas vacuoles in Trichodesmium Ehrenberg. Br Phycol J 13:103–116

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Wanner G, Vogl K, Overmann J (2008) Ultrastructural characterization of the prokaryotic symbiosis in "*Chlorochromatium aggregatum*". J Bacteriol 190:3721–3730

Ward DM, Weller R, Shiea J, Castenholz RW, Cohen Y (1989) Hot springs microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Cohen Y, Rosenberg E (eds) Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 3–15

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

Waterbury JB, Wiley JM, Franks DG, Valois FW, Watson SW (1985) A cyanobacterium capable of swimming motility. Science 230:74–76

Waterbury JB, Watson SW, Valois FW, Franks DG (1986) Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. In: Platt T, Li WKW (eds) Photosynthetic picoplankton. Can Bull Fish Aquat Sci 214:71–120

Watson SW, Novitsky TJ, Quinby HL, Valois FW (1977) Determination of bacterial number and biomass in the marine environment. Appl Environ Microbiol 33:940–946

Weller R, Bateson MM, Heimbuch BK, Kopczynski ED, Ward DM (1992) Uncultivated cyanobacteria. Chloroflexus-like inhabitants, and spirochete-like inhabitants of a hot spring microbial mat. Appl Environ Microbiol 58:3964–3969

Wellington CL, Bauer CE, Beatty JT (1992) Photosynthesis gene superoperons in purple non-sulfur bacteria: the tip of the iceberg? Can J Microbiol 38:20–27

Wenter R, Hütz K, Dibbern D, Reisinger V, Li T, Plöscher M, Eichacker L, Eddie B, Hanson T, Bryant D, Overmann J (2010) Expression-based identification of genetic determinants of the bacterial symbiosis in "*Chlorochromatium aggregatum*". Environ Microbiol 12:2259–2276

Wessels DCJ, Büdel B (1995) Epilithic and cryptoendolithic cyanobacteria of Clarens Sandstone Cliffs in the Golden Gate Highlands National Park. South Africa Bot Acta 108:220–226

Whittaker RH, Likens GE (1975) The biosphere and man. In: Lieth H, Whittaker RH (eds) Primary productivity of the biosphere. Springer, New York, pp 305–328

Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B (1993) Ferrous iron oxidation by anoxygenic phototrophic bacteria. Nature 362:834–836

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

Woese CR, Gibson J, Fox GE (1980) Do genealogical patterns in photosynthetic bacteria reflect interspecific gene transfer? Nature 283:212–214

Wraight CA, Clayton RK (1973) The absolute quantum efficiency of bacteriochlorophyll photooxidation in reaction centres of Rhodopseudomonas spheroides. Biochim Biophys Acta 333:246

Wu H, Green M, Scranton MI (1997) Acetate cycling in the water column and surface sediment of Long Island Sound following a bloom. Limnol Oceanogr 42:705–713

Xiong J (2006) Photosynthesis: what color was its origin? Genome Biol 7:245

Xiong J, Bauer CE (2002) Complex evolution of photosynthesis. Annu Rev Plant Biol 53:503–521

Xiong J, Fischer W, Inoue K, Nakahara M, Bauer CE (2000) Molecular evidence for the early evolution of photosynthesis. Science 289:1724–1730

Yeager CM, Kornosky JL, Morgan RL, Cain EC, Belnap J, Garcia-Pichel F, Kuske CR (2007) Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiol Ecol 60:85–97

Yildiz FH, Gest H, Bauer CE (1992) Conservation of the photosynthesis gene cluster in Rhodospirillum centenum. Mol Microbiol 6:2683–2691

Yurkov W, Beatty JT (1998) Isolation of aerobic anoxygenic photosynthetic bacteria from black smoker plume waters of the Juan de Fuca Ridge in the Pacific Ocean. Appl Environ Microbiol 64:337–341

Yurkov V, Csotonyi JT (2009) New light on aerobic anoxygenic phototrophs. In: Hunter CN, Daldal F, Thurnauer M, Beatty JT (eds) The purple phototrophic bacteria. Springer, Dordrecht, pp 31–55

Yurkov V, van Gemerden H (1993) Impact of light/dark regime on growth rate, biomass formation and bacteriochlorophyll synthesis in Erythromicrobium hydrolyticum. Arch Microbiol 159:84–89

Yurkov V, Gad'on N, Angerhofer A, Drews G (1994) Light-harvesting complexes of aerobic bacteriochlorophyll-containing bacteria Roseococcus thiosulfatophilus, RB3 and Erythromicrobium ramosum, E5 and the transfer of excitation energy from carotenoids to bacteriochlorophyll. Z Naturforsch 49(c):579–586

Zehnder AJB, Stumm W (1988) Geochemistry and biogeochemistry of anaerobic habitats. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley-Liss, New York, pp 1–38

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourtit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Zevenboom W, Mur LR (1984) Growth and photosynthetic response of the cyanobacterium Microcystis aeruginosa in relation to photoperiodicity and irradiance. Arch Microbiol 139:232–239

Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. Science 334:1551–1553

Zuber H, Cogdell RJ (1995) Structure and organization of purple bacterial antenna complexes. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 315–348

Zucconi AP, Beatty JT (1988) Posttranscriptional regulation by light of the steady state levels of mature B800-850 light-harvesting complex in *Rhodobacter capsulatus*. J Bacteriol 170:877–882

13 The Anaerobic Way of Life

Ruth A. Schmitz[1] · *Rolf Daniel*[2] · *Uwe Deppenmeier*[3] · *Gerhard Gottschalk*[4]
[1]Institut für Allgemeine Mikrobiologie, Christian-Albrechts-Universität Kiel, Kiel, Germany
[2]Department of Genomic and Applied Microbiology, Georg-August-Universität Göttingen, Göttingen, Germany
[3]Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, USA
[4]Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Göttingen, Germany

Introduction

Molecular oxygen in appreciable amounts is found only in those areas on earth that are in direct contact with air or are inhabited by organisms carrying out oxygenic photosynthesis. The solubility of oxygen in water is low. In equilibrium with air at 1.013 bar and at 20 °C, pure water will contain approximately 9 mg/l of dissolved oxygen. In aqueous systems, aerobic organisms rapidly consume dissolved oxygen so that deeper layers of many waters and soils (especially if they are rich in organic compounds), as well as mud and sludge, are practically anaerobic. Nevertheless, these areas are inhabited by numerous organisms that fulfill the important ecological role of converting insoluble organic material to soluble compounds and gases that can circulate back into aerobic regions. Other important anaerobic habitats are the rumen, the intestinal tract, and man-made anaerobic digesters of sewage treatment plants.

Anaerobic prokaryotes that can live in the above-mentioned environments are either phototrophs, which, of course, can only flourish if light is available, or chemotrophs. With respect to their relationship to aerobic metabolism, three groups of organisms capable of growth in an anaerobic environment can be identified:

1. Organisms that are aerobes but can use alternate electron acceptors such as nitrate or nitrite when exposed to an anaerobic environment. The electron transport from NADH to these acceptors is coupled to the phosphorylation of ADP, as is the electron transport to oxygen.
2. Organisms that are facultative aerobes. The enterobacteria are the most prominent representatives of this group. These organisms grow as typical aerobes in the presence of oxygen; in its absence, they carry out fermentations.
3. Obligately anaerobic bacteria that are characterized by the inability to synthesize a respiratory chain with oxygen as terminal electron acceptor. They are restricted to life without oxygen.

The diversity of microorganisms able to thrive under anaerobic conditions is overwhelming. Up to now, more than 200 genera of obligate anaerobic microorganisms have been described. Obligate anaerobes are found in all three domains. The eukaryotes are represented by anaerobic fungi, ciliates, and flagellates; the archaea by the methanogens, which comprise 23 genera; and by the most hyperthermophilic genera *Pyrolobus*, *Pyrodictium*, and *Pyrococcus*. Most genera of the obligate anaerobes belong to the bacteria. Especially prominent are the 32 genera characterized by their ability of dissimilatory reduction of sulfate, sulfite, or sulfur. Sporeformers are well represented, for example, by the genera *Clostridium*, *Sporomusa*, *Desulfotomaculum*, *Moorella*, and *Thermoanaerobacterium*. There are halophiles such as the genera *Haloanaerobacter* and *Sporohalobacter* and alkaliphiles like *Anaerobranca*. A few genera comprise more than a dozen species: *Bacteroides*, *Bifidobacterium* and *Clostridium* (the genus which by far contains the most species), *Desulfotomaculum*, *Desulfovibrio*, *Eubacterium*, and *Thermococcus*. Quite a few genera are represented just by one species, for example, *Acetitomaculum*, *Acetonema*, *Chrysiogenes*, *Desulfobacula*, *Hippea*, *Stetteria*, and *Succinispira*. Autotrophic CO_2 fixation is widespread among the acetogenic anaerobes such as *Acetobacterium woodii*, *Clostridium aceticum*, and *Moorella thermoautotrophica* and especially among the methanogens of which only a few representatives are unable

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_52,

to grow with CO_2 plus H_2, for example, *Methanosaeta concilii*, *Methanosarcina acetivorans*, and the *Methanosphaera* species. A few sulfate-reducing bacteria utilize CO_2, such as *Desulfobacterium autotrophicum* and *Desulfosarcina variabilis*. The ability to fix molecular nitrogen is probably more common among anaerobes than known at the moment. Several clostridia are able to do so, with *Clostridium pasteurianum* being the first species demonstrated to have nitrogenase activity. Methanogens express active nitrogenase under nitrogen-limited growth conditions as has been demonstrated for *Methanosarcina barkeri*, *Methanosarcina mazei*, and *Methanococcus maripaludis*. Many more anaerobes can be expected to do so.

So, obligate anaerobes are known now for all-important anaerobic habitats on earth. Because of their inability to utilize oxygen, they had to develop their strategies to conserve energy in the form of ATP, to metabolize substrates, and to cope with some of their own products such as ethanol, lactate, butyrate, or acetate. Some of the characteristic features of the anaerobes will be outlined.

Novel Ion Translocation Reactions Involved in Energy Conservation

It is a fact that several anaerobic microorganisms produce ATP only by substrate-level phosphorylation. Growth on sugars or on amino acids coupled to the formation of ethanol, lactate, butyrate, or acetate very often indicates that substrate-level phosphorylation is involved (Thauer et al. 1977). This holds true for lactic acid bacteria and also for many clostridia. Some of the reactions employed for ATP synthesis by these bacteria and by other anaerobes are listed in ❯ *Table 13.1*. It can be seen that the reactions 1–4 listed in ❯ *Table 13.1* are part of the glycolytic pathway of acetate and butyrate formation. Carbamoyl phosphate is formed in the conversion of arginine to ornithine and thereby becomes available for ATP synthesis. The conversion of N^{10}-formyl FH_4 (N^{10}-formyl tetrahydrofolic acid, an intermediate of methyl group oxidation) to formate and FH_4 gives rise to ATP synthesis. Glycine reductase is involved in the reductive part of the Stickland reaction, the pairwise fermentation of amino acids. This interesting reaction will be discussed in detail below.

There are fermentations in which at first sight, reactions giving rise to ATP synthesis cannot be identified. Such processes are, for instance, hydrogen-dependent fermentations; some are summarized in ❯ *Table 13.2*. Here, it has been assumed for quite some time that electron transport processes might be coupled to ion translocation and that the ion-motive force generated might support ATP synthesis. Experimental proof for this assumption has been provided in recent years.

Wolinella succinogenes grows on fumarate and H_2 according to the equation given in ❯ *Table 13.2*. Clearly, this organism must gain ATP by electron transport phosphorylation. The electron transport chain that catalyzes this reaction (❯ *Fig. 13.1b*) consists of hydrogenase, menaquinone, and fumarate reductase (Lancaster and Kröger 2000). Using vesicles and reconstituted liposomal systems, the generation of a proton-motive force could be demonstrated in the course of H_2-dependent menaquinone reduction as catalyzed by the hydrogenase

Table 13.1
Reactions yielding ATP by substrate-level phosphorylation in anaerobes

Reaction	Enzyme	ΔG_{abs}^{0} (kJ/mol)
1,3-Bisphosphoglycerate + ADP ⇔ 3-phosphoglycerate + ATP	Phosphoglycerate kinase	−24.1
Phosphoenolpyruvate + ADP ⇔ pyruvate + ATP	Pyruvate kinase	−23.7
Acetyl phosphate + ADP ⇔ acetate + ATP	Acetate kinase	−12.9
Butyryl phosphate + ADP ⇔ butyrate + ATP	Butyrate kinase	−12.9
Carbamoyl phosphate + ADP ⇔ carbamate + ATP	Carbamate kinase	−7.5
N^{10}-Formyl FH_4[a] + ADP + P_i ⇔ formate + FH_4 + ATP	Formyl-FH_4 synthetase	+8.32
Glycine + 2H + ADP + P_i ⇔ acetate + NH_3 + ATP	Glycine reductase	About −46.0

[a] FH_4, tetrahydrofolic acid

Table 13.2
H_2-dependent fermentations

Reaction			Change of free energy
Fumarate + H_2	→	Succinate	$\Delta G^{o\prime} = -86$ kJ/mol
CO_2 + 4 H_2	→	CH_4 + 2 H_2O	$\Delta G^{o\prime} = -131$ kJ/mol
2 CO_2 + 4 H_2	→	$CH_3{\cdot}COO^-$ + H^+ + 2 H_2O	$\Delta G^{o\prime} = -95$ kJ/mol
SO_4^{2-} + 4 H_2 + H^+	→	HS^- + 4 H_2O	$\Delta G^{o\prime} = -152$ kJ/mol
2 FeOOH + H_2 + 4 H^+	→	2 Fe^{2+} + 4 H_2O	$\Delta G^{o\prime} = -110$ kJ/mol

(Gross et al. 1998). A number of other bacteria also can take advantage of ion-translocating electron transport system using fumarate as a terminal electron acceptor (Kröger et al. 1992). Formate, NADH, and H_2 are typical electron donors, and succinate and propionate are formed as catabolic end products.

The pathway (as employed by the methanogens) for CO_2 reduction to methane by H_2 is depicted in ❯ *Fig. 13.2*. It has been demonstrated in recent years that one reaction, the methyl group transfer from methyltetrahydromethanopterin to coenzyme M, is coupled to the translocation of sodium ions (Deppenmeier et al. 1996). This system represents a novel type of sodium ion pump, which will be discussed below in connection with other sodium ion pumps. Some methanogens (e.g., *Methanosarcina* spp.) employ two novel membrane-bound electron transport systems generating an electrochemical proton gradient. The systems are composed of the heterodisulfide reductase and either a membrane-bound hydrogenase or an $F_{420}H_2$ dehydrogenase (Bäumer et al. 2000), which is

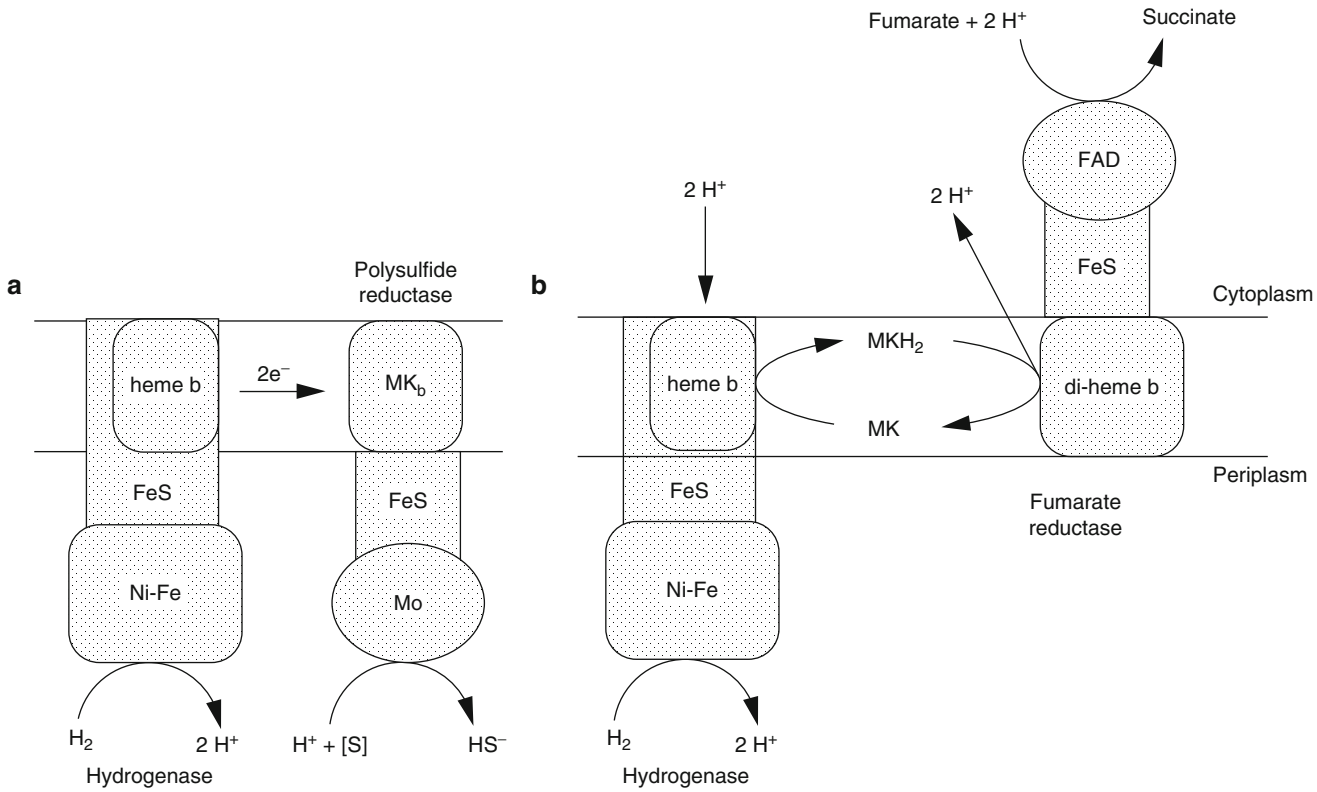


Fig. 13.1

Anaerobic respiration in *Wolinella succinogenes*. (a) Polysulfide reduction: The membrane-bound hydrogenase is composed of three subunits (HydABC) and contains heme *b*, iron-sulfur (*FeS*) clusters, and the nickel/iron (*Ni-Fe*) center for hydrogen oxidation. The gene products PsrA, B, and C form the polysulfide reductase which contains a molybdopterin guanine dinucleotide (*Mo*), iron-sulfur (*FeS*) clusters. A menaquinone (*Mkb*) is tightly bound to the protein. Electron transfer is probably mediated by diffusion and collision of the enzymes. (b) Fumarate reduction: The hydrogenase is identical to the one shown in a. The fumarate reductase consists of three subunits (*frdCAB*). A diheme cytochrome *b* anchors the enzyme in the membrane (di-heme *b*). The catalytic subunit carries a covalently bound FAD. These subunits are connected by an iron-sulfur (*FeS*) protein. Electron transfer from the hydrogenase to the fumarate reductase is mediated by menaquinone

functionally homologous to the proton-translocating NADH dehydrogenase (complex I of the respiratory chain). It has been shown that all of these enzymes are involved in proton translocation. Interestingly, the electron transport systems of these organisms contain electron carriers (such as cytochromes and the novel redox carrier methanophenazine), not found in methanogens utilizing only H_2 + CO_2 (Deppenmeier et al. 1999).

A number of archaea as well as of bacteria reduce elemental sulfur with H_2 to H_2S (Hedderich et al. 1999). Examples are *Pyrodictium occultum*, *Stetteria hydrogenophila*, and *Desulfurobacterium thermolithotrophum* but also the already-mentioned *Wolinella succinogenes* in which an H_2: polysulfide reductase was characterized consisting of a nickel-iron hydrogenase, a menaquinone, and a molybdenum iron sulfide-containing polysulfide reductase (*Fig. 13.1a*). Because the solubility of elemental sulfur in water is extremely low, it is believed that polysulfide is the actual electron acceptor (Hedderich et al. 1999). It is formed in an H_2S environment according to

$$nS^0 + HS^- \rightarrow S^{2-}{}_{n+1} + H^+ \qquad (13.1)$$

Proton gradients are also established in the process of dissimilatory sulfate reduction. Here, the electron transfer from H_2 to sulfite is coupled to ATP synthesis via a chemiosmotic mechanism (Badziong and Thauer 1980). Shewanella putrefaciens (not an obligate anaerobe) can grow with Fe^{3+} and H_2. The mode of energy conservation is not known as yet.

Diffusion gradients may also be exploited for the generation of a proton-motive force. As long as the intercellular lactate concentration is high as compared to the extracellular one, it can be exported accompanied by two protons:

$$\text{Lactate}_{\text{inside}} + 2H^+{}_{\text{inside}} \rightarrow \text{lactate}_{\text{outside}} + 2H^+{}_{\text{outside}} \qquad (13.2)$$

Thus, the proton/product symport helps lactic acid bacteria to increase their ATP yield (Konings et al. 1997).

Sodium Ion Pumps

Cells have the tendency to expel sodium ions from the interior. Usually, expulsion is catalyzed by sodium-proton antiporters,

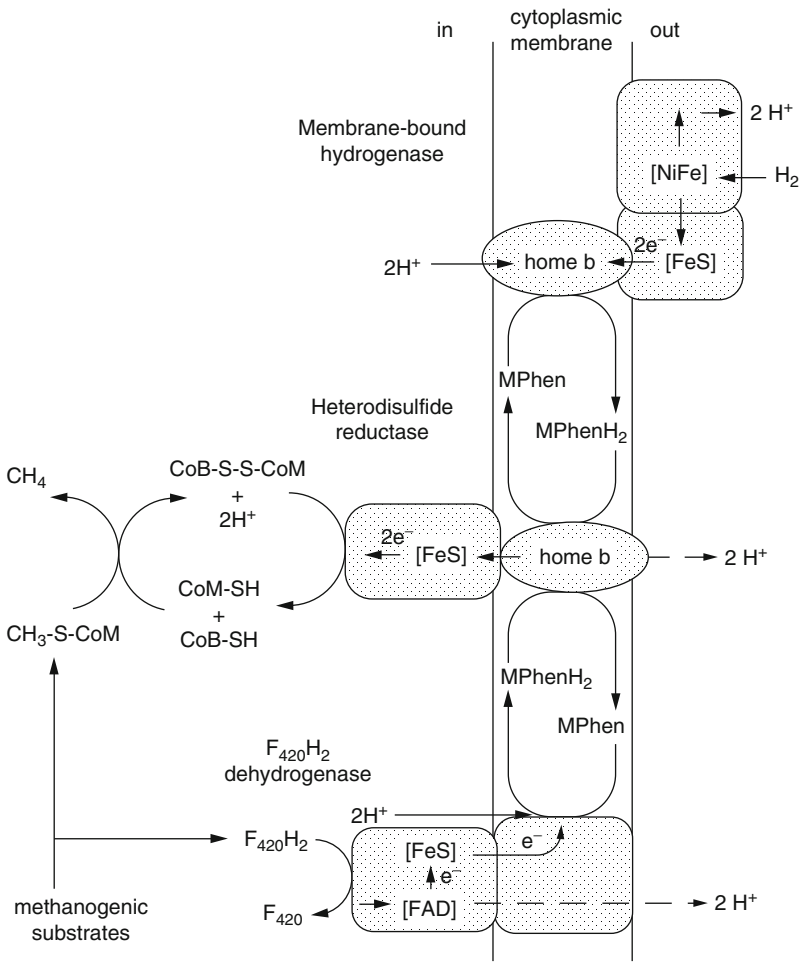


Fig. 13.2

Membrane-bound electron transport chain in *Methanosarcina mazei*. In the course of methanogenesis, methyl-coenzyme M (CH3-S-CoM) is formed and is reductively cleaved by the methyl-CoM reductase which uses coenzyme B (HS-CoB) as electron donor. The reaction results in the formation of methane and a heterodisulfide (CoB-S-S-CoM) from HS-CoM and HS-CoB. The disulfide functions as electron acceptor of the anaerobic respiratory chain. Molecular hydrogen or reduced coenzyme F_{420} ($F_{420}H_2$) serves as electron donors. The $F_{420}H_2$ dehydrogenase contains FAD and FeS clusters and is responsible for the oxidation of $F_{420}H_2$. Electrons are transferred to methanophenazine (*MPhen*). The reduced form of this novel cofactor is the electron donor of the heterodisulfide reductase. This enzyme contains heme b and iron-sulfur clusters. It catalyzes the reduction of CoM-S-S-CoB. The H_2-dependent electron transport system is composed of a membrane-bound hydrogenase which is very similar to the corresponding enzyme from *Wolinella* (Fig. 13.1). Methanophenazine functions as mediator of electron transport to the heterodisulfide reductase

but a number of obligately anaerobic microorganisms have primary sodium ion pumps at their disposal. In these organisms, certain exergonic reactions are coupled with Na^+ translocation across the cytoplasmic membrane. One example was given already – the methyltetramethanopterin: coenzyme M methyltransferase reaction which is present in all methanogens and which is responsible for the Na^+ dependence of growth and methane formation of this group of archaea. This enzyme system is an extremely complex one consisting of eight different subunits and containing B_{12} as cofactor (Gottschalk and Thauer 2001). A related enzyme system may occur in *Acetobacterium woodii* and related organisms that are Na^+ dependent and generate a sodium ion-motive force during acetogenesis (Heise et al. 1989). This, however, is not true for all acetogens. Organisms such as *Clostridium aceticum* and *Moorella thermoautotrophica* are not Na^+ dependent; they contain cytochromes and apparently generate a proton gradient instead of a sodium ion gradient (Hugenholtz and Ljungdahl 1990).

Certain decarboxylases have been found to function as primary Na^+ pumps. They are membrane bound and they contain biotin. These enzymes occur in organisms such as *Propionigenium modestum*, *Acidaminococcus fermentans*, or *Klebsiella pneumoniae*, and the acids that are decarboxylated with Na^+ extrusion are oxaloacetate, methylmalonyl-CoA, glutaconyl-CoA, or malonyl-acyl carrier protein (malonyl-ACP; Dimroth 1997; Dimroth and Schink 1998). A scheme is depicted in Fig. 13.3.

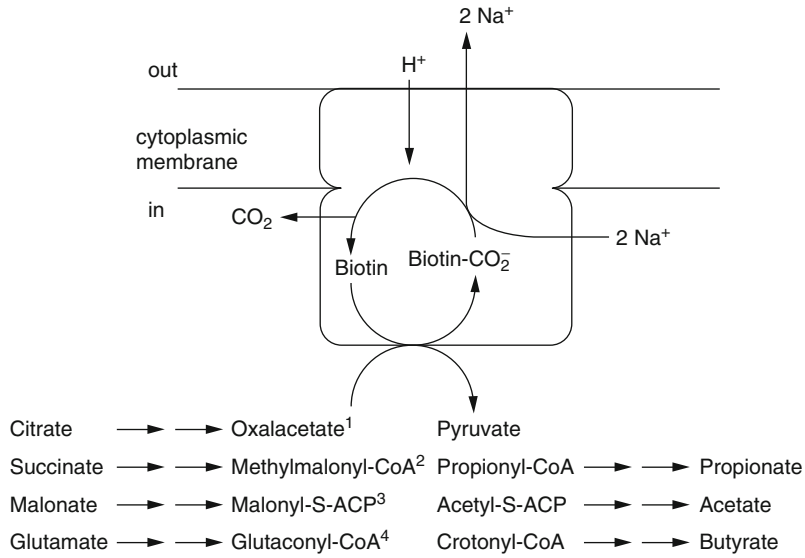


Fig. 13.3

Decarboxylation reactions coupled to sodium ion translocation: *1* oxaloacetate decarboxylase (e.g., is used by *Klebsiella pneumoniae* to ferment citrate), *2* methylmalonyl-coenzyme A (CoA) decarboxylase (e.g., is used by *Propionigenium modestum* for succinate metabolism), *3* malonyl-S-acyl carrier protein (ACP) decarboxylase (e.g., is used by *Malonomonas rubra* growing on malonate), and *4* glutaconyl-CoA decarboxylase (e.g., is used by *Acidaminococcus fermentans* to ferment glutamate)

Degradative Pathways

With respect to the degradation of substrates, the anaerobes have disadvantages and advantages. One difficulty is that in the absence of an external electron acceptor, anaerobes must balance their oxidation and reduction reactions. The electron donors and acceptors are derived from organic molecules of medium redox states such as sugars, organic acids, heterocyclic compounds, and amino acids. Often more reduced (e.g., ethanol) and more oxidized (e.g., CO_2) products are formed. In a few fermentations, the redox state of the substrate and the product is the same, for example, the fermentation of hexoses to two lactates or three acetates. Highly oxidized or reduced compounds such as carbon dioxide or hydrocarbons, respectively, are only suitable for fermentation together with inorganic electron donors or acceptors.

Another disadvantage of anaerobes is, of course, that oxygen cannot be employed for the initial attack of certain substrates such as hydrocarbons. On the other hand, there are a number of advantages. Oxygen-sensitive systems can be taken advantage of radical reactions or even of radical enzymes. So under the dictate of balanced redox reactions and with the involvement of unique enzymes and reactions, a fascinating array of unusual fermentations have evolved; some will be discussed now.

Coenzyme B_{12}-Dependent Pathways

When *Clostridium tetanomorphum* or *Clostridium cochlearium* grows on L-glutamate, the substrate is prepared for a cleavage into a two-carbon and a three-carbon compound in an interesting way. Under the catalysis of glutamate mutase (a B_{12}-containing enzyme), L-glutamate is converted to L-*threo*-β-methylaspartate (Buckel and Golding 1996b). This carbon-skeleton rearrangement facilitates the elimination of ammonia and formation of mesaconate by β-methylaspartase. Subsequently, mesaconate is hydrated to citramalate, which then is cleaved into acetate and pyruvate (Buckel 1980; *Fig. 13.4*). Oxidative decarboxylation of pyruvate results in the formation of acetyl-CoA and reduced ferredoxin, which is reoxidized during the synthesis of butyryl-CoA from two moles of acetyl-CoA. Then, ATP is synthesized in the acetate and butyrate kinase reactions (Barker 1981). By this pathway, a degradation of glutamate via the tricarboxylic acid cycle is circumvented; the latter would not be feasible because of an unbalanced generation of reducing equivalents in the form of NADH and $FADH_2$.

Coenzyme $B1_2$-dependent rearrangements like the glutamate mutase reaction proceed via radical intermediates; they are per se oxygen sensitive although another reaction of this type, the methylmalonyl-CoA mutase reaction, proceeds in higher eukaryotes such as man. This reaction is also of key importance in propionic acid bacteria and many other anaerobes because it allows the interconversion of succinate and propionate (*Fig. 13.5*).

A fermentation that involves a coenzyme B_{12}-dependent reaction and proceeds only under anaerobic conditions is the glycerol conversion to 1,3-propanediol. This fermentation was discovered in enteric bacteria such as *Citrobacter freundii* and *Klebsiella pneumoniae*; it proceeds as depicted in *Fig. 13.6*. Glycerol is oxidized to dihydroxyacetone, which is converted further to dihydroxyacetone phosphate. To balance

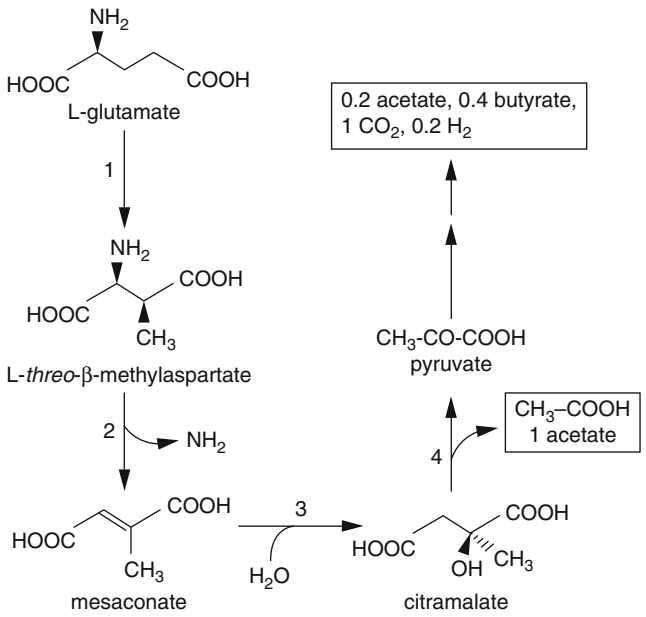


Fig. 13.4
Pathway of L-glutamate fermentation by *Clostridium tetanomorphum*: *1* glutamate mutase (coenzyme B_{12} dependent), *2* β-methylaspartase, *3* citramalate dehydratase, and *4* citramalate lyase

the fermentation, a portion of glycerol is dehydrated to 3-hydroxypropionaldehyde in a coenzyme B_{12}-dependent reaction. Subsequently, the aldehyde is reduced to the major fermentation product 1,3-propanediol, which is of great biotechnological interest. The bottleneck of the pathway is the coenzyme B_{12}-dependent glycerol dehydratase that is rapidly inactivated during glycerol dehydration (Daniel et al. 1998).

Degradation of Amino Acids and α-Hydroxy Carboxylic Acids

Novel reactions occur in a number of anaerobes for the utilization of α-amino acids and α-hydroxy carboxylic acids. If redox balance allows, these acids can be oxidized, of course, to the corresponding α-keto acids and then very easily metabolized further. So lactate or alanine can be oxidized to pyruvate and further to acetyl-CoA. This often is not possible because an acceptor for the electrons generated is not available. A commonly used pathway involves the reduction of the α-keto acids generated by deamination of amino acids to the corresponding hydroxy carboxylic acids, followed by activation to the CoA ester and dehydration to an enoyl-CoA (*Fig. 13.7*). A simple dehydration of α-hydroxy carboxylic acids is not feasible because it would have to proceed against the rule of Markovnikov (Jones 1961). A well-studied example is the dehydration of α-hydroxyglutaryl-CoA to glutaconyl-CoA carried out by *Acidaminococcus fermentans*. The enzyme, α-hydroxyglutaryl-CoA dehydratase, is extremely oxygen sensitive and contains (Fe-S) clusters, reduced riboflavin, and $FMNH_2$. The activation of the dehydratase is catalyzed by an activator protein and requires a reducing agent and catalytic amounts of ATP and Mg^{2+}. A novel mechanism involving thiol ester-derived radical anions (ketyls) has been postulated for these dehydrations (Buckel 1996a).

Another way to deal with certain α-amino acids is reductive deamination. Such deaminations are part of the Stickland reaction in which amino acids are fermented pairwise. Alanine, for instance, is oxidized, and the reducing equivalents generated are transferred to glycine:

$$1\ \text{Alanine} \rightarrow 1\ \text{acetate} + 1\ CO_2 + 1\ \text{ammonia} + 4H$$
$$2\ \text{Glycine} + 4H \rightarrow 2\ \text{acetate} + 2\ \text{ammonia}$$

The structurally related compounds betaine and sarcosine can also serve as hydrogen acceptors (Naumann et al. 1983; Hormann and Andreesen 1989), methylamines being formed instead of ammonia:

$$\text{Betaine} + 2H \rightarrow \text{trimethylamine} + \text{acetate}$$
$$\text{Sarcosine} + 2H \rightarrow \text{methylamine} + \text{acetate}$$

Acetate formation from glycine proceeds via acetyl phosphate, and the last step of acetate formation is catalyzed by acetate kinase giving rise to ATP synthesis by substrate-level phosphorylation. The key enzyme of glycine fermentation (glycine reductase) was well studied in *Eubacterium acidaminophilum* (Andreesen 1994). The enzyme consists of four proteins including one selenoprotein (enzyme A), a pyruvoyl protein (enzyme B), enzyme C, and thioredoxin. The reaction mechanism is depicted in *Fig. 13.8*. The pyruvoyl residue of enzyme B forms a Schiff base with glycine, which then reacts with the Se^- anion of protein A to yield a carboxymethyl selenocysteine residue linked to protein A and the iminopyruvoyl protein. Subsequently, ammonia is released by hydrolysis or in the next turnover. Elimination of ketene yields the oxidized protein A-Se-S intermediate, which is reduced by thioredoxin. Reduction of thioredoxin is catalyzed by thioredoxin reductase with NADH or another electron donor. The hypothetical ketene intermediate adds to the cysteine residue of protein C. An acetylcysteine is formed, which is cleaved by phosphate (P_i) to form acetyl phosphate. Again, this is a complex reaction, which only can be visualized to occur in anaerobes.

Degradation of Aromatic Compounds and Hydrocarbons

Most of the aromatic compounds studied to date are first transformed to benzoyl-CoA, the central intermediate of the best-studied pathway for anaerobic degradation of aromatic compounds (Harwood et al. 1999). Benzoyl-CoA then undergoes a reductive attack (Schink et al. 2000). The key enzyme for this attack is the benzoyl-CoA reductase, which

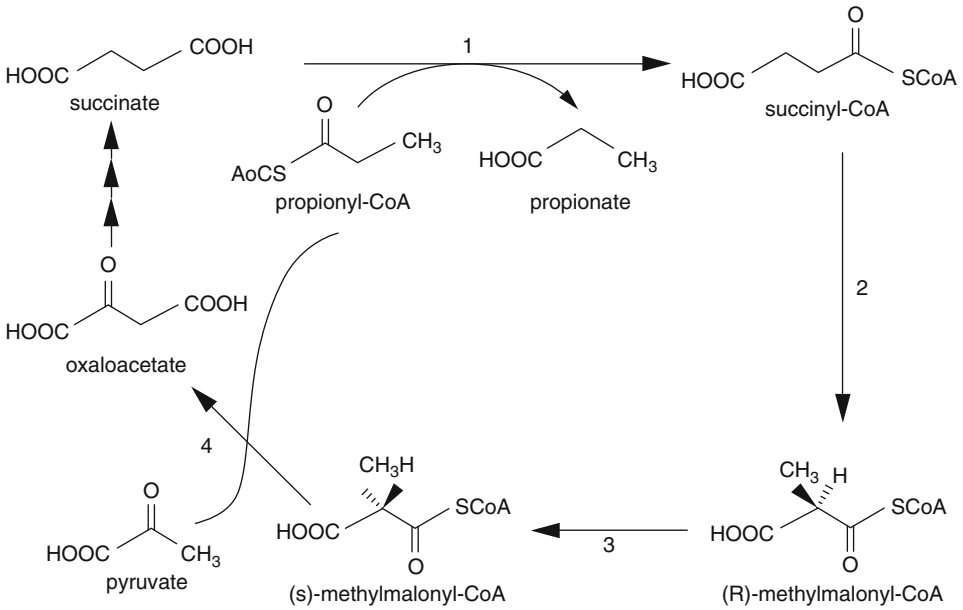


Fig. 13.5

Interconversion of succinate and propionate by methylmalonyl-CoA mutase: *1* propionate CoA-transferase, *2* methylmalonyl-CoA mutase (coenzyme B_{12} dependent), *3* methylmalonyl-CoA epimerase, and *4* transcarboxylase (biotin containing)

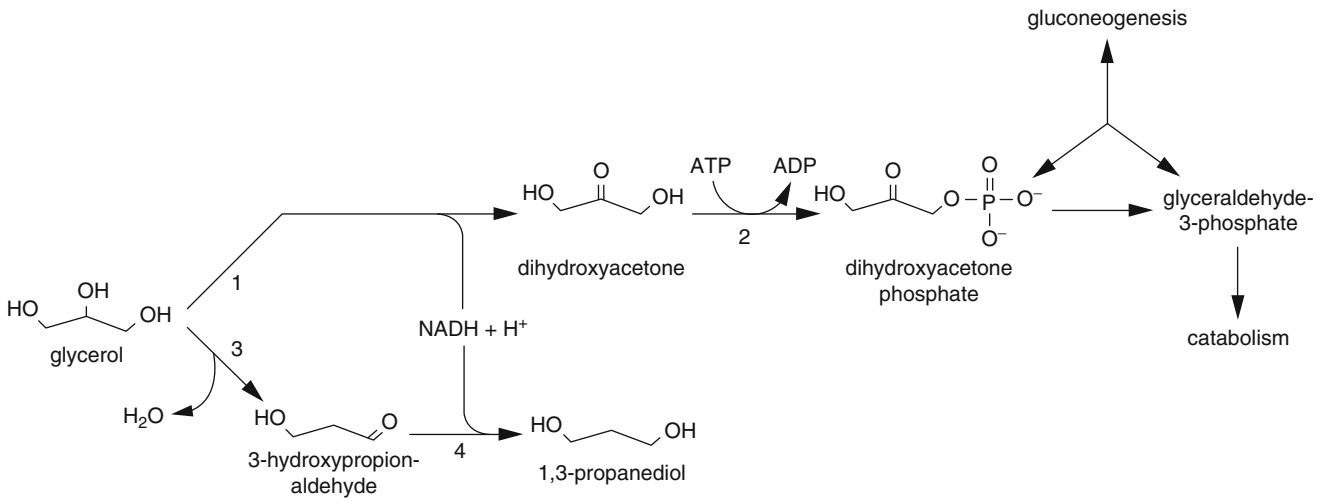


Fig. 13.6

Pathway of glycerol fermentation by *Citrobacter freundii*: *1* glycerol dehydrogenase, *2* dihydroxyacetone kinase, *3* glycerol dehydratase (coenzyme B_{12} dependent), and *4* 1,3-propanediol dehydrogenase

was purified from the denitrifying bacterium *Thauera aromatica* and characterized as an FAD- and iron-sulfur cluster-containing enzyme complex (Boll and Fuchs 1995). Under hydrolysis of ATP, one electron is added to the thiol ester carbonyl of benzoyl-CoA, and the resulting radical intermediate is reduced further to cyclohexa-1,5-dienecarboxyl-CoA (Buckel and Golding 1999; *Fig. 13.9*). This reaction may be of general importance for the anaerobic degradation of aromatic compounds. Recently, it was shown that the reductive strategy for destabilization of the ring is not the only one used in anaerobic degradation of aromatic compounds. Anaerobic degradation of 3,5-dihydroxybenzoate by *Thauera aromatica* (Philipp and Schink 2000) and 1,3-dihydroxybenzene by *Azoarcus anaerobius* (Philipp and Schink 1998) proceeds by a novel mechanism. Phenolic compounds with their hydroxyl groups in *meta* position to each other are hydroxylated by membrane-bound enzymes yielding hydroxyhydroquinone, which is later dehydrogenated to the nonaromatic compound hydroxybenzoquinone. Thus, oxidation rather than reduction is used to overcome the stability of the aromatic ring.

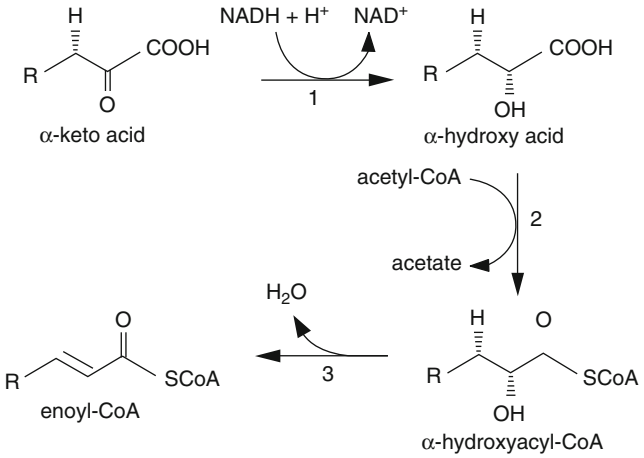


Fig. 13.7
α-Hydroxy acid pathway: *1* α-hydroxy acid dehydrogenase, *2* CoA transferase, and *3* α-hydroxyacyl-CoA dehydratase

Radical Enzymes

Glycyl radical enzymes are involved in a number of anaerobic reactions. Well-studied examples are the pyruvate formate lyase (Knappe et al. 1984), the anaerobic ribonucleotide reductase (Licht et al. 1996), and the benzyl succinate synthase (Leuthner et al. 1998). The latter initiates the breakdown of toluene under anaerobic conditions. These glycyl radical enzymes are formed from their precursor enzyme in a reaction, which requires S-adenosyl methionine. The pyruvate formate lyase of *Escherichia coli* is synthesized as an inactive and coenzyme-free protein. The enzyme is posttranslationally modified by S-adenosyl methionine and a reduced flavodoxin in a reaction catalyzed by an activase. A hydrogen atom is abstracted from a specific glycine residue, yielding methionine and 5′-deoxyadenosine from S-adenosyl methionine. The formed free radical (HS-enzyme) is involved in a two-step reaction:

$$\text{Pyruvate} + \text{HS-enzyme} \rightarrow \text{acetyl-S-enzyme} + \text{formate}$$
$$\text{Acetyl-S-enzyme} + \text{CoASH} \rightarrow \text{acetyl-SCoA} + \text{HS-enzyme}$$

Pyruvate formate lyase, like the other glycyl radical enzymes, is rapidly inactivated by oxygen.

Anaerobic alkane-degrading bacteria have also been isolated recently. Alkanes are used as substrates by several species of sulfate-reducing microorganisms (Aeckersberg et al. 1998). Another group of anaerobic hydrocarbon-degrading bacteria is dependent on syntrophic associations with methanogens. The biochemistry of the process is still poorly understood, but it can be speculated that again radicals are generated to initiate this breakdown (Zengler et al. 1999).

A number of potentially hazardous compounds in our environment are halogenated (e.g., pentachlorophenol or perchloroethene). These compounds can be partially or completely degraded under anaerobic conditions. This degradation occurs by reductive dehalogenations. Organisms such as *Desulfitobacterium dehalogenans*, *Dehalobacter restrictus*, or *Dehalospirillum multivorans* contain corrinoid proteins, which exhibit dehalogenase activities (Holliger et al. 1999). There is evidence that these H_2-dependent fermentations are also coupled with the generation of a proton-motive force.

Anaerobic Food Chains

The anaerobic degradation of complex organic matter depends on the cooperation of various trophic groups of anaerobic bacteria and archaea. Two possible schemes for anaerobic food chains, as they occur in nature in the absence or in the presence of sulfate, are presented in ❯ *Fig. 13.10*. Polymers such as polysaccharides, proteins, and nucleic acids are initially converted to oligomers and monomers and subsequently fermented by the "classical" primary fermentative bacteria. In the absence of sulfate, the products acetate, methanol, methylamines, CO_2, and H_2 can be used directly by methanogenic bacteria to convert them to methane and carbon dioxide. Alcohols longer than one carbon atom, fatty acids longer than two carbon atoms, and branched or aromatic fatty acids are degraded by the secondary fermenters to acetate, C1 compounds, and H_2, which are subsequently used by the methanogens. Because the reactions catalyzed by the secondary fermentative bacteria are mostly endergonic under standard conditions, they depend on a very efficient cooperation with the subsequent partners. Such cooperations are called syntrophic relationships, in which the pool size of shuffling intermediate has to be kept small to allow efficient degradation. In sulfate-rich anaerobic habitats, such as marine sediments, sulfate-reducing bacteria further degrade the primary fermentation products. As many sulfate reducers are metabolically more versatile than methanogenic bacteria, they can use and oxidize all classical fermentation products to carbon dioxide, simultaneously reducing sulfate to sulfide (Hansen 1994; Jansen and Hansen 1998; Zengler et al. 1999; ❯ *Fig. 13.10b*). In addition to the primary fermentations that have already been mentioned, three important points should be briefly discussed here: the fate of acetate under anaerobic conditions, production of H_2, and the syntrophic relationships.

Acetate is the end product of a number of fermentations starting from substrates with two (e.g., ethanol) or more carbon atoms (e.g., glucose), but it is also produced by acetogenic organisms from one-carbon compounds (e.g., methanol) and from $H_2 + CO_2$. Because so many pathways lead to the formation of acetate under anaerobic conditions, the further degradation of acetate is of great importance for carbon flow under anaerobic conditions. Among the methanogenic archaea, only species of the genera *Methanosarcina*, *Methanosaeta*, and *Methanothrix* are able to utilize and degrade acetate to methane and carbon dioxide (e.g., *Methanosarcina barkeri*, *Methanothrix thermophila*, and *Methanosaeta concilii*). The degradation occurs according to the following equation (Thauer et al. 1989):

$$CH_3\text{-}COOH \rightarrow CH_4 + CO_2 \Delta G^{0'} = -36\ \text{kJ/mol}$$

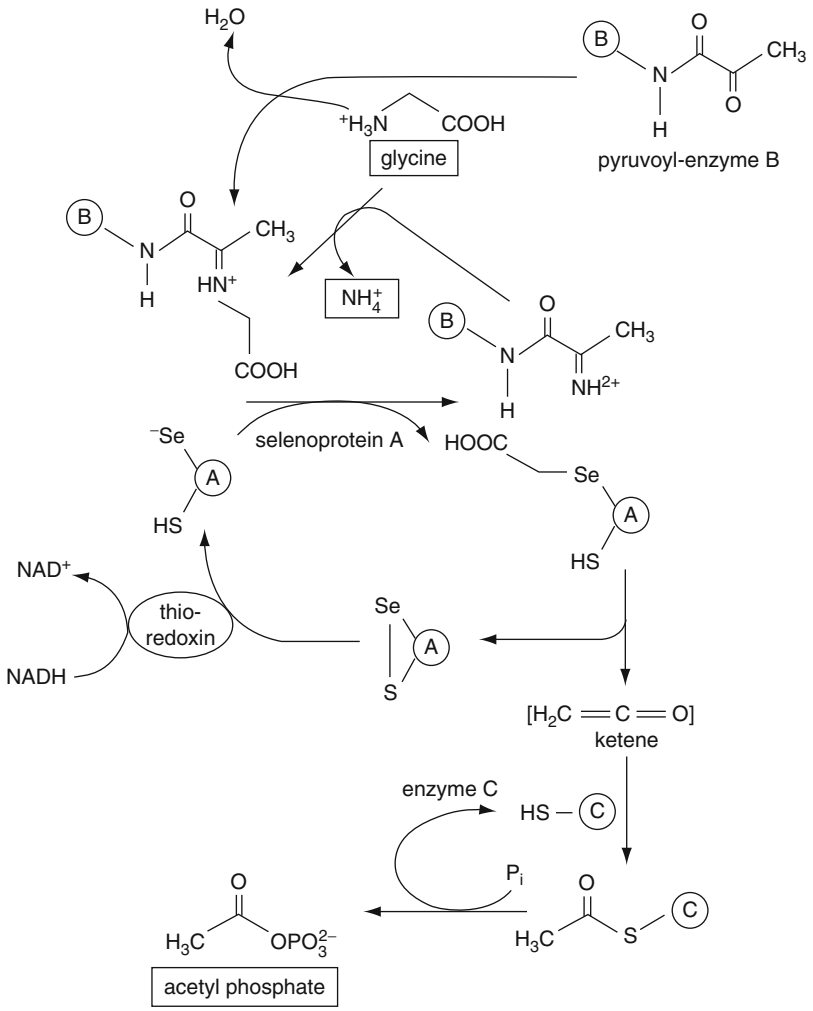


Fig. 13.8
Mechanism of glycine reductase

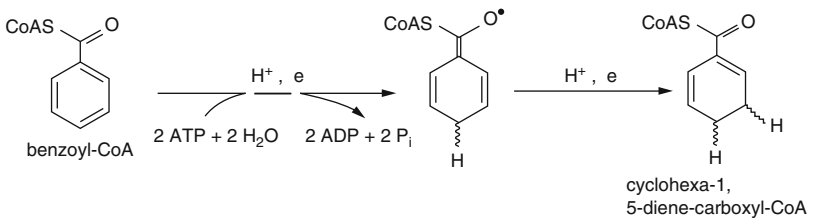


Fig. 13.9
Mechanism of benzoyl-CoA reductase

Initially, acetate is activated to acetyl-CoA by acetate kinase and phosphotransacetylase or directly by acetyl-CoA synthetase (*Methanosaeta*). Acetyl-CoA is subsequently bound to the carbon monoxide (CO) dehydrogenase complex, at which it is decarbonylated by cleavage of the carbon-carbon bond. The methyl group is subsequently transferred via tetrahydromethanopterin (THMP) to coenzyme M, and CO is oxidized to CO_2, providing the reducing equivalents for the reduction of the methyl-coenzyme M to methane by the pathway shown in *Fig. 13.11* (Thauer 1998; Ferry 1997, 1999). It is interesting that the CO dehydrogenase complex, which catalyzes the decarbonylation of acetyl-CoA to methyl-THMP and CO and the oxidation of CO, also catalyzes the reactions mentioned in a reversible manner. In methanogens utilizing acetate, the direction of decarbonylation predominates; when, however, organisms such as *Methanobacterium thermoautotrophicum* grow with $H_2 + CO_2$, they use this enzyme system to synthesize acetyl-CoA from methyl-coenzyme M and CO for

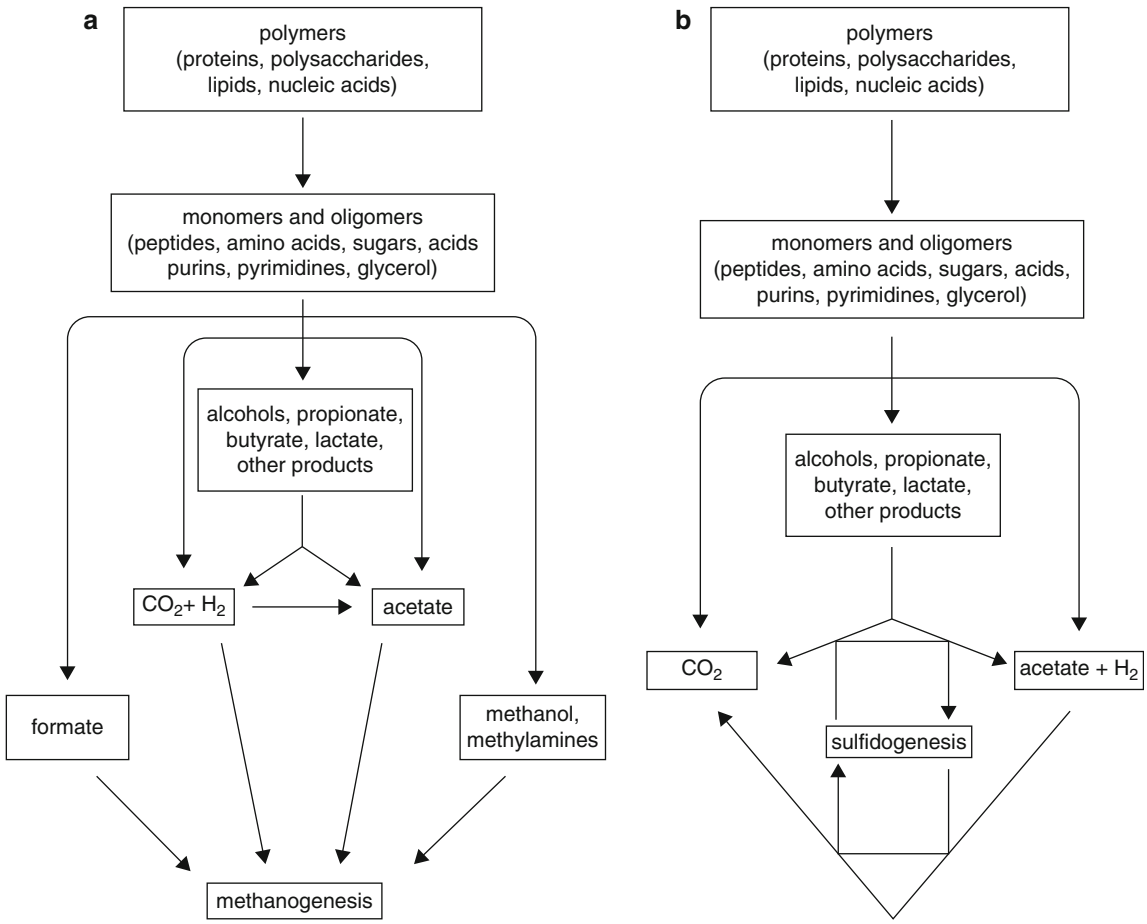


Fig. 13.10

Anaerobic food chains. (a) Methanogenesis. As a terminal process, all organic material is metabolized to methane via a few methanogenic substrates: CO_2 + H_2, acetate, formate, methanol, and methylamines. (b) Sulfidogenesis. As a terminal process, incomplete oxidizers convert various products to CO_2 and acetate, and the complete oxidizers couple sulfate reduction with acetate oxidation to CO_2. In addition, H_2 can be used for sulfate reduction

autotrophic growth (Zeikus 1983; Fuchs 1986; Shieh and Whitman 1988; Huber and Wächtershäuser 1997). Similarly, acetogenic bacteria such as *Acetobacterium woodii* and *Moorella thermoacetica* produce acetyl-CoA from methyl-tetrahydrofolate and CO (Wood et al. 1986; Ljungdahl 1986; Shanmugasundaram et al. 1988; Menon and Ragsdale 1999).

A number of sulfate-reducing bacteria are also able to oxidize acetate completely to CO_2 under anaerobic conditions:

$$CH_3\text{-}COOH + SO_4^{2-} + H^+ \rightarrow 2CO_2 + HS^- + 2H_2O$$

Most of them also take advantage of the described C1 pathway with the CO dehydrogenase complex for decarbonylating acetyl-CoA. The pathway is investigated in more detail in *Desulfotomaculum acetoxidans*, *Desulfobacterium autotrophicum*, and in the archaeon *Archaeoglobus fulgidus* (Spormann and Thauer 1988; Hansen 1994; Möller-Zinkhahn et al. 1989; Brüggemann et al. 2000). Only a small number of acetate-oxidizing sulfate reducers (e.g., *Desulfobacter postgatei*) use the tricarboxylic acid cycle to carry out acetyl-CoA oxidation (Brandis-Heep 1983; Möller et al. 1987; Thauer 1988; Thauer et al. 1989). Activation of acetate in *D. postgatei* occurs by a succinyl-CoA: acetate CoA-transferase; acetate kinase and phosphotransacetylase are lacking.

Many fermentation reactions are associated with the evolution of molecular hydrogen, H_2. This allows a shift from producing alcohols and lactate to acetate and butyrate, a shift beneficial to the organisms because the ATP yield is increased. Important precursors of H_2 are formate and reduced ferredoxin, and H_2 formation is catalyzed by formate hydrogenlyase and hydrogenase, respectively. There are two important reactions coupled to ferredoxin reduction and ultimately to H_2 formation:

1. The pyruvate-ferredoxin oxidoreductase reaction:

$$\text{Pyruvate} + Fd_{ox} \Leftrightarrow Fd_{red} + \text{acetyl-CoA} + CO_2 \Delta G^{0'} = -19.2\ \text{kJ/mol}$$

The reaction is exergonic so that it can drive H_2 formation even at a hydrogen partial pressure (PH_2) of 1.013 kPa. The enzyme was first purified from *Clostridium acidiurici* (Uyeda and Rabinowitz 1971; Charon et al. 1999).

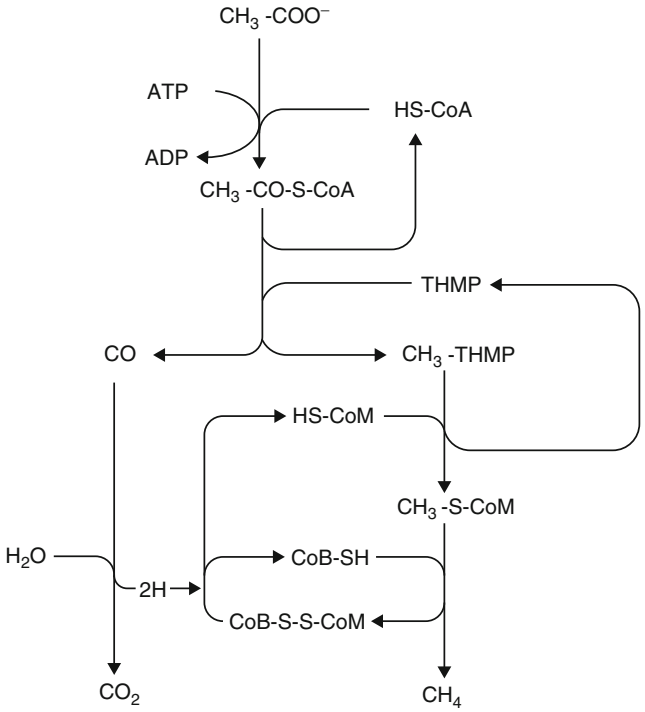


Fig. 13.11
Conversion of acetyl-CoA to methane and carbon dioxide. *THMP*, tetrahydromethanopterin; *HS-CoM*, coenzyme M

2. The NADH-ferredoxin oxidoreductase:

$$\mathrm{NADH} + \mathrm{Fd_{ox}} \Leftrightarrow \mathrm{Fd_{red}} + \mathrm{NAD^+} + \mathrm{H^+} \Delta G^{0'} = +18.8\ \mathrm{kJ/mol}$$

This reaction was discovered in *C. kluyveri* (Jungermann et al. 1969; Gottschalk and Chowdhury 1969); it is endergonic and will only proceed at a largely reduced P_{H2}. In anaerobic habitats, the P_{H2} is kept as low as 10 Pa by H_2-consuming organisms such as the methanogenic archaea and acetogenic and sulfidogenic bacteria. Hydrogen consumption by these microorganisms results in the phenomenon of interspecies hydrogen transfer, which has two consequences. First, the product patterns of saccharolytic fermentations as carried out by many clostridia are changed; for example, glucose can be fermented to acetate and CO_2. The second consequence of the generation of a low P_{H2} by the hydrogen-consuming bacteria is that it opens up an ecological niche for a fascinating group of anaerobes, the obligate proton-reducing bacteria. These organisms were first described in 1967, when a culture called "*Methanobacillus omelianskii*" was found to consist of two different organisms carrying out two different fermentations (Bryant et al. 1967):

1. The "S" organism carries out ethanol oxidation:

$$\mathrm{CH_3\text{-}CH_2\text{-}OH} + \mathrm{H_2O} \rightarrow \mathrm{CH_3\text{-}COOH} + 2\mathrm{H_2}$$

2. A methanogenic archaeon consumes molecular hydrogen for methane production:

$$2\mathrm{H_2} + 1/2\mathrm{CO_2} \rightarrow 1/2\mathrm{CH_4} + \mathrm{H_2O}$$

Cocultures of this type were termed "syntrophic" cultures because the organisms involved mutually depend on one another. Molecular H_2 evolution allows fermentative growth of the "S" organism but only if the PH_2 is kept low enough by the methanogenic bacterium. The term "interspecies hydrogen transfer" was coined for this kind of connection between H_2 evolution and H_2 consumption. Other examples for syntrophically ethanol-oxidizing bacteria known today are *Thermoanaerobacterium brockii* (Ben-Bassat et al. 1981), Pelobacter species (Schink 1984, 1985), and, in the absence of sulfate, *Desulfovibrio vulgaris* (Bryant et al. 1977). Not only alcohols but also organic acids can be oxidized to acetate and H_2 this way, such as propionate by *Syntrophobacter pfennigii* (Wallrabenstein et al. 1995) and butyrate by *Syntrophomonas* species (Roy et al. 1986; McInerney et al. 1981). As these oxidations are more endergonic than alcohol oxidations, PH_2 has to be decreased to significantly lower values (<10 Pa) than, for example, for ethanol (<100 Pa). Syntrophic degradation of aromatic compounds, amino acids, and glycolate also has been discovered (Mountfort and Bryant 1982; Friedrich et al. 1991; Feigel and Knackmuss 1993). In addition to methanogens, H_2 consumption can also occur by sulfur and sulfate reducers and homoacetogenic fumarate-reducing or glycine-reducing bacteria (Schink 1997). In this respect, one species is of special interest, the homoacetogenic strain, named "acetate-oxidizing rod-shaped eubacterium" (AOR), which can either oxidize or synthesize acetate depending on the PH_2 (Lee and Zinder 1988a, b; Zinder and Koch 1984). Based on interspecies hydrogen transfers, metabolically different bacteria are connected in syntrophic relationships, which are a speciality of obligate anaerobes, and are the basis for the formation of consortia and aggregates in which various types of microorganisms are in close physical contact (Schink and Thauer 1987; Stams 1994; Schink 1997). One recent example is a marine consortium consisting of archaea and sulfate-reducing bacteria, which apparently mediate the anaerobic oxidation of methane (Boetius et al. 2000). However, the elusive microorganisms responsible for this conversion have not yet been isolated, and the pathway of anaerobic oxidation of methane is not characterized yet.

Anaerobes and Molecular Oxygen: Oxygen Sensitivity and Sensing

Oxygen Sensitivity

Metabolic reduction of oxygen results in the production of highly toxic and reactive oxygen species ("ROS"), such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$) (Fridovich 1995; Cannio et al. 2000b). The latter is most likely responsible for the toxic effects of molecular oxygen. Its production can occur nonenzymatically, when superoxide reacts with hydrogen peroxide in the presence of transition metals (Haber-Weiss reaction (Eq. 13.6); Haber and Weiss 1934). The most probable sites involved in the

production of superoxide and hydrogen peroxide at the level of the electron transport chain are cytochrome b_{566} and ubiquinone (Eqs. 13.3 and 13.4). In addition, superoxide can be generated from O_2 by nonspecific oxidations of reduced flavines, catecholamines, and tetrahydrofolates or chemically:

$$O_2 + 1e^- \rightarrow O_{2^-\cdot} \quad (13.3)$$

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (13.4)$$

$$O_{2^-\cdot} + e^- + 2H^+ \rightarrow H_2O_2 \quad (13.5)$$

$$O_{2^-\cdot} + H_2O_2 \rightarrow OH^- + \cdot OH + \cdot O_2 \quad (13.6)$$

Most aerobes have developed appropriate protective mechanisms to overcome the toxic effects of hydrogen peroxide and the superoxide anion. Nonenzymic detoxification is effected by glutathione, which is present in high concentration in many bacteria. The main detoxification, however, involves the enzymes superoxide dismutase (Eq. 13.7), catalase (Eq. 13.8), and nonspecific peroxidases (Eq. 13.9) (Fridovich 1995; Niimura et al. 2000):

$$O_{2^-\cdot} + O_{2^-\cdot} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 \quad (13.7)$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \quad (13.8)$$

$$H_2O_2 + RH_2 \xrightarrow{\text{peroxidase}} 2H_2O + R \quad (13.9)$$

Four classes of superoxide dismutases have been identified based on the metal cofactor, which can be either dinuclear Cu/Zn or monomeric Fe, Mn, or Ni (Whittaker and Whittaker 1998). Most bacteria contain only one superoxide dismutase with either Fe or Mn as cofactor. However, *E. coli* contains both types, the Mn type only induced during aerobiosis and the Fe type under both conditions, in the presence and absence of oxygen (Kargalioglu and Imlay 1994). The novel type of superoxide dismutase with Ni as a cofactor has been recently discovered in several *Streptomyces* species (Youn et al. 1996a, b). Interestingly, a few examples for extracellular superoxide dismutases are known, for example, in *Sulfolobus solfataricus* (Cannio et al. 2000a) and *Streptococcus pyogenes* (Gerlach et al. 1998). Catalases are generally present in aerobic and facultative anaerobic bacteria.

Among the obligate anaerobic bacteria, organisms are found that are more or less aerotolerant. Many of these organisms (e.g., a number of lactic acid bacteria) have been shown to contain superoxide dismutase and lack catalase (Morris 1976; Archibald and Fridovich 1981). A number of obligate anaerobes, however, are extremely oxygen sensitive. Most noteworthy in this respect are the methanogenic archaea, clostridial species, and sulfate-reducing bacteria. Cultivation of those anaerobes in the laboratory requires special precautions (Hungate 1969). Simple exclusion of molecular oxygen is not sufficient to provide good conditions of growth. In addition, they require a low redox potential in their environment and growth media supplemented with compounds such as ascorbate, hydrogen sulfide, sodium thioglycolate, or cysteine. Curiously, despite their catalytic capacity for producing molecular oxygen, Fe-containing superoxide dismutases have been discovered in the methanogenic archaea *Methanobacterium bryantii* (Kirby et al. 1981), *Methanobacterium thermoautotrophicum* (Takao et al. 1991; Meile et al. 1995), and *Methanosarcina mazei* Gö1 (G. Gottschalk, unpublished observation). Further examples of Fe-containing superoxide dismutases in strict anaerobes have been reported for sulfate-reducing bacteria, for example, *Desulfovibrio desulfuricans* (Hatchikian and Henry 1971) and *Desulfoarculus boarsii* (Pianzzola et al. 1996). Interestingly, a superoxide reductase from the hyperthermophilic anaerobic *Pyrococcus furiosus* has been discovered, which reduces superoxide without the production of oxygen and therefore confers a selective advantage for anaerobes (Jenney et al. 1999). The physiological role of superoxide dismutases and superoxide reductases in anaerobes that supposedly evolved in ecosystems lacking oxygen, however, has to be elucidated.

Oxygen Sensing

Adaptation of facultative anaerobic microorganisms to anaerobic growth conditions is accompanied by dramatic changes in metabolic gene expression. To make these adaptations, those microorganisms have to be able to sense changes in the environmental oxygen availability. Various sensory and regulatory systems control the expression of aerobic and anaerobic metabolism in response to oxygen. Most of the oxygen sensor proteins known today contain heme, iron-sulfur clusters, or iron as cofactors, for example, FixL from *Sinorhizobium meliloti* (Gilles-Gonzalez et al. 1995), the fumarate nitrate regulator (Fnr) and SoxR from *E. coli* (Hidalgo et al. 1995), and rhizobial NifA proteins (Fischer 1994, 1996), respectively. The Fnr from *E. coli*, which is one of the prominent examples for oxygen sensing and redox control of gene expression in prokaryotes, will be briefly discussed here.

The global regulator Fnr controls transcription of genes, whose functions facilitate adaptation to growth under oxygen limitation (Spiro 1994; Bauer et al. 1999). Under anaerobic conditions, it contains a [4Fe4S] cluster, which is required for the oxygen-sensing function. Recent data suggest that this [4Fe4S] cluster is sufficiently unstable toward oxygen and apparently mediates the sensitivity of the transcriptional activator to oxygen (Khoroshilova et al. 1997; Kiley and Beinert 1998; Beinert and Kiley 1999). The presence of the [4Fe4S] cluster favors dimerization of Fnr, which is correlated with increased site-specific DNA binding of the transcriptional activator Fnr. Upon the presence of oxygen, the [4Fe4S] cluster is disrupted, resulting in the conversion of transcriptionally active Fnr dimers into inactive monomers (Lazazzera et al. 1996; Melville and Gunsalus 1996; Beinert and Kiley 1999). Homologues of Fnr have been identified in several facultative anaerobic bacteria,

some of which differ with respect to the cysteine residues and the coordination of the iron-sulfur clusters (reviewed in Spiro 1994; Cruz Ramos et al. 1995; Saunders et al. 1999; Vollack et al. 1999).

References

Aeckersberg F, Rainey FA, Widdel F (1998) Growth, natural relationships, cell fatty acids and metabolic adaptation of sulfate-reducing bacteria that utilize long-chain alkanes under anoxic conditions. Arch Microbiol 170:361–369

Andreesen JR (1994) Glycine metabolism in anaerobes. Ant v Leeuwenhoek 66:223–227

Archibald FS, Fridovich I (1981) Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. J Bacteriol 146:928–936

Badziong W, Thauer RK (1978) Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy sources. Arch Microbiol 117:209–214

Barker HA (1981) Amino acid degradation by anaerobic bacteria. Ann Rev Biochem 50:23–40

Bauer CE, Elsen S, Bird TH (1999) Mechanisms for redox control of gene expression. Ann Rev Microbiol 53:495–523

Bäumer S, Ide T, Jacobi C, Johann A, Gottschalk G, Deppemeier U (2000) The $F_{420}H_2$ dehydrogenase from *Methanosarcina mazei* Gö1 is a redox-driven proton pump closely related to NADH dehydrogenases. J Biol Chem 275:17968–17973

Beinert H, Kiley PJ (1999) Fe-S proteins in sensing and regulatory functions. Curr Opin Chem Biol 3:152–157

Ben-Bassat A, Lamed R, Zeikus JG (1981) Ethanol production by thermophilic bacteria: metabolic control of end product formation in *Thermoanaerobium brockii*. J Bacteriol 146:192–199

Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jörgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Boll M, Fuchs G (1995) Benzoyl-coenzyme A reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism. ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. Eur J Biochem 234:921–933

Brandis-Heep A, Gebhardt NA, Thauer RK, Widdel F, Pfennig N (1983) Anaerobic acetate oxidation to CO_2 by *Desulfobacter postgatei*. 1: demonstration of all enzymes required for the operation of the citric acid cycle. Arch Microbiol 36:222–229

Brüggemann H, Falinski F, Deppenmeier U (2000) Structure of the $F_{420}H_2$: quinone oxidoreductase of *Archaeoglobus fulgidus* identification and overproduction of the $F_{420}H_2$-oxidizing subunit. Eur J Biochem 267:5810–5814

Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch Microbiol 59:20–31

Bryant MP, Campbell LL, Reddy CA, Crabill MR (1977) Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl Environ Microbiol 33:1162–1169

Buckel W (1980) Analysis of fermentation pathways of clostridia using double labelled glutamate. Arch Microbiol 127:167–169

Buckel W (1996) Unusual dehydrations in anaerobic bacteria: considering ketyls (radical anions) as reactive intermediates in enzymatic reactions. FEBS Lett 389:20–24

Buckel W, Golding BT (1996) Glutamate and 2-methyleneglutarate mutase: from microbial curiosities to paradigms for coenzyme B_{12}-dependent enzymes. Chem Soc Rev 25:329–337

Buckel W, Golding BT (1999) Radical species in the catalytic pathways of enzymes from anaerobes. FEMS Microbiol Rev 22:523–541

Cannio R, D'angelo A, Rossi M, Bartolucci S (2000a) A superoxide dismutase from the archaeon *Sulfolobus solfataricus* is an extracellular enzyme and prevents the deactivation by superoxide of cell-bound proteins. Eur J Biochem 267:235–243

Cannio R, Fiorentino G, Morana A, Rossi M, Bartolucci S (2000b) Oxygen: friend or foe? Archaeal superoxide dismutases in the protection of intra- and extracellular oxidative stress. Front Biosci 5:768–779

Charon MH, Volbeda A, Chabriere E, Pieulle L, Fontecilla-Camps JC (1999) Structure and electron transfer mechanism of pyruvate: ferredoxin oxidoreductase. Curr Opin Struct Biol 9:663–669

Cruz Ramos H, Boursier L, Moszer I, Kunst F, Danchin A, Glaser P (1995) Anaerobic transcription activation in *Bacillus subtilis*: identification of distinct FNR-dependent and-independent regulatory mechanisms. EMBO J 14:5984–5994

Daniel R, Bobik TA, Gottschalk G (1998) Biochemistry of coenzyme B_{12}-dependent glycerol and diol dehydratases and organization of the encoding genes. FEMS Microbiol Rev 22:553–566

Deppenmeier U, Müller V, Gottschalk G (1996) Pathways of energy conservation in methanogenic Archaea. Arch Microbiol 165:149–163

Deppenmeier U, Lienard T, Gottschalk G (1999) Novel reactions involved in energy conservation by methanogenic archaea. FEEBS Lett 457:291–297

Dimroth P (1997) Primary sodium ion translocating enzymes. Biochim Biophys Acta 1318:11–51

Dimroth P, Schink B (1998) Energy conservation in the decarboxylation of dicarboxylic acids by fermenting bacteria. Arch Microbiol 170:69–77

Feigel BJ, Knackmuss HJ (1993) Syntrophic interactions during degradation of 4-aminobenzenesulfonic acid by a two species bacterial culture. Arch Microbiol 159:124–130

Ferry JG (1997) Enzymology of the fermentation of acetate to methane by *Methanosarcina thermophila*. Biofactors 6:25–35

Ferry JG (1999) Enzymology of one-carbon metabolism in methanogenic pathways. FEMS Microbiol Rev 23:13–38

Fischer HM (1994) Genetic regulation of nitrogen fixation in rhizobia. Microbiol Rev 58:352–386

Fischer HM (1996) Environmental regulation of rhizobial symbiotic nitrogen fixation genes. Trends Microbiol 4:317–320

Fridovich I (1995) Superoxide radical and superoxide dismutases. Ann Rev Biochem 64:97–112

Friedrich M, Laderer U, Schink B (1991) Fermentative degradation of glycolic acid by defined syntrophic cocultures. Arch Microbiol 156:398–404

Fuchs G (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol Rev 39:181–213

Gerlach D, Reichardt W, Vettermann S (1998) Extracellular superoxide dismutase from *Streptococcus pyogenes* type 12 strain is manganese-dependent. FEMS Microbiol Lett 160:217–224

Gilles-Gonzalez MA, Gonzalez G, Perutz MF (1995) Kinase activity of oxygen sensor FixL depends on the spin state of its heme iron. Biochemistry 34:232–236

Gottschalk G, Chowdhury AA (1969) Pyruvate synthesis from acetyl coenzyme A and carbon dioxide with $NADH_2$ or $NADPH_2$ as electron donors. FEBS Lett 2:342–344

Gottschalk G, Thauer RK (2001) The Na^+ translocating methyltransferase complex from methanogenic archaea. Biochim Biophys Acta 1505:28–36

Gross R, Simon J, Theis F, Kröger A (1998) Two membrane anchors of *Wolinella succinogenes* hydrogenase and their function in fumarate and polysulfide respiration. Arch Microbiol 170:50–58

Haber F, Weiss J (1934) The catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc London Ser A 147:332–352

Hansen TA (1994) Metabolism of sulfate-reducing prokaryotes. Ant v Leeuwenhoek 66:165–185

Harwood CS, Burchhardt G, Herrmann H, Fuchs G (1999) Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. FEMS Microbiol Rev 22:439–458

Hatchikian EC, Henry YA (1977) An iron-containing superoxide dismutase from the strict anaerobe *Desulfovibrio desulfuricans* (Norway 4). Biochimie 59:153–161

Hedderich R, Klimmek O, Kröger A, Dirmeier R, Keller M, Stetter KO (1999) Anaerobic respiration with elemental sulfur and with disulfides. FEMS Microbiol Rev 22:353–381

Heise R, Müller V, Gottschalk G (1989) Sodium dependence of acetate formation by the acetogenic bacterium *Acetobacterium woodii*. J Bacteriol 171:5473–5478

Hidalgo E, Bollinger JM Jr, Bradley TM, Walsh CT, Demple B (1995) Binuclear [2Fe-2S] clusters in the *Escherichia coli* SoxR protein and role of the metal centers in transcription. J Biol Chem 270:20908–20914

Holliger C, Wohlfarth G, Diekert G (1999) Reductive dechlorination in the energy metabolism of anaerobic bacteria. FEMS Microbiol Rev 22:383–398

Hormann K, Andreesen JR (1989) Reductive cleavage of sarcosine and betaine by *Eubacterium acidaminophilum* via enzyme systems different from glycine reductase. Arch Microbiol 153:50–59

Huber C, Wächtershäuser G (1997) Activated acetic acid by carbon fixation on (Fe, Ni)S under primordial conditions. Science 276:245–247

Hugenholtz J, Ljungdahl LG (1990) Metabolism and energy generation in homoacetogenic clostridia. FEMS Microbiol Rev 87:383–390

Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. In: Norris JB, Ribbons DW (eds) Methods in microbiology, vol 3B. Academic, New York/London, pp 117–132

Jansen M, Hansen TA (1998) Tetrahydrofolate serves as a methyl acceptor in the demethylation of dimethylsulfoniopropionate in cell extracts of sulfate-reducing bacteria. Arch Microbiol 169:84–87

Jenney FE Jr, Verhagen MF, Cui X, Adams MW (1999) Anaerobic microbes: oxygen detoxification without super oxide dismutase. Science 286:306–309

Jones G (1961) The Markovnikov rule. J Chem Educ 38:297–300

Jungermann K, Thauer RK, Rupprecht E, Ohrloff C, Decker K (1969) Ferredoxin-mediated hydrogen formation from NADH in a cell-free system of *Clostridium kluyveri*. FEBS Lett 3:144–146

Kargalioglu Y, Imlay JA (1994) Importance of anaerobic superoxide dismutase synthesis in facilitating outgrowth of *Escherichia coli* upon entry into an aerobic habitat. J Bacteriol 176:7653–76538

Khoroshilova N, Popescu C, Munck E, Beinert H, Kiley PJ (1997) Iron-sulfur cluster disassembly in the FNR protein of *Escherichia coli* by O_2: [4Fe-4S] to [2Fe-2S] conversion with loss of biological activity. Proc Natl Acad Sci USA 94:6087–6092

Kiley PJ, Beinert H (1998) Oxygen sensing by the global regulator, FNR: the role of the iron-sulfur cluster. FEMS Microbiol Rev 22:341–352

Kirby TW, Lancaster JR Jr, Fridovich I (1981) Isolation and characterization of the iron-containing superoxide dismutase of *Methanobacterium bryantii*. Arch Biochem Biophys 210:140–148

Knappe J, Neugebauer FA, Blaschkowski HP, Gänzler M (1984) Post-translational activation introduces a free radical into pyruvate formate-lyase. Proc Natl Acad Sci USA 81:1332–1335

Konings WN, Lokema SJ, van Veen HW, Poolman B, Driessen AJM (1997) The role of transport processes in survival of lactic acid bacteria. Ant v Leeuwenhoek 71:117–128

Kröger A, Geißler V, Lemma E, Theis F, Lenger R (1992) Bacterial fumarate respiration. Arch Microbiol 158:311–314

Lancaster RCD, Kröger A (2000) Succinate: quinone oxidoreductases: new insights from X-ray crystal structures. Biochim Biophys Acta 1459:422–431

Lazazzera BA, Beinert H, Khoroshilova N, Kennedy MC, Kiley PJ (1996) DNA binding and dimerization of the Fe-S-containing FNR protein from *Escherichia coli* are regulated by oxygen. Biol Chem 271:2762–2768

Lee MJ, Zinder SH (1988a) Carbon monoxide pathway enzyme activities in a thermophilic anaerobic bacterium grown acetogenically and in a syntrophic acetate oxidizing coculture. Arch Microbiol 150:513–518

Lee MJ, Zinder SH (1988b) Isolation and characterization of a thermophilic bacterium, which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H_2-CO_2. Appl Environ Microbiol 54:124–129

Leuthner B, Leutwein C, Schulz H, Hörth P, Hachnel W, Schlitz E, Schägger H, Heider J (1998) Biochemical and genetic characterisation of benzylsuccinate synthase from *Thauera aromatica*: a new glycyl-radical enzyme catalysing the first step in anaerobic toluene degradation. Molec Microbiol 28:615–628

Licht S, Gerfen GJ, Stubbe J (1996) Thiyl radicals in ribonucleotide reductases. Science 271:477–481

Ljungdahl LG (1986) The autotrophic pathway of acetate synthesis in acetogenic bacteria. Ann Rev Microbiol 40:415–450

McInerney MJ, Bryant MP, Hespell RB, Costerton JW (1981) Synthrophomonas wolfei gen. nov. sp. nov., an anaerobic, synthrophic, fatty acid-oxidizing bacterium. Appl Environ Microbiol 41:1029–1039

Meile L, Fischer K, Leisinger T (1995) Characterization of the superoxide dismutase gene and its upstream region from *Methanobacterium thermoautotrophicum* Marburg. FEMS Microbiol Lett 128:247–253

Melville SB, Gunsalus RP (1996) Isolation of an oxygen-sensitive FNR protein of *Escherichia coli*: interaction at activator and repressor sites of FNR-controlled genes. Proc Natl Acad Sci USA 93:1226–1231

Menon S, Ragsdale SW (1999) The role of an iron-sulfur cluster in an enzymatic methylation reaction. Methylation of CO dehydrogenase/acetyl-CoA synthase by the methylated corrinoid iron-sulfur protein. J Biol Chem 274:11513–11518

Möller D, Schauder R, Fuchs G, Thauer RK (1987) Acetate oxidation to CO_2 via a citric acid cycle involving an ATP-citrate lyase: a mechanism for the synthesis of ATP via substrate level phosphorylation in Desulfobacter postgatei growing on acetate and sulfate. Arch Microbiol 148:202–207

Möller-Zinkhahn D, Börner G, Thauer RK (1989) Function of methanofuran, tetrahydromethanopterin, and coenzyme F420 in *Archaeoglobus fulgidus*. Arch Microbiol 152:362–368

Morris JG (1976) Oxygen and the obligate anaerobes. J Appl Bacteriol 40:229–244

Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch Microbiol 133:249–256

Naumann E, Hippe H, Gottschalk G (1983) Betaine: New oxidant in the Stickland reaction and methanogenesis from betaine and L-alanine by a *Clostridium sporogenes* Methanosarcina barkeri coculture. Appl Environ Microbiol 45:474–483

Niimura Y, Nishiyama Y, Saito D, Tsuji H, Hidaka M, Miyaji T, Watanabe T, Massey V (2000) A hydrogen peroxide-forming NADH oxidase that functions as an alkyl hydroperoxide reductase in *Amphibacillus xylanus*. J Bacteriol 182:5046–5051

Philipp B, Schink B (1998) Evidence of two oxidative reaction steps initiating anaerobic degradation of resorcinol (1,3-dihydroxybenzene) by the denitrifying bacterium *Azoarcus anaerobius*. J Bacteriol 180:3644–3649

Philipp B, Schink B (2000) Two distinct pathways for anaerobic degradation of aromatic compounds in the denitrifying bacterium *Thauera aromatica* strain AR-1. Arch Microbiol 173:91–96

Pianzzola MJ, Soubes M, Touati D (1996) Overproduction of the rbo gene product from Desulfovibrio species suppresses all deleterious effects of lack of superoxide dismutase in *Escherichia coli*. J Bacteriol 178:6736–6742

Roy F, Samain E, Dubourgier HC, Albagnac G (1986) Synthrophomonas sapovorans sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. Arch Microbiol 145:142–147

Saunders NF, Houben EN, Koefoed S, deWeert S, Reijnders WN, Westerhoff HV, DeBoer AP, VanSpanning RJ (1999) Transcription regulation of the nir gene cluster encoding nitrite reductase of *Paracoccus denitrificans* involves NNR and NirI, a novel type of membrane protein. Molec Microbiol 34:24–36

Schink B (1984) Fermentation of 2.3-butanediol by *Pelobacter carbinolyticus* sp. nov. and *Pelobacter propionicus*, sp. nov., and evidence for propionate formation from C_2 compounds. Arch Microbiol 137:33–41

Schink B (1985) Fermentation of acetylene by an obligate anaerobe. *Pelobacter acetylenicus* sp. nov. Arch Microbiol 142:295–301

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Molec Biol Rev 61:262–280

Schink B, Thauer RK (1987) Energetics of syntrophic methane formation and the influence of aggregation. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hilshoff CW (eds) Granular anaerobic sludge: microbiology and technology. Proceedings of the GASMAT-Workshop, Lunteren, The Netherlands Puduc Wageningen, The Netherlands, pp 5–17

Schink B, Philipp B, Müller J (2000) Anaerobic degradation of phenolic compounds. Naturwissenschaften 87:12–23

Shanmugasundaram T, Ragsdale SW, Wood HG (1988) Role of carbon monoxide dehydrogenase in acetate synthesis by the acetogenic bacterium *Acetobacterium woodii*. Biofactors 1:147–152

Shieh J, Whitman WB (1988) Autotrophic acetyl coenzyme A biosynthesis in *Methanococcus maripaludis*. J Bacteriol 170:3072–3079

Spiro S (1994) The FNR family of transcriptional regulators. Ant v Leeuwenhoek 66:23–36

Spormann AM, Thauer RK (1988) Anaerobic acetate oxidation to CO_2 by Desulfotomaculum acetoxidans. Demonstration of enzymes required for the operation of an oxidative acetyl-CoA/carbon monoxide dehydrogenase pathway. Arch Microbiol 150:374–380

Stams AJ (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. Ant v Leeuwenhoek 66:271–294

Takao M, Yasui A, Oikawa A (1991) Unique characteristics of superoxide dismutase of a strictly anaerobic archaebacterium *Methanobacterium thermoautotrophicum*. J Biol Chem 266:14151–14154

Thauer RK (1988) Citric-acid cycle, 50 years on: modifications and an alternative pathway in anaerobic bacteria. Eur J Biochem 176:497–508

Thauer RK (1998) 1998 Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Marjory Stephenson Prize Lecture. Microbiology 144: 2377–2406

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Thauer RK, Möller-Zinkhan D, Spormann AM (1989) Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. Ann Rev Microbiol 43:43–67

Uyeda R, Rabinowitz JC (1971) Pyruvate-ferredoxin oxidoreductase. III: purification and properties of the enzyme. J Biol Chem 246:3111–3119

Vollack KU, Härtig E, Korner H, Zumft WG (1999) Multiple transcription factors of the FNR family in denitrifying *Pseudomonas stutzeri*: characterization of four fnr-like genes, regulatory responses and cognate metabolic processes. Molec Microbiol 31:1681–1694

Wallrabenstein C, Hauschild E, Schink B (1995) Synthrophomonas pfennigii sp. nov., a new syntrophically propionate-oxidizing anaerobe growing in pure culture with propionate and sulfate. Arch Microbiol 164:346–352

Whittaker MM, Whittaker JW (1998) A glutamate bridge is essential for dimer stability and metal selectivity in manganese superoxide dismutase. J Biol Chem 273:22188–22193

Wood HG, Ragsdale SW, Pezacka E (1986) The acetyl-CoA pathway or autotrophic growth. FEMS Microbiol Rev 39:345–362

Youn HD, Kim EJ, Roe JH, Hah YC, Kang SO (1996a) A novel nickel-containing superoxide dismutase from *Streptomyces* spp. Biochem J 318:889–896

Youn HD, Youn H, Lee JW, Yim YI, Lee JK, Hah YC, Kang SO (1996b) Unique isozymes of superoxide dismutase in *Streptomyces griseus*. Arch Biochem Biophys 334:341–348

Zeikus JG (1983) Metabolism of one-carbon compounds by chemotrophic anaerobes. Adv Microbiol Physiol 24:215–293

Zengler K, Richnow HH, Rossello-Mora R, Michaelis W, Widdel F (1999) Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401:266–269

Zinder SH, Koch M (1984) Non-acetoclastic methanogenesis from acetate: acetate oxidation by a thermophilic synthrophic coculture. Arch Microbiol 138:263–272

14 The Chemolithotrophic Prokaryotes

Donovan P. Kelly[1] · *Ann P. Wood*[2]
[1]School of Life Sciences, The University of Warwick, Coventry, UK
[2]School of Biomedical Sciences, Department of Biochemistry
King's College London, London, UK

> *Ihre Lebensprozesse spielen sich nach einem viel einfacheren Schema ab; durch einen rein anorganischen chemischen Prozess...werden alle ihre Lebensbewegungen im Gange erhalten.*
>
> *["Their life processes are played out in a very simple fashion; all their life activities are driven by a purely inorganic chemical process."]*
>
> —Winogradsky, 1887

Introduction

Such was Winogradsky's (1887) description of the ability of certain bacteria to use energy from inorganic chemicals. Winogradsky's (1887) name for such organisms was "Anorgoxydanten" (literally "inorganic oxidizers"). Today the term chemolithotrophy is used to describe the energy metabolism of bacteria that use the oxidation of inorganic substances, in the absence of light, as a source of energy for cell biosynthesis and maintenance (Rittenberg 1969; Brock and Schlegel 1989; Kelly 1990). Chemolithotrophs exhibit extraordinary diversity of substrates, modes of carbon nutrition, morphology, and habitat. Grouping chemolithotrophs into some kind of homogeneous taxonomic unit is thus at least as artificial as grouping by most taxonomic devices in that virtually every possible morphology and physiology among bacteria (including the archaebacteria) is represented. Such taxonomic "lumping" does have value because some fundamental aspects of carbon and energy metabolism unify many of the chemolithotrophs into groups that are useful for physiological comparison.

The fundamental process in energy-conserving metabolism and in all respiratory processes is the transfer of hydrogen from a state more electronegative than that of the H^+/H_2O couple to that of water. Classically, "heterotrophs" or "chemoorganotrophs" obtain reducing potential from the dehydrogenation of organic compounds. Although a great variety of organic substrates are available and many are oxidized by heterotrophs, only a few principal metabolic processes exist whereby the hydrogen equivalents are fed into energy-conserving electron transport. Chief among these are processes that use the dehydrogenases of sugar phosphates and of organic acids, especially those of the tricarboxylic acid cycle. Diversity among substrates is thus merely a peripheral aspect of organism function; the central energy-generating and energy-conserving processes are fundamentally the same and involve the same kinds of components. This observation applies equally to the chemolithotrophs. Their electron-transporting and energy-trapping mechanisms are essentially the same as those of chemoorganotrophs (Kelly 1978, 1982, 1989, 1990).

From the time of its inception, the concept of chemolithotrophy was linked with the autotrophic assimilation of carbon dioxide. Thus, Pfeffer (1897) coined "chemosynthesis," the term that was used for many years to describe the metabolism of bacteria that use inorganic oxidations to support autotrophic carbon dioxide assimilation (Kiesow 1963; Jannasch and Wirsen 1979; Brock and Schlegel 1989). And Winogradsky's definition of the "anorgoxydant" uncompromisingly coupled energy generation from inorganic oxidation with not only cell synthesis exclusively from carbon dioxide but also the concept of the general toxicity of organic nutrients (Winogradsky 1922; Rittenberg 1969, 1972; Schlegel 1975; Whittenbury and Kelly 1977). This concept is now known to be too restrictive because organisms (subsequently isolated) proved to be facultatively chemolithoautotrophs (growing on organic media as heterotrophs), mixotrophic (obtaining energy or carbon from both inorganic and organic sources), or chemolithotrophic heterotrophs (using inorganic energy substrates to effect assimilation of organic growth substrates).

The concepts of autotrophy (the assimilation of carbon dioxide as the major or sole source of biosynthetic carbon) and

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_63,

chemolithotrophy (growth with inorganic energy sources) thus were accepted as separate processes obligatorily linked in some specialized types of organisms. Paradoxically, these both clarified and blurred the boundaries between the "autotrophic" and "heterotrophic" bacteria (Rittenberg 1972; Whittenbury and Kelly 1977; Kelly 1990). Clarification resulted from the fact that energy generated from an inorganic source does not have to be coupled exclusively to autotrophy (equally, the "organic oxidation" of formate or methanol can be coupled to autotrophic growth on carbon dioxide). Thus, physiological classification was in terms of either energy or carbon nutrition (Kelly 1971). "Blurring" came from the possibility of extending the concept of autotrophy from a restricted definition. Thus, autotrophy could be defined in ever broader terms as metabolic processes that obtain (1) most carbon for biosynthesis from carbon dioxide by the action of ribulose bisphosphate carboxylase enzyme and derive energy chemolithotrophically, (2) carbon from carbon dioxide by the Calvin-Bassham-Benson cycle (which we shall call the "Calvin cycle"), (3) most carbon from carbon dioxide by any biochemical means, or (4) one-carbon compounds for all biosynthesis by processes fundamentally akin to those involved in the autotrophic fixation of carbon dioxide (Smith and Hoare 1977; Whittenbury and Kelly 1977). It is clearly preferable to distinguish carbon and energy metabolism, especially among chemolithoautotrophs, but there is no merit in restricting the definition of autotrophy to those organisms using the Calvin cycle. Studies in recent years have established that distinct pathways, in addition to the Calvin cycle pathways from which they differ, operate in some groups of autotrophs. The term autotrophy should be applied without question to all organisms capable of basing biosynthesis on one-carbon compounds. In our view, it is useful to separate "methylotrophy" from "autotrophy" when considering the pathways by which organisms growing on one-carbon compounds as sole source of energy convert those one-carbon units into biomass. This enables a clear distinction between methylotrophically based energy and carbon metabolism to be made. There can be methylotrophic autotrophs, which use methanol or methylamine oxidation to drive carbon dioxide fixation by the Calvin cycle, and non-autotrophic methylotrophs, which use from C1-compound oxidation to drive the serine pathway or the Quayle (ribulose monophosphate) cycle to assimilate formaldehyde.

Inorganic Oxidations as Sources of Energy

In principle, any inorganic exergonic oxidation reaction might be expected to be the basis of the energy-conserving metabolism of a chemolithotroph if (1) the reaction creates sufficient energy to support ATP synthesis and electron transport, enabling proton translocation and phosphorylation, and (2) conditions during geological time favor evolution and selection of suitable enzyme systems and organisms. The latter criterion was not always met inasmuch as chemolithotrophs have not been shown to exploit some potentially energy-yielding processes. This absence probably reflects low concentrations or unavailability of the reaction components in the natural environment and no selective pressure or advantage leading to the survival of any organisms evolving such oxidation capacities.

The reactions unequivocally established as sources of chemolithotrophic energy are the oxidation of hydrogen, ammonia, nitrite, sulfur and its reduced compounds, ferrous iron, and possibly cuprous copper, antimony, and uranium (IV). Detailed consideration of the organisms involved, the mechanisms and types of reactions catalyzed, and the mechanisms of energy trapping are given in the specialist chapters in *The Prokaryotes* and in specialist reviews. This chapter attempts to present views of the chemolithotrophs from the perspective of energy yields of known chemolithotrophic oxidations, possible origins of chemolithotrophic processes, factors limiting the distribution and diversity of chemolithotrophs, and the potential for chemolithotrophy among known bacteria and those which still may remain to be discovered.

Energy Yields from Inorganic Oxidations

Estimates of the efficiency of energy production from inorganic oxidations in chemolithotrophs have been made from thermodynamic calculations and the measurement of growth and carbon dioxide assimilation (e.g., Baas Becking and Parks 1927; Fromageot and Senez 1960; Kelly 1978, 1982, 1990, 1999). Estimates of ATP production for oxidative phosphorylation in nitrifying bacteria and for sulfur, hydrogen, and iron oxidation are available and generally reflect P/O ratios of about 1.0, except for hydrogen where a normal complete electron transport chain, possibly allowing P/O=3.0, is present. Some studies using chemostat culture with chemolithotrophs have been reported, and in some cases, maximum theoretical growth yields for the oxidation of different inorganic substrates can be compared and related to probable oxidation pathways and the theoretically available free energy. Chemostat culture methods with thiobacilli growing on sulfur compounds or iron have proved useful to the interpretation of oxidation and energy-coupling mechanisms (Hempfling and Vishniac 1967; Timmer-ten-Hoor 1976; Kelly et al. 1977; Eccleston and Kelly 1978; Justin and Kelly 1978; Kelly 1982, 1990; Jones and Kelly 1983). Overall oxidation reactions exploited by the different groups of known chemolithotrophs are given in ❯ *Table 14.1*, and our current knowledge of the energy calculated to be available from these reactions and the observed growth or energy yields achieved by some chemolithotrophic bacteria are given in ❯ *Table 14.2*. Kelly (1990, 1999) has reviewed this topic in more detail.

Except in the case of hydrogen oxidation, where the electrode potential is more negative than the NAD+/NADH couple, all these oxidations couple electron transport to the cytochrome system of the bacteria, and NAD+ reduction requires energy-dependent electron flow from cytochromes (Kelly 1978, 1990). This dependence is a biochemical hindrance to the growth of such chemolithotrophs because their energy metabolism is often largely concerned with the generation of NADH. Less of the energy available from an oxidation can be coupled more directly

Table 14.1
Known chemolithotrophic oxidation reactions

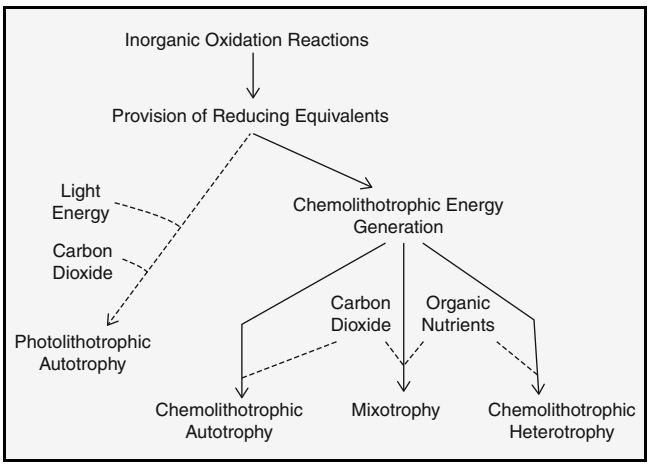


Table 14.2
Energetics and growth yields from the chemolithotrophic oxidations of *Table 14.1*

$S^0 + 6Fe^{3+} + 4H_2O = HSO_4^- + 7H^+$ (pH 2; $\Delta F^0 - 314$ kJ)
$S^0 + 3NO_3^- + H_2O = 3NO_2^- + SO_4^{2-} + 2H^+$ (pH 7; $\Delta F^0 - 352$ kJ)
$5S^0 + 6NO_3^- + 2H_2O = 5SO_4^{2-} + 3N_2 + 4H^+$ ($\Delta F^0 - 515$ kJ/atom S^0)
$2S^0 + 3O_2 + 2H_2O = 2HSO_4^- + 2H^+$ (pH 2; $\Delta F^0 - 519$ kJ/atom S^0)

to biosynthesis than would occur, for example, during growth on hydrogen or pyruvate as an energy substrate. In addition, many of the most-studied chemolithotrophs use the Calvin cycle to fix carbon dioxide as a main source of carbon, and more than 80 % of the total energy budget of non-hydrogen-oxidizing chemolithotrophs is indicated for use in converting carbon dioxide to an amount of carbohydrate, equivalent to that found in the growth nutrient of most heterotrophs. This combination of energy requirements explains why the growth yields of chemolithotrophs (already limited by the relatively low molar energy yield of their substrates) are generally apparently rather meager. In fact, they convert energy with reasonable efficiency in spite of the biochemical problems to be surmounted (Justin and Kelly 1978; Kelly 1990, 1999).

Chemolithotrophy and Autotrophy Among Heterotrophs

A consequence of the historical development of the concepts of chemolithotrophy and "chemoautotrophy" was that any capacity for heterotrophic growth in an organism isolated originally as an autotroph tended to be regarded as an additional property of an organism that was primarily an "autotroph." Thus, the first isolates of the facultatively autotrophic thiobacilli were classified as *Thiobacillus novellus* rather than as a *Pseudomonas* that could grow chemolithoautotrophically. This procedure of slotting such organisms into "filing boxes" on the basis of their autotrophy, essentially devaluing their heterotrophic potential as a secondary character, was introduced by Winogradsky and used for most of the following 100 years. The practice has had both good and bad consequences for our understanding of the chemolithotrophs and their place in the natural environment. It has been good because it focused attention on the "obligate chemolithotroph" as an accepted physiological phenomenon, exemplified by clearly definable genera (*Thiobacillus*, some strains of *Beggiatoa*, *Hydrogenobacter*, *Hydrogenovibrio*, *Aquifex*, and the nitrifying bacteria being good examples; Kelly and Harrison 1989; Nishihara et al. 1990, 1991, 1998; Huber et al. 1992; Shima and Suzuki 1993; Nelson and Hagen 1996), and it has enabled and motivated numerous investigators to probe the biochemistry of chemolithotrophic processes. It has been a hindrance to the broader understanding of chemolithotrophy in the natural environment because for many years minds were closed to the idea that chemolithotrophy could be a property of heterotrophs.

The period of microbiologists' reluctance to seek "new" chemolithotrophs (Schlegel 1975) ended with a new understanding that mixed physiology and metabolic flexibility involving chemolithotrophy and autotrophy is the basis of successful growth and survival in more organisms than was dreamed previously. Even *Beggiatoa*, so central in the formulation of the concept of the anorgoxydant, is proving a bizarre physiological kaleidoscope.

One of the first cracks in the defense of the concept that chemolithotrophy is a unique and essential taxonomic character was the reclassification of the hydrogen bacteria. For years, autotrophic growth with hydrogen oxidation as a source of energy justified the grouping of these bacteria (all known examples of them were also heterotrophic) into an artificial taxon known as "hydrogen bacteria." In 1969, these were scattered by new thinking and reclassified into diverse standard genera of heterotrophs, on the basis of their morphology and heterotrophic physiology, with their hydrogen-based chemolithotrophy and autotrophy being regarded simply as additional physiological properties (Davis et al. 1969).

The other genus of questionable validity is *Thiobacillus*, whose members obtain energy from oxidizing inorganic sulfur. As currently constituted, this genus contains diverse obligate chemolithotrophs, which differ from each other so widely that they should be reclassified into new genera (Kelly 1989; Kelly and Harrison 1989; Kelly and Wood 1999), as well as facultatively heterotrophic species, which should be reassigned to existing genera of chemoorganotrophs or to new genera, created for them using heterotrophic metabolism and molecular biological criteria. For example, some species, such as those described in the previous edition (Kelly 1991) as *T. novellus* and *T. versutus*,

are extremely versatile heterotrophs that can exhibit mixotrophy (in which heterotrophic and chemolithoautotrophic mechanisms operate simultaneously) and are taxonomically remote from obligately chemolithotrophic species. Since the previous edition of *The Prokaryotes*, *T. versutus* has been recognized as a species of *Paracoccus* (Katayama et al. 1995), and 16 S rRNA gene sequence analysis has placed *T. novellus* in the α-proteobacteria, whereas the type species, *T. thioparus*, is a member of the β-proteobacteria (Lane et al. 1992). Other species, including the obligate chemolithotrophs *T. tepidarius*, *T. neapolitanus*, *T. halophilus*, *T. hydrothermalis*, and *T. thiooxidans*, have been found to be members of the γ-proteobacteria and unlikely to be related even at the genus level to the type species of *Thiobacillus* (McDonald et al. 1997; Kelly and Wood 1999).

Among the early isolates of autotrophic organisms that had become regarded as probable heterotrophs, or at best mixotrophs, were *Gallionella* and *Beggiatoa*, but their ability to grow respectively as iron- or sulfide-oxidizing chemolithotrophs is now well established (Keil 1912; Hanert 1981; Nelson and Jannasch 1983; Nelson et al. 1986a, b, 1989a; Kelly 1988). There have been unequivocal demonstrations of an obligately autotrophic, sulfide-oxidizing marine strain of *Beggiatoa* that used a biosynthetic citric acid cycle, lacked 2-oxoglutarate dehydrogenase, and showed virtually no regulatory effect by organic substrates on its constitutive ribulose bisphosphate carboxylase (Hagen and Nelson 1996). An intriguing observation was that growth of *Beggiatoa alba* on acetate was greatly stimulated by the availability of sulfide, which was apparently used as an energy source enabling surplus assimilation of acetate over that obtainable heterotrophically (Güde et al. 1981). Similarly intriguing is the presence of the autotrophic enzyme, ribulose bisphosphate carboxylase in non-autotrophic strains of *Beggiatoa*, the enzyme seemingly being under repression control by acetate (Nelson et al. 1989b). *B. leptomitiformis* grows mixotrophically on succinate medium with thiosulfate or tetrathionate, which are oxidized to generate ATP by oxidative phosphorylation (Grabovich et al. 1998), further extending the range of strains of filamentous sulfur bacteria exhibiting this kind of mixed energy generation. Another filamentous genus believed to be capable only of mixotrophic growth with inorganic sulfur compounds was *Thiothrix*, but then *T. ramosa* was shown to be able to grow autotrophically using thiosulfate oxidation as sole energy source (Odintsova et al. 1993). Interestingly, while *Thiothrix* and *Beggiatoa* are both members of the γ-proteobacteria, *Thiothrix* was not part of the novel monophyletic lineage comprising *Beggiatoa* and *Thioploca* (Teske et al. 1996).

Among the unicellular sulfur bacteria, recent work has shown just how blurred is the physiological distinction between the classical definition of *Thiobacillus* and heterotrophs capable of chemolithotrophy. Thus, obligately heterotrophic marine pseudomonads showing thiosulfate-stimulated growth (and carbon dioxide fixation) have been isolated and shown to produce sulfate, as would thiobacilli (Ruby et al. 1981). Even *Pseudomonas aeruginosa* shows increased growth yields in chemostat culture on glucose, when oxidizing thiosulfate to tetrathionate (Mason and Kelly 1988). Chemolithoautotrophy on thiosulfate is also known in new and old genera of heterotrophs, including *Thiosphaera* (Robertson and Kuenen 1983; now reclassified as *Paracoccus pantotrophus*, Ludwig et al. 1993; Kelly et al. 1999), *Paracoccus denitrificans*, and other hydrogen-oxidizing heterotrophs (Friedrich and Mitrenga 1981; Kelly 1988, 1989). An organism, called *Thiobacillus* Q, isolated as the predominant organism from ditch water, using a chemostat limited by acetate plus thiosulfate, proved to be incapable of autotrophic growth and thus to be another example of a heterotroph capable also of chemolithoheterotrophy (Gommers and Kuenen 1988). It thus bears some similarity to *Thiobacillus intermedius* (London 1963; now reclassified as *Thiomonas* intermedia; Moreira and Amils 1997) and the original description of *Thiobacillus perometabolis* (now *Thiomonas perometabolis*), which grew best as chemolithotrophic heterotrophs (London and Rittenberg 1967; Katayama-Fujimura and Kuraishi 1983). Mixotrophy and chemolithotrophic heterotrophy have been shown also in the extreme thermoacidophile, *Sulfolobus*, and in iron-oxidizing mesophiles and thermophiles (Wood and Kelly 1983; Barros et al. 1984; Wood et al. 1987). Metabolic flexibility exhibited by such bacteria and the facultatively heterotrophic thiobacilli is of great survival significance to such organisms (Whittenbury and Kelly 1977; Robertson and Kuenen 1991) during competition in the natural environment. Thus, mixotrophy can enable these bacteria to dominate in mixed populations when both chemolithotrophic and chemoorganotrophic nutrients are present (Gottschal et al. 1979; Kelly and Kuenen 1984). The observation of such flexibility should stimulate us to ask just how adaptable the physiology of the lithotrophs may be: whether, for example, the photolithotrophs function significantly as sulfur-oxidizing chemolithotrophs in darkness when given access to low concentrations of oxygen. Thus, *Thiocapsa* can grow in darkness using aerobic oxidation of thiosulfate or sulfide to support carbon dioxide fixation (Kondratieva et al. 1976; Kondratieva 1989). Pigmentation is lost under such conditions, but ribulose bisphosphate carboxylase-specific activity is similar under all photo- or chemolithotrophic conditions. The bacteria thus have the adaptive ability to behave physiologically like thiobacilli. Phenomena of this kind could prove to have considerable ecological and biogeochemical significance in the natural environment. Their widespread demonstration would further prove the undesirability of establishing dogmatic definitions of physiological types of organisms without the possibility of overlap areas. Thus, organisms of seemingly very different fundamental physiology could, under appropriate conditions, behave similarly. Indeed the converse question should be addressed: are any missing links of physiological types of obligate or facultative autotrophs possible? What of obligate hydrogen oxidizers and facultatively heterotrophic nitrifying bacteria, neither of which was proved to exist when one of us wrote the equivalent chapter in the first edition of *The Prokaryotes* (Kelly 1981). There is now also no doubt that numerous organisms exist that have no autotrophic potential but use energy from lithotrophic oxidations to support growth on organic carbon sources. A remarkably versatile example is *Sulfurospirillum deleyianum*,

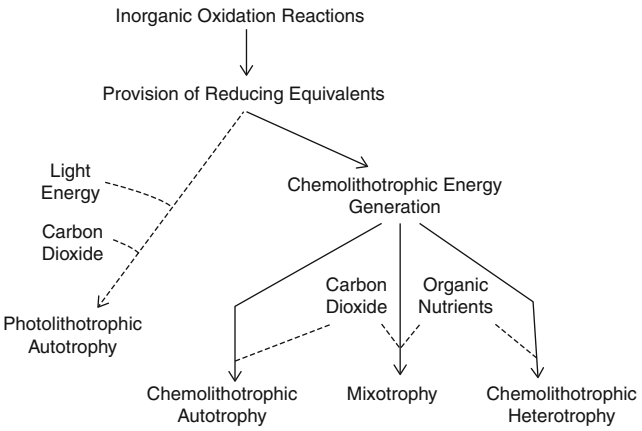


Fig. 14.1
Scheme illustrating the relation of inorganic oxidations to chemolithotrophic and photolithotrophic metabolism

which can use hydrogen as an electron donor, with its oxidation coupled to the reduction of inorganic or organic acceptors (oxygen, nitrate, nitrite or reduced sulfur compounds, or dimethyl sulfoxide or C4-organic acids). This organism also uses sulfide as an electron donor (with acetate as carbon source), reducing nitrate and nitrite to ammonia to generate metabolic energy (Eisenmann et al. 1995). Some of these concepts are summarized in *Fig. 14.1*.

For many decades, the species of the nitrifying bacteria were uniformly believed to be obligate chemolithoautotrophs, in accordance with Winogradsky's original description of them and use of their properties to define the anorgoxydant. Subsequently, facultative heterotrophy was proved in nitrite-oxidizing species, in which acetate or pyruvate was used to support aerobic and anaerobic growth (Smith and Hoare 1968; Bock 1976; Freitag et al. 1987; Bock et al. 1988). Also, one of the most abundant of the nitrite-oxidizing bacteria in the natural environment, *Nitrobacter vulgaris*, has been shown to grow faster heterotrophically than autotrophically and can reduce nitrate (to nitrite, ammonia, NO, and N_2O) during anaerobic growth on acetate or pyruvate (Bock et al. 1990). The complexity of habitat niches and complexity of the involvement of nitrite-oxidizing bacteria in soil ecosystems are thus greater than were long believed. As yet, nitrite- (or ammonia-) dependent chemolithotrophic heterotrophy has not been shown, but mixotrophic growth of *N. vulgaris* can be faster than during heterotrophy (Bock et al. 1990).

The Overlap of Autotrophy, Methylotrophy, and Chemolithotrophy

The methane-oxidizing bacteria, such as *Methylococcus*, are dependent on methane oxidation (or in some cases also methanol) for energy and carbon, but even in these, the autotrophic enzyme ribulose bisphosphate carboxylase has been found (Taylor 1977) and could contribute to the synthesis of phosphoglycerate during growth on methane (Stanley and Dalton 1982). Interestingly, the amount of the carboxylase in *M. capsulatus* in chemostat culture decreased with oxygen limitation (Khmelenina et al. 1992), possibly suggesting its function as an oxygenase has a greater importance. The carbon monoxide-oxidizing bacteria also fix carbon dioxide by means of the Calvin cycle, as do *Thiobacillus novellus* and *Paracoccus* versutus during growth on methylamine, methanol, or formate as substrates (Kelly et al. 1979; Kelly and Wood 1982, 1984; Meyer 1989). Thus, autotrophy can occur in organisms growing on methylotrophic or other one-carbon energy substrates and does not have to be linked to chemolithotrophic energy sources. Growth of some thiobacilli and hyphomicrobia has been shown using one-carbon sulfur compounds as sole substrates (for review, see Kelly and Smith 1990). These substrates, including dimethyl sulfoxide, dimethyl sulfide, dimethyl disulfide, and methanethiol, provide a somewhat remarkable meeting place or common feeding ground for sulfur-dependent chemolithoautotrophs and truly methylotrophic organisms. Thus, some strains of *Thiobacillus thioparus* oxidize both the methyl and the sulfur groups and obtain energy from their oxidation. The thiobacilli cannot grow on one-carbon compounds, such as methylamine, which is used by the hyphomicrobia, and the latter cannot grow on sulfide or thiosulfate unless a compound such as methylamine is also supplied. Remarkably, the hyphomicrobia can grow mixotrophically on methylamine and thiosulfate, when they derive energy from oxidizing the latter, and use this to increase the proportion of methylamine-carbon incorporated by the serine pathway. They are thus chemolithotrophic methylotrophs that show no capacity for autotrophy and serve to illustrate further that chemolithotrophic energy-generating processes can underpin or enhance the metabolism and growth not only of diverse autotrophs but also of methylotrophs and heterotrophs. It also illustrates that substrates such as inorganic sulfide, methylated sulfides, methylamines, and other intermediates of sulfur or one-carbon metabolism may be competed for in the natural environment by organisms of diverse basic physiology, including specialist thiobacilli and hyphomicrobia, as well as some chemolithotrophic heterotrophs.

Chemoorganotrophic Potential among Obligate Chemolithotrophs

So far we have dwelt on the diversity of organisms, both autotrophs and heterotrophs, in which the potential for chemolithotrophic energy conservation from inorganic oxidations resides. In writing such an introduction 20 or 50 years ago, the emphasis would have been placed on the uniqueness of the chemolithotrophic mode of growth and the puzzle of why in some organisms the chemolithoautotrophic mode of growth is obligatory (van Niel 1943; Umbreit 1947; Kelly 1967, 1971). The reason why some bacteria exhibit this obligate chemolithotrophy is still inadequately explained (Kelly 1971; Zavarzin 1989), and it is probable that there is no single, simple, and universal explanation. Certainly, impermeability to organic

nutrients is not the reason, as many obligate thiobacilli and nitrifying bacteria incorporate organic compounds at the expense of chemolithotrophically generated energy. Also it has long been realized that the hypothesis that fundamental differences exist between the central metabolic processes of chemolithotrophs and heterotrophs was not tenable. Indeed, the problem is made more intriguing by the evidence for storage polymers in obligate strains of thiobacilli and *Nitrobacter* (van Gool et al. 1971). *Thiobacillus neapolitanus* has been shown not only to accumulate polyglucose to levels exceeding 20 % of the cellular protein content (Beudeker et al. 1981a) but also to consume this under conditions of (aerobic) carbon dioxide starvation and to degrade it under anaerobic conditions by a heterolactic fermentation pathway (Beudeker et al. 1981b). A wide range of organic-nitrogen compounds, such as purines and urea, can be used to provide nitrogen for the chemolithoautotrophic growth of *Thiobacillus thiooxidans* (Brierley and Brierley 1968; Metzdorf and Kaltwasser 1988), and some obligate strains exhibit requirements for trace compounds such as vitamins. Thus, the explanation for obligate chemolithotrophy must lie in the nature of the central regulatory processes that control carbon flow to biosynthesis in such bacteria, and this also must determine their seeming inability to oxidize exogenously supplied organic nutrients as sources of energy. The significant chemolithotrophic energy-dependent incorporation of some compounds (such as amino acids) indicates that in the natural environment the obligate chemolithotroph, while likely to be mainly autotrophic, must exhibit a degree of chemolithotrophic heterotrophy. They are thus at one end of a continuum of physiological behavior, as discussed by Kelly and Kuenen (1984).

It has become clear that chemolithotrophy is not a restricted property of a few quaint but ecophysiologically specialized bacteria, but it is a metabolic mode shared among many heterotrophs and may enable them to prevail over less versatile species in the competition for resources in nutrient-restricted natural environments.

Some Novel Chemolithotrophic Reactions and Some "New" Chemolithotrophs

Potentially energy-yielding novel reactions have been found in sulfur-oxidizing thiobacilli and *Sulfolobus* and in iron-oxidizing thermophiles. *Thiobacillus ferrooxidans* and *T. thiooxidans* can oxidize sulfur at the expense of ferric iron reduction as the respiratory oxidant (Brock and Gustafson 1976). *T. ferrooxidans* does this anaerobically and thus may be capable of anaerobic growth using this system. It is noteworthy that previous reports of ferric iron reduction by heterotrophs have attributed the process to nitrate reductase (Thauer et al. 1977), but the probable inability of these bacteria to denitrify may suggest the existence of a specialist enzyme system, possibly a relict of a metabolic process that evolved before the appearance of nitrate respiration in early microorganisms. The amount of energy available from sulfur oxidation coupled to iron reduction is comparable with that from nitrate reduction or from aerobic sulfur or iron oxidation:

$$S^0 + 6Fe^{3+} + 4H_2O = HSO_4^- + 7H^+ \quad (\text{pH } 2;\ \Delta F^0 - 314 \text{ kJ})$$

$$S^0 + 3NO_3^- + H_2O = 3NO_2^- + SO_4^{2-} + 2H^+ \quad (\text{pH } 7;\ \Delta F^0 - 352 \text{ kJ})$$

$$5S^0 + 6NO_3^- + 2H_2O = 5SO_4^{2-} + 3N_2 + 4H^+ \quad (\Delta F^0 - 515 \text{ kJ/atom } S^0)$$

$$2S^0 + 3O_2 + 2H_2O = 2HSO_4^- + 2H^+ \quad (\text{pH } 2;\ \Delta F^0 - 519 \text{ kJ/atom } S^0)$$

Iron-oxidizing, moderately thermophilic, chemolithotrophic heterotrophs were described by Brierley et al. (1978, 1980), Clark and Norris (1996), growing on ferrous iron or pyrite as a substrate, but incapable of sustained growth on sulfur. Those isolates did, however, grow on copper sulfide (CuS), but only if small amounts (e.g., 1 mM) of iron (Fe^{2+} or Fe^{3+}) were present. Such organisms might simply be exhibiting an unusually high requirement for assimilable iron to grow on sulfur compounds. It is also possible that their main source of energy could be the oxidation of ferrous iron, with its constant regeneration by either biological oxidation of the CuS with Fe^{3+} or purely chemical reaction of CuS with ferric sulfate, according to the following processes:

1. Biological, energy-yielding aerobic ferrous iron oxidation:

$$4FeSO_4 + O_2 + 2H_2SO_4 = 2Fe_2(SO_4)_3 + 2H_2O$$

2. Sulfide oxidation at the expense of ferric iron reduction:

$$CuS + Fe_2(SO_4)_3 = CuSO_4 + 2FeSO_4 + S^0$$

3. Chemical reduction of ferric iron:

$$S^{2-} + 8Fe^{3+} + 4H_2O = SO_4^{2-} + 8Fe^{2+} + 8H^+$$

Measurement of growth yields or carbon dioxide fixation in such an organism should elucidate the main energy-yielding processes because considerably greater yields should result if both sulfide and iron oxidation were energy coupled, rather than solely the latter.

Earlier, we noted that all then known hydrogen-oxidizing autotrophs could be classified as heterotrophs (Davis et al. 1969). The vacant metabolic niche of the obligately chemolithoautotrophic hydrogen oxidizer was filled in 1980 with the demonstration that such bacteria exist in some hot springs (Kawasumi et al. 1988). Several species of the thermophilic (and one case of a halophilic) *Hydrogenobacter* genus have been described (Kristjansson et al. 1985; Nishihara et al. 1990)

and found growing on hydrogen optimally at 70 °C, fixing carbon dioxide by a reductive tricarboxylic acid cycle, but shown to be incapable of growth on a wide range of simple and complex organic materials. Nishihara et al. (1989) isolated another obligately chemolithoautotrophic, halophilic hydrogen bacterium that grew best at 37 °C (with no growth at 45 °C) and used the Calvin cycle to fix carbon dioxide. This bacterium represented a further metabolic signature on the bewildering palimpsest of microbial physiology. Interestingly, it also grows on inorganic sulfur compounds, and if originally it had been isolated from enrichment culture (for example on thiosulfate), it would have been classifiable as "*Thiobacillus*," but only if its ability to use hydrogen had not been tested. It is interesting to note the degree of overlap among the obligate and facultative hydrogen oxidizers in their ability also to use inorganic sulfur oxidation for energy.

This metabolic overlap and presence of versatile physiology is perhaps nowhere more remarkably emphasized than in *Acidianus* (Segerer et al. 1986; Zillig et al. 1986; Fuchs et al. 1996). *Acidianus* species are extremely thermophilic (85–90 °C) and acidophilic (pH 2.0–2.5) archaebacteria. They are capable of aerobic autotrophic growth on elemental sulfur, which they oxidize to sulfate, while fixing carbon dioxide by a not yet fully resolved mechanism that resembles the 3-hydroxypropionate pathway postulated in *Chloroflexus* (Holo 1989; Ishii et al. 1996). These bacteria are also capable of strictly anaerobic autotrophic growth during which they reduce elemental sulfur to hydrogen sulfide at the expense of hydrogen oxidation (Kelly 1985, 1988; Segerer et al. 1985, 1986; Zillig et al. 1985, 1986). Thus, chemolithotrophy can exist in the same organisms using aerobic sulfur oxidation or anaerobic hydrogen oxidation, while simultaneously dissolving the previous distinction of sulfur oxidation and reduction as properties of quite distinct physiological groups of organisms.

This distinction has been weakened further by the work of Cypionka (e.g., Bak and Cypionka 1987) on sulfate-reducing bacteria that can grow using "sulfur fermentation" as a means of generating chemolithotrophic energy (Kelly 1987, 1988, 1990a). Thus, *Desulfovibrio sulfodismutans* and *Desulfobacter curvatus* are among organisms able to conserve energy from the anaerobic disproportionation of thiosulfate or sulfite to produce mixtures of sulfate and sulfide as end products; thus, both sulfur (or sulfite) reduction to sulfide and anaerobic oxidation to sulfate occur in their energy-generating mechanism. The overall process is a fermentation, in that the combined oxidation state of the end products equals that of the substrate (Bak and Cypionka 1987; Bak and Pfennig 1987; Kelly 1987, 1988):

$$S_2O_3^{2-} \rightarrow SO_4^{2-} + HS^- + H^+ \ (\Delta G^{0'} = -21.9 \text{ kJ/mol})$$

Recent studies have shown that there is differential fractionation of the sulfur isotopes (^{32}S and ^{34}S) within thiosulfate, when this is the sulfur compound being disproportionated by *Desulfovibrio desulfuricans*. Disproportionation of thiosulfate yielded sulfate that was isotopically heavier than the inner sulfur (sulfonate, $-SO_3-$) of thiosulfate and sulfide which was isotopically lighter than the outer (sulfane, -S-) of thiosulfate (Cypionka et al. 1998; Smock et al. 1998). This means that the disproportionation of thiosulfate does not result in its direct conversion to sulfate and sulfide, but that intermediate reactions occur during which there is fractionation of ^{32}S and ^{34}S (Cypionka et al. 1998).

Enzymological studies have partially resolved the probable mechanism of these processes and how the free energy is conserved (Krämer and Cypionka 1989). The overall reaction, and three component steps leading to sulfate and sulfide formation from sulfite, with ATP formation, may be

$$4HSO_3^- \rightarrow 3SO_4^{2-} + HS^- + 3H^+ \ (\Delta G^{0'} = -58.3 \text{ kH/mol } HSO_3^-)$$

$$3HSO_3^- + 3AMP + 3H^+ = 3APS + 6[H] \ (E^{0'} = -60\text{mV}) \quad (14.1)$$

$$3APS + 3PPi = 3ATP + 3SO_4^{2-} \quad (14.2)$$

$$HSO_3^- + 6[H] = HS^- + 3H_2O \ (E^{0'} = -116 \text{ mV}) \quad (14.3)$$

(where APS is adenylyl sulfate and PPi is pyrophosphate).

The problem implicit in this scheme is that the reductant-donating reaction (Eq. 14.1) is less electronegative than that producing sulfide (Eq. 14.3). This led Krämer and Cypionka (1989) to postulate energy-dependent reversed electron transport as part of the overall process. This would be needed if an alternative means of oxidizing sulfite to sulfate were not found in these anaerobes. Such an oxidation could well involve energized membrane functions that lead to further ATP synthesis (in a coupled sulfite oxidation and sulfite reduction) and possibly to $NAD(P)^+$ reduction. These sulfite- or thiosulfate-fermenting chemolithotrophs are in fact chemolithotrophically heterotrophic in that they have been grown on sulfur compounds as sole energy source and on acetate as carbon source. The sulfur fermentation continued also when hydrogen was provided as an oxidizable substrate (Krämer and Cypionka 1989).

Hydrogen oxidation is of course a source of energy not only to many aerobes and the sulfur-reducing archaebacteria (already discussed) but also to the sulfate-reducing bacteria and the methanogens (Table 3, Eq. 14.1). The chemolithotrophy and autotrophy of the sulfate-reducing bacteria have a long and somewhat bizarre history. For some time, *Desulfovibrio* was regarded as a facultative autotroph, oxidizing hydrogen to reduce sulfate and fixing carbon dioxide as a source of carbon. The autotrophic status of *Desulfovibrio* species (Butlin and Adams 1947) was demoted to that of "not to be true autotrophs" (Mechalas and Rittenberg 1960; Postgate 1979), and then the genus was reinstated as having strains truly capable of chemolithotrophy on hydrogen and sulfate (Badziong et al. 1978; Thauer 1989). Now some sulfate reducers also are known to be capable of chemolithotrophic autotrophy, such as *Desulfobacterium autotrophicum*, which uses the reductive acetyl CoA pathway to fix carbon dioxide, and *Desulfobacter hydrogenophilus*, which uses the reductive tricarboxylic acid cycle (Fuchs 1989).

Clearly, the physiological types of chemolithotrophs, and metabolic combinations involving chemolithotrophy, are many and various, and many more novelties can be expected as *The Prokaryotes* is progressively updated.

Evolutionary Aspects of the Origin of Chemolithotrophy

For perhaps 2 billion years, since the origin of life on earth, metabolic evolution occurred in a globally anaerobic environment. Before the appearance of the oxygen atmosphere, the widely accepted view of life processes was that of a fermentative metabolism acting at the expense of organic materials (the "prebiotic soup") accumulated during an era of prebiotic chemical evolution (Oparin 1957; Maden 1995). Thus, the first living entity was regarded as a completely heterotrophic unit using abiotically preformed organic molecules (Horowitz 1945). Metabolism during this period might have included the development of some steps of the tricarboxylic acid cycle as an anaerobic process and of bacterial photosynthesis at the expense of organic compounds, with hydrogen and sulfide as reductants. During this time, the earliest chemolithotrophs were postulated to have appeared: these were proposed to be methanogens, which reduced carbon dioxide with hydrogen, and the sulfate-reducing bacteria, which used hydrogen as well as other reductants.

There is, however, no longer a universal acceptance of the view that the earliest organisms were heterotrophs (Maden 1995; Edwards 1998). One view gaining strong support is that the earliest self-sustaining metabolism was rooted in chemolithoautotrophy (Wachtershauser 1988, 1990a, b, 1992). All of the most deeply rooted lines of Bacteria and Archaea are hyperthermophiles, and many of these are autotrophs (Woese 1987; Burggraf et al. 1992; Stetter 1992). Indeed, Stetter (1992) has proposed that the deepest branches of the phylogenetic tree comprise chemolithotrophic autotrophs, thus indicating autotrophy as a primordial metabolic process. It is also possible that chemolithotrophy predated autotrophy and that the first chemolithotrophs were chemolithotrophic heterotrophs, using inorganic energy sources as well as prebiotic organic molecules.

There is a widely accepted view that the first autotrophic processes were akin to those now seen in the sulfur- and sulfate-reducing bacteria and archaea (i.e., cyclic processes involving organic acids). The process once regarded as typifying autotrophy, namely, the Calvin reductive pentose phosphate cycle, is regarded as being of much later origin and as having evolved from earlier sugar-metabolizing pathways (Maden 1995). The Calvin cycle is apparently not functional in carbon dioxide fixation by Archaea and hydrogen- or sulfur-oxidizing hyperthermophiles (Fuchs and Stupperich 1985; Maden 1995; Schönheit and Schäfer 1995), and this is used as evidence that it cannot have been a primordial pathway. It is noteworthy, however, that genes apparently coding for a novel (or even primordial) form of ribulose bisphosphate carboxylase occur in *Pyrococcus* and other Archaea (Ezaki et al. 1998), but their origin and whether they are the result of later lateral gene transfer is not known. If these genes are relict rather than a result of lateral transfer, the Calvin cycle could be of much more ancient origin, arising in chemolithotrophic heterotrophs in which biosynthesis using simple exogenous sugars had already evolved. An argument for a later origin also has been applied to the carbon monoxide dehydrogenase pathway (acetyl coenzyme A pathway), which requires additional carbon dioxide fixing processes to support biosynthesis beyond the level of two-carbon units (Maden 1995). Wachtershauser (1990a, b, 1992) postulates that the first pathway of autotrophic carbon fixation was an archaic precursor of the reductive citric acid cycle, whose descendent is found in examples of modern eubacteria, green sulfur bacteria, hyperthermophilic hydrogen bacteria, and archaea (Evans et al. 1966; Schauder et al. 1987; Wachtershauser 1990a, b; Shima and Suzuki 1993; Beh et al. 1993; Maden 1995).

Studies of anciently duplicated genes encoding components of the protein-targeting machinery have been suggested to help identify the root of the universal tree of life (Gribaldo and Cammarano 1998). The key protein of the signal recognition particle (SRP54 in Eukarya and Fm in Bacteria) and the protein involved in the recognition and binding of the ribosome SRP nascent polypeptide complex (SR alpha in Eukarya and Ftsy in Bacteria) are products of ancient gene duplication that appears to predate the divergence of all extant taxa (Gribaldo and Cammarano 1998). This leads to the reasoning that the first bifurcation in the tree of life separated the lineage leading to Bacteria from a common ancestor to Archaea and Eukarya (Gogarten and Taiz 1992; Gogarten 1995; Gribaldo and Cammarano 1998). Phylogenetic studies on dissimilatory sulfite reductases and adenylyl sulfate (APS) reductase (Hipp et al. 1997; Wagner et al. 1998), using organisms as diverse as *Chromatium*, *Archaeoglobus*, and *Desulfovibrio*, showed gene homology for these reductase enzymes. The degree of similarity of the DNA sequences for the bacterial and archaeal genes was such that the most likely explanation was a common origin from ancestral reductase genes. This suggests that the genes for the ancestral reductases were present before the split between the domains of Bacteria, Archaea, and Eukarya took place. This kind of evidence lends considerable weight to a view of primordial organisms that were chemolithotrophs capable of the dissimilatory processing of inorganic sulfur compounds, including the earliest sulfur- and sulfate-reducing organisms.

While the dating of early evolutionary processes is exceedingly conjectural, the consensus view at present is that chemolithotrophic and autotrophic processes existed early in the development of living systems. Currently, however, there is debate about (1) the validity of the concept of the divergence of the Bacteria and the Archaea (Gupta 1998a, b) and (2) the nature and dating of the progenitor organisms that led to modern life forms (Gogarten-Boeckels et al. 1995; Gogarten et al. 1996). The first argument does not greatly affect our view of a very ancient origin of chemolithoautotrophy, but the theory advanced by the latter authors may push back the origin of these processes to a time in Earth's history even prior to the cessation of heavy meteoritic bombardment (Chyba 1992; Gogarten-Boeckels et al. 1995). Their hypothesis is that prokaryotic forms evolved and

could have reached a sophisticated state of development by about 3.7 billion years (3.7 Gyr) ago. These primordial prokaryotes would have colonized extremely hot environments but did not arise necessarily in such environments. The deep phylogenetic separation of modern Archaea and Bacteria, and their fundamental differences in cell biology, could be evidence of their descent from distinct primordial ancestors, each extant at 3.7 Gyr, rather than a common cenancestor. If a mass-extinction-scale extraterrestrial impact event occurred at 3.7 Gyr, resulting in near-boiling of oceans, then only isolated examples of ancient primordial and hyperthermophilic prokaryotic types might have survived, and two of these might have led by subsequent separate evolution to the Bacteria and Archaea (and presumably the Eukarya). This scenario would make the common ancestor of all current life forms a chemolithotrophic autotroph, which originated little more than 0.5 Gyr after the formation of the Earth.

If one accepts the view that elemental oxygen and nitrate are both relatively very recent additions to the natural environment (Broda 1977a; Gautier 1992), the latter only appearing when the biosphere became less reducing, then the modern chemolithotrophs, dependent on oxygen or nitrate respiration, must be regarded as relatively recent evolutionary products. Their origins, however, are clearly traced from the types of inorganic oxidative metabolism (e.g., of hydrogen by sulfate-reducers and of both hydrogen and sulfide by photolithotrophs) that evolved in the anaerobic phase of evolution. The basic patterns for chemolithotrophic and autotrophic biochemistry could well have been laid in this period; thus, the principle for sulfur-compound oxidation now seen in thiobacilli could have developed in ancient phototrophs. Interestingly, in relation to the idea of primordial organisms being anaerobic hyperthermophiles, there are several examples of modern thermophiles or hyperthermophiles capable of hydrogen oxidation either aerobically or by nitrate reduction to dinitrogen. Examples are *Hydrogenobacter acidophilus* (optimum temperature 65 °C and pH 3–4; Shima and Suzuki 1993) and the archeon *Pyrobaculum aerophilum* (optimum growth at 100 °C; Volkl et al. 1993). Better known are the examples of *Sulfolobus* and *Acidianus* (some with temperature optima approaching 100 °C), capable of aerobic sulfur oxidation or anaerobic hydrogen oxidation coupled to sulfur reduction (Fuchs et al. 1996); however, such organisms and *Pyrobaculum* are clearly unlike any primordial archeon in being facultative aerobes, but their unusual combination of chemolithotrophic potentials could be relict indicators of the earliest chemolithotrophic biochemical processes.

The mechanisms for ammonia (and possibly nitrite) oxidation could stem from more ancient methane-oxidizing organisms: methane oxidation by ammonia oxidizers supports this view, and the similarities of the DNA sequences for genes encoding ammonia and methane mono-oxygenases (Holmes et al. 1995) are consistent with evolution from common ancestral enzyme forms. The major changes were, of course, the development of enzyme systems enabling the reaction with oxygen and in some cases the coupling of energy conservation to the oxidation of inorganic substances.

The questions remain: are other lithotrophs to be discovered in nature, or are such theoretically possible chemolithotrophs missing from nature? If so, did they once exist?

As already stated, any exergonic oxidation reaction with a reasonably electronegative potential might support metabolism and growth. The oxidation of ferrous iron is a good example of a process of rather low energy yield and unfavorable electrode potential (relative to NAD^+ reduction) that has been very successfully exploited in the evolution of organisms like *Thiobacillus ferrooxidans*. Among some possible reactions would be the oxidation of metals that show several valence states. Thus, manganese oxidation (possibly a source of energy for ill-defined organisms like *Metallogenium*) could well prove to be the basis of a chemolithotrophic process, and the demonstration of ribulose 1,5-bisphosphate carboxylase genes in a marine manganese-oxidizing bacterium suggests that autotrophy driven by manganese oxidation may be possible (Caspi et al. 1996). There is also reasonable evidence that cytochrome-mediated oxidation of cuprous copper and stannous tin occurs in *T. ferrooxidans* (Lewis and Miller 1977), which appears anyway to obtain energy from the former. Other metal oxidations mentioned in the literature are of antimony (for which there is evidence of an organism; Lyalikova 1972), selenium, and uranium, for which possible reactions are

$$Sb_2O_3 + O_2 = Sb_2O_5 \qquad (\Delta F^0 - 276\ \text{kJ})$$
$$Se^0 + O_2 + H_2O = H_2SeO_3 \qquad (\Delta F^0 - 189\ \text{kJ})$$
$$H_2Se + 0.5O_2 = Se^0 + H_2O \qquad (\Delta F^0 - 75\ \text{kJ})$$
$$HSe^- + 0.5O_2 + H^+ = Se^0 + H_2O \qquad (\Delta F^0 - 80\ \text{kJ})$$
$$UO_2 + 0.5O_2 = UO_3 \qquad (\Delta F^0 - 109\ \text{kJ})$$
$$UO_2 + 0.5O_2 + H_2SO_4 = UO_2SO_4 + H_2O \qquad (\Delta F^0 - 151\ \text{kJ})$$

All of these oxidations yield at least as much energy as iron oxidation, presuming the ΔF0 values to reflect free energy changes that could be trapped metabolically.

Oxidation of anions also could be energy yielding: the organisms using sulfur oxyanions and nitrite are well known. Other energy substrates could be compounds of tellurium, molybdenum, vanadium, or any salt of a multivalent element capable of further oxidation. Another possible substrate might be phosphine (PH_3), which could be oxidized through several intermediates to orthophosphate, with an overall large change in free energy:

$$PH_3 + 2O_2 = H_3PO_4 \qquad (\Delta F^0 - 1166\ \text{kJ})$$

Broda (1977b) discussed the apparently unfulfilled expectation that two further types of ammonia-oxidizing bacteria might exist. The first of these was anaerobic, photosynthetic ammonia bacteria, which use NH_4^+ in a way analogous to sulfide, use light-dependent CO_2 fixation, and generate dinitrogen from ammonia:

$$1.3NH_4^+ + CO_2 = (CH_2O) = 0.65N_2 + H_2O + 1.3H^+ \ (\Delta F^0 + 50\ \text{kJ})$$

Such phototrophic organisms, if they ever existed, could have been the precursors of the modern aerobic nitrifying bacteria.

Anaerobic ammonia oxidation had not been shown in known species of modern nitrifying bacteria, and this was attributed to the presumed need for an oxygenation of ammonia to hydroxylamine which was obligatorily dependent on elemental oxygen. Broda (1977b) speculated that anaerobic ammonia oxidation using nitrite as oxidant is feasible, with dinitrogen as the end product. Similarly, aerobic ammonia oxidation to N_2 is theoretically possible as a chemolithotrophic process (Broda 1977b):

$$NH_4^+NO_2^- = N_2 + 2H_2O\ (\Delta F^0 - 360\ \text{kJ})$$

$$NH_4^+ + 0.75O_2 = 0.5N_2 + 1.5H_2O + H^+ \quad (\Delta F^0 - 314\ \text{kJ})$$

Both these reactions are more exergonic overall than aerobic ammonia oxidation to nitrite. That comparable processes may indeed be catalyzed by anaerobic ammonia-oxidizing bacteria is indicated by the observation in a commercial denitrification plant that the overall nitrogen balance of the system could be explained only if ammonia oxidation with nitrate as oxidant was occurring (Mulder 1989). Novel anaerobic, ammonium-oxidizing ("Anammox"), bacteria were shown to be highly enriched in this plant, apparently growing autotrophically by obtaining energy from the conversion of ammonia and nitrite (via nitrite, hydroxylamine, and hydrazine) to nitrogen gas (van der Graaf et al. 1996, 1997). The well-known aerobic nitrifier, *Nitrosomonas*, has been shown to couple anaerobic ammonia oxidation to the reduction of nitrogen dioxide, with the formation of NO and nitrite (Schmidt and Bock 1997).

The sulfur-oxidation-dependent reduction of ferric iron referred to earlier also could be a relict of a primordial, energy-yielding, proto-chemolithotrophic process. In the pre-oxic biosphere, most iron was probably in the ferrous state, but because of the absence of auto-oxidation, ferrous iron may have been a substrate for a form of photosynthesis. In any case, it was oxidized to ferric iron over possibly 10^9 years following the appearance of O_2 photosynthesis, but before free oxygen began to accumulate in the atmosphere. Abundant sulfide and iron could thus have made a selective "niche" for such chemolithotrophy.

Probably many of the theoretically feasible reactions for energy generation will not be found in any organisms, past or present, because the substrates of the reactions were never sufficiently abundant on earth for any chance evolution of an enzyme system that used the reaction to have survived. The modern chemolithotrophs seem to be so successful by one of two physiological specializations. Either they are specialist organisms, like the obligately chemolithotrophic sulfur oxidizers, which do not compete with heterotrophs because they use an inorganic substrate not available to most of the latter and are not dependent on organic carbon, or they are versatile facultative organisms, capable in organic, nutrient-rich environments of "switching off" their chemolithotrophic autotrophic metabolism and competing as successful heterotrophs. The place of mixotrophy and chemolithotrophic heterotrophy in the natural environment is less certain but probably confers advantage on such organisms, as they can compete simultaneously for distinct and unrelated sources of energy and carbon. In rather selective environments (such as acid, hot, or metal-rich habitats) where the chemolithotroph is best adapted to survive (e.g., the sulfur-oxidizing thermophiles of hot springs or the metal-tolerant, acidophilic, iron-oxidizing organisms of mineral leaching systems), mixotrophy could be a great selective advantage, where there is perhaps minimal competition with pure heterotrophs. Mixotrophy, in particular, potentially enables an organism to exploit as many nutritional facets of its environment as possible.

Our growing understanding of the complexity of the metabolic possibilities among chemolithotrophs and heterotrophs with chemolithotrophic potential, as well as the realization of the extreme antiquity of chemolithoautotrophic processes, not only helps explain why so many organisms can live together in seeming contradiction of the competitive exclusion principle but also means that even greater metabolic variety can be expected to be discovered.

References

Baas Becking LGM, Parks GS (1927) Energy relations in the metabolism of autotrophic bacteria. Physiol Rev 7:85–106

Badziong W, Thauer RK, Zeikus JG (1978) Isolation and characterization of *Desulfovibrio* growing on hydrogen plus sulfate as the sole energy source. Arch Microbiol 116:41–49

Bak F, Cypionka H (1987) A novel type of energy metabolism involving fermentation of inorganic sulfur compounds. Nature 326:891–892

Bak F, Pfennig N (1987) Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. Arch Microbiol 147:184–189

Barros MEC, Rawlings DE, Woods DR (1984) Mixotrophic growth of a *Thiobacillus ferrooxidans* strain. Appl Environ Microbiol 47:593–595

Beh M, Strauss G, Huber R, Stetter KO, Fuchs G (1993) Enzymes of the reductive citric acid cycle in the autotrophic eubacterium *Aquifex neutrophilus*. Arch Microbiol 160:306–311

Beudeker RF, Kerver JWM, Kuenen JG (1981a) Occurrence, structure, and function of intracellular polyglucose in the obligate chemolithotroph *Thiobacillus neapolitanus*. Arch Microbiol 129:221–226

Beudeker RF, De Boer W, Kuenen JG (1981b) Heterolactic fermentation of intracellular polyglucose by the obligate chemolithotroph *Thiobacillus neapolitanus* under anaerobic conditions. FEMS Microbiol Lett 12:337–342

Bock E (1976) Growth of Nitrobacter in the presence of organic matter. II. Chemoorganotrophic growth of *Nitrobacter agilis*. Arch Microbiol 108:305–312

Bock E, Wilderer PA, Freitag A (1988) Growth of Nitrobacter in the absence of dissolved oxygen. Water Res 22:245–250

Bock E, Koops H-P, Möller UC, Rudert M (1990) A new facultatively nitrite-oxidizing bacterium, *Nitrobacter vulgaris* sp. nov. Arch Microbiol 153:105–110

Brierley JA, Brierley CL (1968) Urea as a nitrogen source of thiobacilli. J Bacteriol 96:573–574

Brierley JA, Norris PR, Kelly DP, Le Roux NW (1978) Characteristics of a moderately thermophilic and acidophilic iron-oxidizing *Thiobacillus*. European J Appl Microbiol Biotechnol 5:291–299

Brierley CL, Brierley JA, Norris PR, Kelly DP (1980) Metal-tolerant microorganisms of hot, acid environments. In: Gould GW, Corry JEL (eds) Microbial growth and survival in extremes of environment, vol 15, Society for applied bacteriology technical series. Academic, London, pp 39–51

Brock TD, Gustafson J (1976) Ferric iron reduction by sulfur-and iron-oxidizing bacteria. Appl Environ Microbiol 32:567–571

Brock TD, Schlegel H (1989) Introduction. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer-Verlag/Science Tech Publishers, Berlin/Madison WI, pp 1–15

Broda E (1977a) The position of nitrate respiration in evolution. Orig Life 8:173–174

Broda E (1977b) Two kinds of lithotrophs missing in nature. Z Allg Mikrobiol 17:491–493

Burggraf S, Olse GJ, Stetter KO, Woese CR (1992) A phylogenetic analysis of *Aquifex pyrophilus*. Syst Appl Microbiol 15:352–356

Butlin KR, Adams ME (1947) Autotrophic growth of sulphate-reducing bacteria. Nature 160:154–155

Caspi R, Haygood MG, Tebo BM (1996) Unusual ribulose-1, 5-biphosphate carboxylase/oxygenase genes from a marine manganese-oxidizing bacterium. Microbiology (UK) 142:2549–2559

Chyba CF (1992) The violent environment of the origin of life. In: Tran Thanh Van J, Tran Thanh Van K, Mounlou JC, Schneider J, McKay C (eds) Frontiers of life. Editions Frontieres, Gif-sur-Yvette France, pp 97–104

Clark DA, Norris PR (1996) *Acidimicrobium ferrooxidans* gen. nov., sp. nov.: mixed cultures ferrous iron. Microbiology (UK) 142:785–790

Cypionka H, Smock AM, Bottcher ME (1998) A combined pathway of sulfur compound disproportionation in *Desulfovibrio desulfuricans*. FEMS Microbiol Lett 166:181–186

Davis OH, Doudoroff M, Stanier RY (1969) Proposal to reject the genus *Hydrogenomonas*. Int J Syst Bacteriol 19:375–390

Eccleston M, Kelly DP (1978) Oxidation kinetics and chemostat growth kinetics of *Thiobacillus ferrooxidans* on tetrathionate and thiosulfate. J Bacteriol 134:718–727

Edwards MR (1998) From a soup or a seed? Trends Ecol Evol 13:178–181

Eisenmann E, Beuerle J, Sulger K, Kroneck PMH, Schumacher W (1995) Lithotrophic growth of *Sulfospirillum deleyianum* with sulfide as electron donor coupled to respiratory reduction of nitrate to ammonia. Arch Microbiol 164:180–185

Evans MCW, Buchanan BB, Arnon DI (1966) A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc Natl Acad Sci USA 55:928–934

Freitag A, Rudert M, Bock E (1987) Growth of *Nitrobacter* by dissimilatory nitrate reduction. FEMS Microbiol Lett 48:105–109

Friedrich C, Mitrenga G (1981) Oxidation of thiosulfate by *Paracoccus denitrificans* and other hydrogen bacteria. FEMS Microbiol Lett 10:209–212

Fromageot C, Senez JC (1960) Aerobic and anaerobic reactions of inorganic substances. In: Florkin M, Mason HS (eds) Comparative biochemistry, vol 1. Academic, New York, pp 347–409

Fuchs G (1989) Alternative pathways of autotrophic CO_2 fixation. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer-Verlag\Science Tech Publishers, Berlin\Madison WI, pp 365–382

Fuchs T, Huber H, Burggraf S, Stetter KO (1996) The 16 S rDNA-based phylogeny of the archaeal order *Sulfolobales* and reclassification of *Desulfurolobus ambivalens* as *Acidanus ambivalens* comb nov. Syst Appl Microbiol 19:56–60

Galtier N, Tourasse N, Gouy M (1999) A nonhyperthermophilic common ancestor to extant life forms. Science 283:220–221

Gautier D (1992) Primitive planetary atmospheres: origin and evolution. In: Tran Thanh Van J, Tran Thanh Van K, Mounlou JC, Schneider J, McKay C (eds) Frontiers of life. Editions Frontieres, Gif-sur-Yvette France, pp 307–315

Gogarten JP (1995) The early evolution of cellular life. Trends Ecol Evol 10:147–151

Gogarten JP, Taiz L (1992) Evolution of proton pumping ATPases rooting the tree of life. Photosynth Res 33:137–146

Gogarten JP, Olendzenski L, Hilario E, Simon C, Holsinger KE (1996) Dating the cenancestor of organisms. Science 274:1750–1751

Gogarten-Boeckels M, Hilario E, Gogarten JP (1995) The effects of heavy meteroritic bombardment on the early evolution—the emergence of the three domains of life. Orig Life Evol Biosph 25:251–264

Gommers PJF, Kuenen JG (1988) *Thiobacillus* strain Q, a chemolithoheterotrophic sulphur bacterium. Arch Microbiol 150:117–125

Gottschal JC, de Vries S, Kuenen JG (1979) Competition between the facultatively chemolithotrophic Thiobacillus A2, an obligately chemolithotrophic Thiobacillus and a heterotrophic spirillum for inorganic and organic substrates. Arch Microbiol 121(3):241–249

Grabovich MY, Dubinina GA, Lebedeva VY, Churikova VV (1998) Mixotrophic and lithoheterotrophic growth of the freshwater filamentous sulfur bacterium *Beggiatoa leptomitiformis* D-402. Microbiology (Moscow) 67:383–388

Gribaldo S, Cammarano P (1998) The root of the universal tree of life inferred from anciently duplicated genes encoding components of the protein-targeting machinery. J Mol Evol 47:508–516

Güde H, Strohl WR, Larkin JM (1981) Mixotrophic and heterotrophic growth of *Beggiatoaalba* in continuous culture. Arch Microbiol 129:357–360

Gupta RS (1998a) Life's third domain (Archaea): an established fact or an endangered paradigm? Theor Popul Biol 54:91–104

Gupta RS (1998b) What are archaebacteria: life's third domain or modern prokaryotes related to Gram-positive bacteria? A new proposal for the classification of prokaryotic organisms. Mol Microbiol 29:695–707

Hagen KD, Nelson DC (1996) Organic carbon utilization by obligately and facultatively autotrophic *Beggiatoa* strains in homogeneous and gradient cultures. Appl Environ Microbiol 62:947–953

Hanert H (1981) The genus *Gallionella*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes, 1st edn. Springer, Berlin, pp 509–515

Hempfling WP, Vishniac W (1967) Yield coefficients of *Thiobacillus neapolitanus* in continuous culture. J Bacteriol 93:874–878

Hipp WM, Pott AS, Thum-Schmirtz N, Faath I, Dahl C, Truper HG (1997) Towards a phylogeny of APS reductases and sirohaem sulfite reductases in sulfate-reducing and sulfur-oxidizing prokaryotes. Microbiology (UK) 143:2891–2902

Holmes AJ, Costello A, Lidstrom ME, Murrell JC (1995) Evidence that particulate methane monooxygenase may be evolutionarily related. FEMS Microbiol Lett 132:203–208

Holo H (1989) *Chloroflexus aurantiacus* secretes 3-hydroxypropionate, a possible intermediate in the assimilation of carbon dioxide and acetate. Arch Microbiol 151:252–256

Horowitz NH (1945) On the evolution of biochemical synteses. Proc Natl Acad Sci USA 31:153–157

Huber R, Wilharm T, Huber D, Trincone A, Burggraf S, Konig H, Rachel R, Rockinger I, Fricke H, Stetter KO (1992) *Aquifex pyrophilus*, gen. nov. sp. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. Syst Appl Bacteriol 15:340–351

Ishii M, Miyake T, Satoh T, Sugiyama H, Oshima Y, Igarashi Y (1996) Autotrophic carbon dioxide fixation in *Acidanus brierleyi*. Arch Microbiol 166:368–371

Jannasch HW, Wirsen CO (1979) Chemosynthetic primary production at East Pacific sea floor spreading centres. Bioscience 29:592–598

Jones CA, Kelly DP (1983) Growth of *Thiobacillus ferrooxidans* on ferrous iron in chemostat culture: influence of product and substrate inhibition. J Chem Tech Biotechnol 33B:241–261

Justin P, Kelly DP (1978) Growth kinetics of *Thiobacillus denitrificans* in anaerobic and aerobic chemostat culture. J Gen Microbiol 107:123–130

Katayama Y, Hiraishi A, Kuraishi H (1995) *Paracoccus thiocyanatus* sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of Thiobacillus versutus to the genus *Paracoccus* as *Paracoccus* versutus comb. nov. with emendation of the genus. Microbiology (UK) 141:1469–1477

Katayama-Fujimura Y, Kuraishi H (1983) Emendation of *Thiobacillus perometabolis* London and Rittenberg, 1967. Int J Syst Bacteriol 33:650–651

Kawasumi T, Igarashi U, Kodama T, Minoda Y (1988) Isolation of strictly thermophilic and obligately autotrophic hydrogen bacteria. Agric Biol Chem 44:1985–1986

Keil F (1912) Beiträge zur Physiologie der farblosen Schwefelbakterien. Beitr Biol Pfl 11:335–365

Kelly DP (1967) Problems of the autotrophic microorganisms. Sci Prog 55:35–51

Kelly DP (1971) Autotrophy: concepts of lithotrophic bacteria and their organic metabolism. Ann Rev Microbiol 25:177–210

Kelly DP (1978) Bioenergetics of chemolithotrophic bacteria. In: Bull AT, Meadow PM (eds) Companion to microbiology. Longman, London, pp 363–386

Kelly DP (1981) Introduction to the chemolithotrophic bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes, 1st edn. Springer, Berlin, pp 997–1004

Kelly DP (1982) Biochemistry of the chemolithotrophic oxidation of inorganic sulphur. Phil Trans R Soc London B298:499–528

Kelly DP (1985) Crossroads for archaebacteria. Nature 313:734

Kelly DP (1987) Sulphur bacteria first again. Nature 326:830–831

Kelly DP (1988) Oxidation of sulphur compounds. Soc Gen Microbiol Symp 42:65–98

Kelly DP (1989) Physiology and biochemistry of unicellular sulfur bacteria. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer-Verlag/Science Tech Publishers, Berlin/Madison WI, pp 193–217

Kelly DP (1990) Energetics of chemolithotrophs. In: Krulwich TA (ed) Bacterial energetics, vol 12. Academic, San Diego, pp 478–503

Kelly DP (1991) The chemolithotrophic prokaryotes. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, New York, pp 331–343

Kelly DP (1999) Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. Arch Microbiol 171:219–229

Kelly DP, Harrison AP (1989) The genus *Thiobacillus*. In: Staley JT (ed) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins, Baltimore, pp 1842–1858

Kelly DP, Kuenen JG (1984) Ecology of the colourless sulphur bacteria. In: Codd GA (ed) Aspects of microbial metabolism and ecology. Academic, London, pp 211–240

Kelly DP, Smith NA (1990) Organic sulfur compounds in the environment. Adv Microbiol Ecol 11:345–385

Kelly DP, Wood AP (1982) Autotrophic growth of *Thiobacillus* A2 on methanol. FEMS Microbiol Lett 15:229–233

Kelly DP, Wood AP (1984) R. L. Crawford. In: Crawford RL, Hanson RS (eds) Microbial growth on C_1-compounds. American Society for Microbiology, Washington D. C, pp 324–329

Kelly DP, Wood AP (2000) The genus *Thiobacillus Beijerinck*. In: Krieg NR, Staley JT, Brenner DJ (eds) Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer, New York NY, in press

Kelly DP, Eccleston M, Jones CA (1977) Evaluation of continuous chemostat cultivation of *Thiobacillus ferrooxidans* on ferrous iron or tetrathionate. In: Schwartz W (ed) Bacterial leaching. Verlag Chemie, Weinheim, pp 1–7

Kelly DP, Wood AP, Gottschal JC, Kuenen JG (1979) Autotrophic metabolism of formate by *Thiobacillus* strain A2. J Gen Microbiol 114:1–13

Khmelenina VN, Gayazov RR, Suzina NE, Doronina VA, Mshenshii YN, Trotsenko YA (1992) Synthesis of polysaccharides by Methylococcus capsulatus under different growth conditions. Microbiology (Moscow) 61:277–282

Kiesow L (1963) Über die Reduktion von Diphospho-pyridinnucleotid bei der Chemosynthese. Biochem Z 338:400–406

Kondratieva EN (1989) Chemolithotrophy of phototrophic bacteria. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer-Verlag/Science Tech Publishers, Berlin/Madison WI, pp 283–287

Kondratieva EN, Zhukov VG, Ivanovsky RN, Petushkova Yu P, Monosov EZ (1976) The capacity of phototrophic sulfur bacterium *Thiocapsa roseopersicina* for chemosynthesis. Arch Microbiol 108:287–292

Krämer M, Cypionka H (1989) Sulfate formation via ATP sulfurylase in thiosulfate-and sulfite-disproportionating bacteria. Arch Microbiol 151:232–237

Kristjansson JK, Ingason A, Alfredsson GA (1985) Isolation of thermophilic, obligately autotrophic hydrogen-oxidizing bacteria, similar to *Hydrogenobacter thermophilus*, from Icelandic hot springs. Arch Microbiol 140:321–325

Lane DJ, Harrison AP, Stahl D, Pace B, Giovannoni SJ, Olsen GJ, Pace NP (1992) Evolutionary relationships among sulfur-and iron-oxidizing eubacteria. J Bacteriol 174:269–278

Lewis AJ, Miller DJD (1977) Stannous and cuprous iron oxidation by *Thiobacillus ferrooxidans*. Can J Microbiol 23:319–324

London J (1963) *Thiobacillus intermedius* nov. sp. A novel type of facultative autotroph. Arch Mikrobiol 46:329–337

London J, Rittenberg SC (1967) *Thiobacillus perometabolis* nov. sp., a non-autotrophic *Thiobacillus*. Arch Mikrobiol 59:218–225

Lyalikova NN (1972) Oxidation of trivalent antimony up to higher oxides as a source of energy for the development of a new autotrophic organism, *Stibiobacter* gen. nov. Russian Doklady Akademii Nauk SSSR 205:1228–1229

Maden BEH (1995) No soup for starters? Autotrophy and the origins of metabolism. Trends Biochem Sci 20:337–341

Mason J, Kelly DP (1988) Thiosulfate oxidation by obligately heterotrophic bacteria. Microb Ecol 15:123–134

McDonald IR, Kelly DP, Murrell JC, Wood AP (1997) Taxonomic relationships of *Thiobacillus halophilus, T. Aquaesulis*, and other species of *Thiobacillus*, as determined using 16 S rRNA sequencing. Arch Microbiol 166:394–398

McFadden BA, Denend AR (1972) Ribulose diphosphate carboxylase from autotrophic microorganisms. J Bacteriol 110:633–642

Mechalas BJ, Rittenberg SC (1960) Energy coupling in *Desulfovibrio desulfuricans*. J Bacteriol 80:501–507

Metzdorf N, Kaltwasser H (1988) Utilization of organic compounds as the sole source of nitrogen by *Thiobacillus thiooxidans*. Arch Microbiol 150:85–88

Meyer O (1989) Aerobic carbon monoxide-oxidizing bacteria. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer/Science Tech Publishers, Berlin/Madison WI, pp 331–350

Moreira D, Amils R (1997) Phylogeny of *Thiobacillus* cuprinus and other mixotrophic thiobacilli: proposal for *Thiomonas* gen nov. Int J Syst Bacteriol 47:522–528

Nelson DC, Hagen DC (1996) Organic carbon utilization by obligately and facultatively autotrophic *Beggiatoa* strains in homogeneous and gradient cultures. Appl Environ Microbiol 62:947–953

Nelson DC, Jannasch HW (1983) Chemoautotrophic growth of a marine *Beggiatoa* in sulfide-gradient cultures. Arch Microbiol 136:262–269

Nelson DC, Revsbech NP, Jørgensen BB (1986a) Microoxic-anoxic niche of *Beggiatoa* spp. microelectrode survey of marine and freshwater strains. Appl Environ Microbiol 52:161–168

Nelson DC, Jørgensen BB, Revsbech NP (1986b) Growth pattern and yield of chemoautotrophic *Beggiatoa* sp. in oxygen-sulfide gradients. Appl Environ Microbiol 52:225–233

Nelson DC, Wirsen CO, Jannasch HW (1989a) Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. Appl Environ Microbiol 55:2909–2917

Nelson DC, Williams CA, Farah BA, Shively JM (1989b) Occurrence and regulation of Calvin cycle enzymes in non-autotrophic *Beggiatoa* strains. Arch Microbiol 151:15–19

Nishihara H, Igarashi Y, Kodawa T (1989) Isolation of an obligately chemolithoautotrophic, halophilic and aerobic hydrogen-oxidizing bacterium from marine environment. Arch Microbiol 152:39–43

Nishihara H, Igarashi U, Kodawa T (1990) A new isolate of *Hydrogenobacter*, an obligately chemolithoautotrophic, thermophilic, halophilic and aerobic hydrogen-oxidizing bacterium from a seaside saline hot spring. Arch Microbiol 153:294–298

Nishihara H, Igarashi Y, Kodama T (1991) *Hydrogenovibrio marinus* gen nov. sp. nov., a marine obligately chemolithotrophic hydrogen-oxidizing bacterium. Int J Syst Bacteriol 41:130–133

Nishihara H, Toshiaki Y, Chung SY, Suzuki K-I, Yanagi M, Yamasata K, Kodama T, Igarashi Y (1998) Phylogenetic position of an obligately chemoautotrophic, marine hydrogen-oxidizing bacterium, *Hydrogenovibrio marinus*, on the basis of 16 S rRNA gene sequences and two form I RuBisCO gene sequences. Arch Microbiol 169:364–368

Odintsova EV, Wood AP, Kelly DP (1993) Chemolithoautotrophic growth of *Thiothrix ramosa*. Arch Microbiol 160:152–157

Oparin A (1957) The origin of life on the Earth (trans. A. Synge). Oliver and Boyd, Edinburgh

Postgate JR (1979) The sulphate-reducing bacteria. Cambridge University Press, Cambridge

Rainey FA, Kelly DP, Stackebrandt E, Burghardt J, Hiraishi A, Katayama Y, Wood AP (1999) A reevaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the creation of *Paracoccus pantotrophus* comb nov. Int J Syst Bacteriol 49:645–651

Rittenberg SC (1969) The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. Adv Microbial Physiol 3:159–196

Rittenberg SC (1972) The obligate autotroph—the demise of a concept. Antonie van Leeuwenhoek J Microbiol Serol 38:457–478

Robertson LA, Kuenen JG (1983) *Thiosphaera pantotropha* gen nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. J Gen Microbiol 129:2847–2855

Robertson LA, Kuenen JG (1991) The colorless sulfur bacteria. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, New York, pp 385–413

Ruby EG, Wirsen CO, Jannasch HW (1981) Chemolithotrophic sulfur-oxidizing bacteria from the Galapagos rift hydrothermal vents. Appl Environ Microbiol 42:317–324

Schauder R, Widdel F, Fuchs G (1987) Carbon assimilation pathways in sulfate-reducing bacteria 2 Enzymes of a reductive citric acid cycle in the autotrophic *Desulfobacter hydrogenophilus.* Arch Microbiol 167:218–225

Schlegel HG (1975) Mechanisms of chemoautotrophy. In: Kinne O (ed) Marine ecology, vol 2, part 1. Wiley, London, pp 9–60

Schmidt I, Bock E (1997) Anaerobic ammonia oxidation with nitrogen dioxide by *Nitrosomonas eutropha.* Arch Microbiol 167:106–111

Schönheit P, Schäfer T (1995) Metabolism of hyperthermophiles. World J Microbiol Biotechnol 11:26–57

Segerer A, Stetter KO, Klink F (1985) Two contrary modes of chemolithotrophy in the same bacterium. Nature 313:787–789

Segerer A, Neuner A, Kristjansson JK, Stetter KO (1986) *Acidianus infernus* gen. nov. sp. nov., and *Acidianus brierleyi* comb. nov. facultatively aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Int J Syst Bacteriol 36:559–564

Shima S, Suzuki KI (1993) *Hydrogenobacter acidophilus* sp. nov., a thermoacidophilic, aerobic, hydrogen-oxidizing bacterium requiring elemental sulfur for growth. Int J Syst Bacteriol 43:703–708

Smith AJ, Hoare DS (1968) Acetate assimilation by *Nitrobacteragilis* in relation to its "obligateautotrophy". J Bacteriol 95:844–855

Smith AJ, Hoare DS (1977) Specialist phototrophs, lithotrophs, and methylotrophs: a unity among a diversity of prokaryotes? Bacteriol Rev 41:419–448

Smock AM, Bottcher ME, Cypionka H (1998) Fractionation of sulfur isotopes during thiosulfate reduction by *Desulfovibrio desulfuricans.* Arch Microbiol 169:460–463

Stanley SH, Dalton H (1982) Role of ribulose-1,5-biphosphate carboxylase/oxygenase in *Methylococcus capsulatus.* J Gen Microbiol 128:2927–2935

Stetter KO (1992) Life at the upper temperature border. In: Tran Thanh Van J, Tran Thanh Van K, Mounlou HC, Schneider J, McKay C (eds) Frontiers of life. Editions Frontieres, Gif-sur-Yvette France, pp 195–219

Taylor S (1977) Evidence for the presence of ribulose 1,5-bisphosphate carboxylase and phosphoribulokinase in *Methylococcuscapsulatus* (Bath). FEMS Microbiol Lett 2:305–307

Teske A, Ramsing NB, Kuever J, Fossing H (1996) Phylogeny of *Thioploca* and related filamentous sulfide-oxidizing bacteria. Syst Appl Microbiol 18:517–526

Thauer RK (1989) Energy metabolism of sulfate-reducing bacteria. In: Schlegel HG, Bowien B (eds) Autotrophicbacteria. Springer-Verlag/Science Tech Publishers, Berlin/Madison WI, pp 397–413

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Timmer-ten-Hoor A (1976) Energetic aspects of the metabolism of reduced sulphur compounds in *Thiobacillus denitrificans.* Antonie van Leeuwenhoek J Microbiol Serol 42:483–492

Umbreit WW (1947) Problems of autotrophy. Bacteriol Rev 11:157–182

van der Graaf AA, de Bruijn P, Robertson LA, Jetten MSM, Kuenen JG (1996) Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. Microbiology (UK) 142:2187–2196

van der Graaf AA, de Bruijn P, Robertson LA, Jetten MSM, Kuenen JG (1997) Metabolic pathway of anaerobic ammonium oxidation on the basis of 15 N studies in a fluidized bed reactor. Microbiology (UK) 143:2415–2421

van Gool A, Tobback PP, Fischer I (1971) Autotrophic growth and synthesis of reserve polymers in *Nitrobacter winogradskyi.* Arch Mikrobiol 76:252–264

van Niel CB (1943) Biochemical problems of the chemoautotrophic bacteria. Physiol Rev 23:338–364

Volkl P, Huber R, Drobner E, Rachel R, Burggraf S, Trincone A (1993) *Pyrobaculum aerophilum* sp. nov., a novel nitrate-reducing hyperthermophilic *Archaeum.* Appl Environ Microbiol 59:2918–2926

Wachtershauser G (1988) Before enzymes and templates: theory of surface metabolism. Microbiol Rev 52:452–484

Wachtershauser G (1990a) The case for the chemo-autotrophic origin of life in an iron-sulfur world. Orig Life Evol Biosph 20:173–176

Wachtershauser G (1990b) Evolution of the first metabolic cycles. Proc Natl Acad Sci USA 87:200–204

Wachtershauser G (1992) Order out of order. In: Tran Thanh Van J, Tran Thanh Van K, Mounlou JC, Schneider J, McKay C (eds) Frontiers of life. Editions Frontieres, Gif-sur-Yvette France, pp 21–39

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J Bacteriol 180:2975–2982

Watson GMF, Yu J-P, Tabita FR (1999) Unusual ribulose 1,5-biphosphate carboxylase/oxygenase of anoxic Archaea. J Bacteriol 181:1569–1575

Whittenbury R, Kelly DP (1977) Autotrophy: a conceptual phoenix. Symp Soc Gen Microbiol 27:121–149

Winogradsky S (1887) Über Schwefelbacterien. Bot Z 45(489–600):606–616

Winogradsky S (1922) Eisenbakterien als Anorgoxydanten. Centralbl Bakteriol Parasitenk Abt 2, 57:1–21

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

Woese CR (1998) The universal ancestor. Proc Natl Acad Sci USA 95:6854–6859

Wood AP, Kelly DP (1983) Autotrophic and mixotrophic growth of three thermoacidophilic iron-oxidizing bacteria. FEMS Microbiol Lett 20:107–112

Wood AP, Kelly DP, Norris PR (1987) Autotrophic growth of four *Sulfolobus* strains on tetrathionate and the effect of organic nutrients. Arch Microbiol 146:382–389

Zavarzin GA (1989) Sergei N. Winogradsky and the discovery of chemosynthesis. In: Schlegel HG, Bowien B (eds) Autotrophic bacteria. Springer-Verlag/Science Tech Publishers, Berlin/Madison WI, pp 17–32

Zillig W, Yeats S, Holz I, Böck A, Gropp F, Rettenberger M, Lutz S (1985) Plasmid-related anaerobic autotrophy of the novel archaebacterium Sulfolobus ambivalens. Nature 313:789–791

Zillig W, Yeats S, Holz I, Böck A, Rettenberger M, Gropp F, Simon G (1986) *Desulfurolobus ambivalens* gen nov., sp. nov., an autotrophic archaebacterium, facultatively oxidizing or reducing sulfur. Syst Appl Microbiol 8:197–203

15 Bacterial Behavior

Judith P. Armitage · Kathryn A. Scott
OCISB, Department of Biochemistry, University of Oxford, Oxford, UK

Introduction

The majority of bacterial species can swim, as evidenced both by the observation of growing cell cultures and the identification of genes encoding motility elements in genome sequences. Swimming uses the most complex protein structure in the bacterial cell, the bacterial flagellum. The flagellum is the product of approximately 50 genes, controling or encoding 25 or so proteins found in multiple copies within a structure spanning from the cytoplasm to the extracellular environment. The extracellular helical flagellum is rotated at 300–1,300 Hz by the movement of protons or sodium ions through a transmembrane rotary motor. The flagellum has an ancient evolutionary history; although there are variations between species, the central core of the flagellum is conserved across all bacteria studied to date. Indeed it may also share a common evolutionary history with the protein complexes used by some species to glide over surfaces without the use of pili, and both may share an origin with proton-motive-force-dependent transmembrane transport systems, such as the Ton-type systems and type 3 secretion systems. Interestingly, although the chemosensory system regulating swimming behavior is common to bacteria and archaea, archaea have a motility system based on an ATP-dependent pilus-like system.

This chapter will concentrate on the swimming behavior of bacterial cells, as less is known about the synthesis and mechanism of action of archaeal flagella (Armitage 2006). Bacterial flagella are metabolically expensive to synthesize due to the large number of proteins in the flagellar filament; therefore, if a bacterium is motile, it is because motility provides a survival advantage. The control of swimming direction moves bacterial cells to optimum environments for growth, for better or worse from a human point of view as it might enable a symbiote to reach a legume root and enhance nitrogen fixation or move a pathogen toward a site for invasion. Recently it has become apparent that most bacteria in the environment may be associated with surface-attached biofilms, but again, motility is important for both getting to a surface and moving from one surface to a new site.

Swimming at Low Reynolds Number

The size of a bacterium means that it will experience very little inertia as it swims and that the dominant physical force is due to viscosity. A bacterium therefore does not displace liquid as it moves and will stop immediately when flagella rotation is halted. Indeed, it has been calculated that the stopping distance is less than the diameter of a proton (Berg 1993). The Reynolds number is a measure of the ratio of inertial to viscous forces for objects of a given size. It is defined as $R = lv\rho/\eta$ where l is the length of the organism, v the velocity of the organism, ρ the density, and η the viscosity of the liquid. While a large organism, such as man, may have a Reynolds number of over 100, a bacterium has one about 10^{-6}. At these low Reynolds numbers, fluid flow is smooth and streamlining unnecessary; bacteria do not displace liquid but carry a shell of medium with them.

In addition to the problems involved in swimming through a highly viscous environment, bacteria have also to cope with the

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_53,

buffeting that comes from the movement of the molecules in the water, Brownian motion. Nonmotile cells observed down a microscope are seen to be constantly moving; swimming bacteria are subject to the same forces and therefore cannot swim in a straight line for more than a few seconds before they are moved off course. This, combined with a gentle curve which results from the rotation of the cell body, means that a sensory system has evolved to frequently reset the swimming direction and allow movement in a positive direction despite the constant bombardment.

The majority of bacterial species are also too small to sense a stimulus gradient along their body length. This means that unlike eukaryotic microbes which can have a head and a tail and swim directly toward the source of an attractant (reviewed in Swaney et al. (2010)), prokaryotes cannot sense spatially but must instead make temporal comparisons (Berg and Turner 1995; Brown and Berg 1974). They compare the strength of a stimulus now with that a few seconds before. This means that their patterns of swimming must be different from eukaryotes rather than steering toward an attractant; they must change direction regularly to "check" if they are going in a positive direction (see section on "Patterns of Swimming" in this chapter).

Flagella

Although the flagella of all swimming prokaryotes perform the same function, it has become apparent that there are marked differences between the structures of bacterial and archaeal flagella and the mechansims by which their rotation is driven (Thomas et al. 2001; Bardy et al. 2004). Bacteria swim by rotating semirigid helical flagellar filaments; these filaments are ~20 nm in thickness and have a central lumen through which flagellin mononers are transported to the distal tip for assembly. Rotation is driven by the electrochemical ion gradient across the cytoplasmic membrane, which is typically a proton-motive force but in some species is a sodium-motive force. In contrast, archaeal flagella show a greater similarity to bacterial type IV pili than to bacterial flagella; archaeal flagellins lack sequence similarity to bacterial flagllelins and homologues of bacterial genes encoding flagellar assembly are not found in archaeal genomes (Ng et al. 2006). The archaeal flagella have a smaller diameter than bacterial flagella and are thought to be assembled from the proximal end. Homologues of bacterial motor components are not seen in the archaea, and it has recently been shown that archaeal flagella rotation can be ATP dependent (Streif et al. 2008).

Patterns of Flagellation

An early method of species classification relied on whether bacteria swam and, if they did, upon the pattern of flagellation. We now know that whether or not a bacterium swims may depend on the medium in which it is grown, with many species only swimming under limited growth conditions or during certain phases of the growth cycle. Some species have a single flagellum which may be polar as in *Pseudomonas aeruginosa* or randomly positioned as in *Rhodobacter sphaeroides*. Many species have multiple flagella arising from apparently random sites all over the cell surface. These peritrichous flagella tend to come together in bundles to cause the cells, such as *Escherichia coli* or *Sinorhizobium meliloti*, to swim. Bundles of flagella are also found at the poles of some species; these might be at both poles as in *Rhodospirillum rubrum* or at one pole as in *Chromatium vinosum*. Some species, such as *Borrelia burgorferi*, have internalized filaments, a characteristic of the spirochetes (Holt 1978).

Interestingly several species of bacteria alter their patterns of flagellation dependent on their physical environment. Many marine *Vibrio* species, including *Vibrio alginolyticus* and *Vibrio parahaemolyticus*, swim using a single flagellum when in an aquatic environment but when in contact with a solid surface induce the synthesis of numerous peritrichous flagella enabling movement along the surface (McCarter 2004). For many years, such environmental control of flagellation was believed to be a defining characteristic of *Proteus* species but is now recognized to occur in *Bacillus, Salmonella, Rhodospirillum*, and many other species (Harshey 1994, 2003). It is noteworthy that *V. alginolyticus* switches between sodium- and proton-driven flagellar motors when cells change from free-swimming to surface movement (Atsumi et al. 1992), while other species, such as *Shewanella oneidensis*, can change the ion used to drive the motor depending on the prevailing environment (Paulick et al. 2009). Genome sequencing has identified many species with regulons for two or more complete flagellar systems, although the conditions under which the different systems are expressed are often unclear. A good example is *R. sphaeroides* which encodes two flagella systems (Mackenzie 2001; Choudhary et al. 2004). Under standard laboratory conditions, it swims by rotating a single randomly positioned flagellum encoded by the *fla*1 genes. Recently however, mutants expressing the *fla*2 genes, which produce a polar tuft of flagella, have been isolated (Poggio et al. 2007). The role of the two flagellar systems in the motility of *R. sphaeroides* in its natural environment, however, remains unclear.

Patterns of Swimming

All flagellar filaments appear to be passive helices, rotated at their base by a transmembrane motor which is driven by either the transmembrane electrochemical proton or sodium gradient. The different patterns of swimming observed in different species arise because of different patterns of motor switching (*Fig. 15.1*). The semirigid helix of the bacterial flagellum has a particular handedness and wavelength when rotated in one direction. Switching rotational direction from counterclockwise (CCW) to clockwise (CW) changes both the wavelength and handedness of the helix and thus the swimming direction. The best-studied swimming pattern is that of the peritrichously flagellate bacteria. The individual flagellar motors

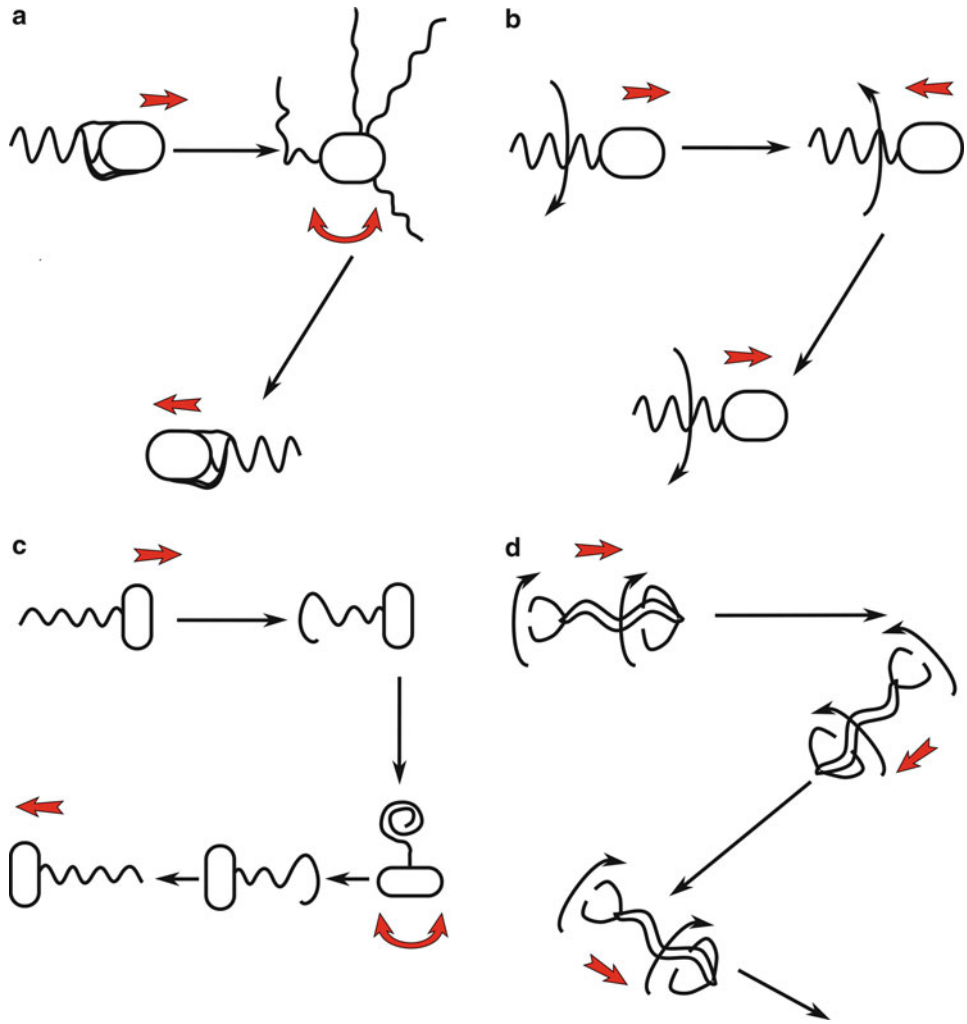


Fig. 15.1
This cartoon shows four different patterns of flagellation and the corresponding mechanisms of direction changing. (a) *Escherichia coli* swims by rotating a bundle of flagella counterclockwise. Periodically, a number of flagellar motors switch to clockwise rotation, and the bundle flies apart causing the cell to tumble. When the majority of filaments return to counterclockwise rotation and the bundle reforms, the cell is usually pointing in a new direction. (b) *Pseudomonas aeruginosa* swims by rotating its single polar flagellum thereby pushing the cell forward. Periodically, the motor reverses its direction of rotation and pulls the cell backward. During this time, Brownian motion tends to reorient the cell. The motor again reverses direction and the flagellum resumes pushing the cell, usually in a new direction. (c) *Rhodobacter sphaeroides* has a single flagellum that rotates clockwise, pushing the cell forward. Periodically, the motor stops and the flagellum relaxes into a short wavelength, large-amplitude, filament. This filament is rotated slowly, reorienting the cell. After a period of time, the motor resumes normal clockwise rotation and the functional filament reforms to push the cell in a new direction. (d) *Rhodospirillum rubrum* has two polar tufts of flagella that rotate to move the spiral-shaped cells through the medium. Periodically, the bundles of flagella synchronously reverse the direction of their rotation to change the direction in which the cell is swimming

can rotate either CCW or CW and switch between the two states (Macnab 1976, 1977; Khan and Macnab 1980). When the majority of flagella are rotating CCW, the helical filaments come together as a bundle, rotate together, and push the cell forward. Periodically, a number of motors switch to CW rotation, causing a polymorphic transition in the flagellar helix such that the handedness and wavelength changes. As a result, the bundle of flagella is forced apart and the cell "tumbles" on the spot (*Fig. 15.2*). The bundle reforms when the majority of motors return to CCW rotation and the cell resumes swimming, usually in a new direction (Berg and Anderson 1973; Turner et al. 2000). This pattern of periods of smooth swimming interspersed every few seconds with a brief period of tumbling results in a three-dimensional random pattern of swimming (Berg and Brown 1972).

Other species achieve a three-dimensional pattern of swimming by a different motor behavior. For example, *R. sphaeroides* has a single flagellum which only rotates CCW. Every few

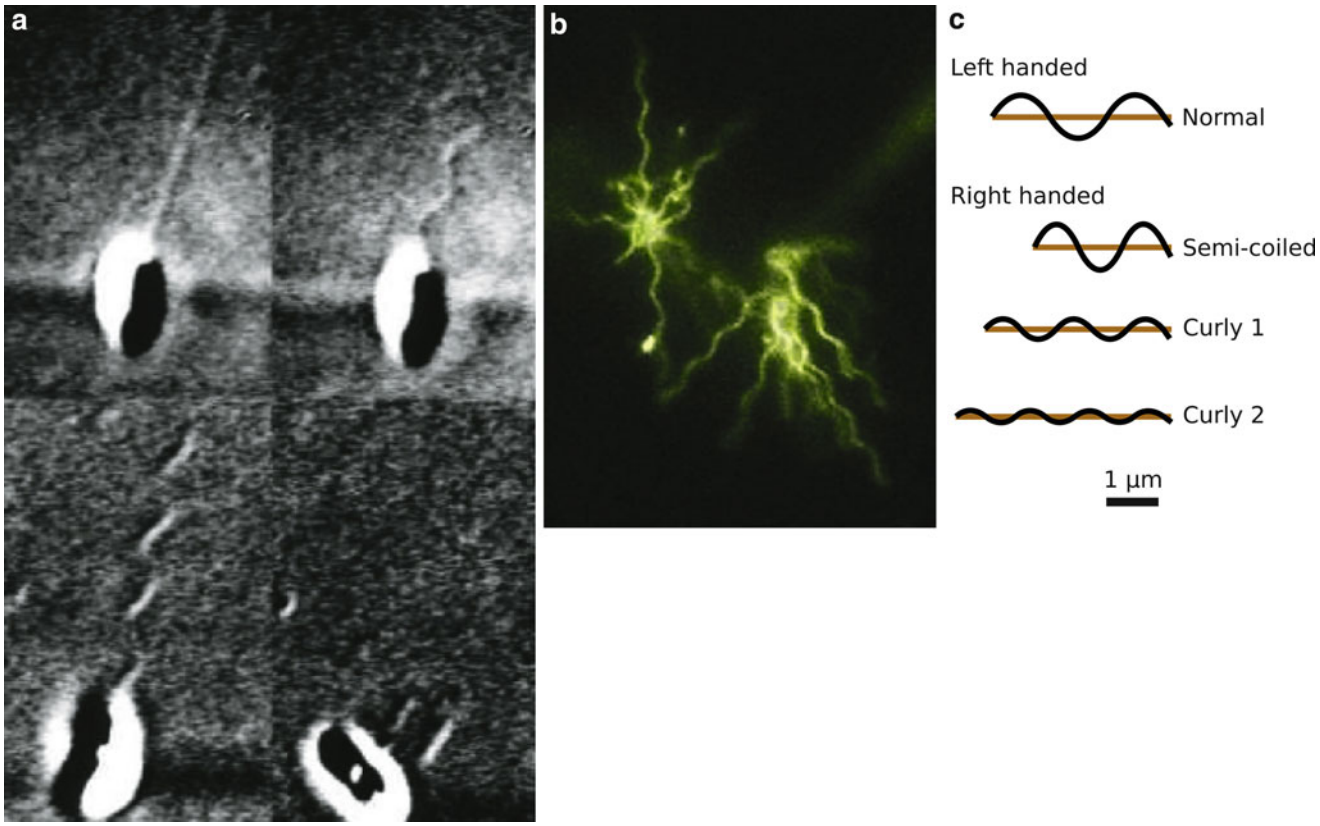


Fig. 15.2

Changes in shape and motion of flagella during swimming, tumbling, and reversal of motor rotation. (a) Still differential interference contrast (DIC) microscope images taken from a video of swimming *Rhodobacter sphaeroides* showing a polymorphic transformation of the flagellar filament. The functional filament can switch to a longer wavelength (apparently straight form) or change to a coiled large amplitude (short wavelength form) when rotation stops (From Armitage et al. 1999). (b) Still image taken from a video of tumbling *Escherichia coli* with filaments labeled with fluorescent dye (From Turner et al. 2000). (c) Polymorphic shapes adopted by flagella during changes in the direction of motor rotation (*Fig. 15.2a, b* are courtesy of H.C. Berg and L. Turner using the Rowland Institute DIC microscope)

seconds, the flagellar motor stops rotating and the flagellar filament changes conformation to a large-amplitude, short wavelength coil against the cell body. The cell is reoriented partly by Brownian motion and partly by the slow rotation of the coiled form of the flagellum. When the motor restarts and a functional flagellar helix reforms, the cell is usually pointing in a new direction (Armitage and Macnab 1987; Armitage et al. 1999). Other species with a single flagellum, such as *Pseudomonas citronellolis*, change direction by briefly switching rotation of the motor from CCW to CW. This causes the flagellum to pull rather than push the cell for a brief period (Taylor et al. 1999; Taylor and Koshland 1974). The low Reynolds number means that a bacterium whose flagellum functions in this way will, with the exception of changes due to Brownian motion, backtrack along its trajectory upon motor reversal. As a result, only small changes in orientation occur with each motor reversal. Recent studies have shown, however, that the change in orientation on motor reversal is much greater when the cell swims near a surface (Magariyama et al. 2005).

Sinorhizobium meliloti swims using a bundle of flagella. Rather than stopping or switching rotational direction, a number of flagella slow their rotational speed. This results in disruption of the bundle and the cell changing direction without either stopping or tumbling. This again effects a random swimming pattern (Scharf et al. 1998; Platzer et al. 1997). Spiral-shaped species such as *Rhodospirillum rubrum* have two counter-rotating polar tufts of flagella, one at each cell pole, spiraling the cells through the medium. Periodically, both bundles simultaneously switch rotational direction and the cell changes the direction of movement (Lee and Fitzsimons 1976). In spirochetes, these polar bundles of flagella are internalized; however, it is thought that the the bundles still rotate. Evidence indicates that the rotation of the filament lying between the outer cell wall and the cell body causes the spiral-shaped cell body to move through the viscous environment. Spiroketes have three different modes of motility, two running modes (with opposite ends of the cell leading) and a nontranslational flexing mode. When the two bundles of flagella rotate antisymmetrically, the bacterium runs; if the direction of only one of the

two bundles of flagella reverses, then the bacterium will stop. This is often referred to as the flexing mode due to the distortion of the cell body caused by the bundles of flagella acting in an opposing manner (Wolgemuth et al. 2006; Wolgemuth and Charon 2005; Berg 1976; Charon and Goldstein 2002). Spiroketes move far more effeciently through viscous media than do flagellated bacteria. As many spiroketes are pathogens, this efficiency of movement is thought to aid their invasion of, for example, mucous membranes (Kaiser and Doetsch 1975).

Swarming

A range of species from three bacterial genera, the firmicutes, the Alphaproteobacteria, and the Gammaproteobacteria, have been shown to differentiate into highly flagellate swarmer cells when inoculated onto surfaces (reviewed in Kearns 2010). In most cases, it is thought that the expression of the genes involved in flagella synthesis is increased in response to some surface stimulus, either cell density or increased viscous drag. Many members of the Gammaproteobacteria can be induced to swarm, including *E. coli*, *Salmonella enterica* Typhimurium, and *Yersinia* species (Harshey and Matsuyama 1994; Young et al. 1999). The best studied are the swarmer cells of the marine vibrio, *V. parahaemolyticus, V. cholerae*, and *V. alginolyticus*, and the enteric proteus species, *Proteus mirabilis* and *P. vulgaris. Proteus* species swim in liquid medium in much the same way as *E. coli.* However, when inoculated onto agar plates, the cells differentiate into long filamentous cells with a severalfold increase in flagella numbers per unit cell surface. The highly flagellate filamentous cells move together in rafts over the surface, the increase in combined cell surface area allowing them to overcome the surface tension (Jones et al. 2004). The marine vibrio species *V. haemolyticus* or *V. alginolyticus* synthesize a completely new type of flagellar motor when grown on surfaces. When free-swimming, they utilize a fast, sodium-driven motor controling a polar flagellum and switch to using large numbers of slower proton-driven motors controling lateral flagella when grown on surfaces (Shinoda and Okamoto 1977; Atsumi et al. 1992). Another species which increases lateral flagella synthesis on surfaces is the Alphaproteobacterium *Rhodospirillum centenum.* In this case, rather than some of the hyperflagellate cells moving away from the colony as rafts, the whole colony of photosynthetic bacteria moves over the agar surface toward red light (Ragatz et al. 1995). *Agrobacterium tumefaciens*, another member of the Alphaproteobacteria, is also known to become hyperflagellate on surfaces. Swarming is not confined to the Gram-negative genera as both *Bacillus* and *Clostridium* species have been shown to swarm.

Environmental signals are believed to control the master regulator transcription factors that govern changes in flagellar biosynthesis during the transition from swimming to swarming behavior (reviewed in detail in Patrick and Kearns (2012)). In *V. parahaemolyticus*, increased drag on the polar motor (which occurs when swimming near surfaces or in viscous media) appears to trigger the expression of the peritrichous proton-driven motor genes (Kawagishi et al. 1996). Increased drag on the motor does not appear to be a universal signal for the switch to the synthesis of peritrichous flagellar, however, as an artificial increase in external viscosity only causes peritrichous expression in some species. The increased expenditure in energy required to sythesize large numbers of flagella suggests that moving over a surface must provide a survival advantage for these species. It has been shown that swarming behavior can facilitate infection by *Proteus* species and it may also help marine *Vibrio* species to maintain themselves on nutrient-rich surfaces (McCarter 2004; Jones et al. 2004).

Flagella Structure

The majority of species investigated have flagellar filaments made from a single protein, flagellin. Flagellins vary in size from species to species, but all have a conserved C- and N-terminal domain which allows the flagellin to polymerize into a helical structure (Fedorov et al. 1984; Macnab and DeRosier 1988; MimoriKiyosue et al. 1997; Vonderviszt et al. 1991). Unusual amino acids, such as ε-N-methyllysine, are often found in flagellin proteins, but the significance of this observation remains unclear. Individual flagellin monomers polymerize through interaction of their N- and C-terminal domains to form protofilaments. In the majority of species characterized to date, the flagellum is composed of 11 protofilaments and contains many thousands of flagellin subunits (Morgan et al. 1995; Namba and Vonderviszt 1997; Namba et al. 1989; Trachtenberg and DeRosier 1987). The 11-strand structure of the flagellum is not conserved across all species; however, for example, the flagellum of the Epsilonproteobacterium *Campylobacter jejuni* has been shown to be composed of only seven protofilaments (Galkin et al. 2008). The flagellin subunits can interact with the neighboring subunits in the protofilament in two defined ways, which leads to two different protofilament conformations which differ in the repeat distance between flagellin monomers. In "short" protofilaments, the flagellin monomers are more closely packed than in "long" protofilaments. Flagellar polymorphs are formed when long and short protofilaments are combined in different ratios. A flagellum that includes both short and long protofilaments will be helical in shape, with the ratio of short to long protofilaments defining the wavelength and handedness of the structure (Turner et al. 2000). Switching of the direction of flagellar motor rotation induces a change in the superhelical structure of the flagellum (Trachtenberg and DeRosier 1991, 1992). The change in helical shape results from the torque imposed on the filament by the rotating motor being transmitted through a junction protein connecting the flagellar filament to the hook (Fahrner et al. 1994). The protein regions involved in the switching of flagellar conformations in the flagellum from *S. enterica* have been putatively identified in the crystal structure of a flagellar fragment (Samatey et al. 2001). The molecular basis for conformational switching has also been investigated using molecular dynamics simulation (Samatey et al. 2001; Kitao et al. 2006; Arkhipov et al. 2006). The helical

filament can be several times longer than the cell body, for example, filaments as long as 7 μm have been seen on *R. sphaeroides*, which is only 2 μm long. Analysis of cells tethered to glass slides by antibodies raised to either their filaments or the hook region, which connects the filament to the cell, suggests that the filament is one of the most rigid protein structures in biology, certainly more rigid than actin (Block et al. 1989).

The central domain of the flagellin protein is highly variable, and it is this region that is exposed on the outside of the flagellar filament. This provides the highly antigenic (H-Ag) domain, used for many years to type bacterial strains such as *Salmonella*. This region is not required for flagellar assembly, and mutants can be created which lack most of the central domain but still assemble functional flagella. *Salmonella* has two genes encoding antigenically different flagellins and alternates expression of these genes in a phenomenom known as "phase shifting." Two DNA invertases can catalyze the inversion of a genetic element which contains the promotor for one of the flagellin genes and also for a negative regulatory protein for expression of the second flagellin gene (Kutsukake et al. 2006). This phenomenon has been suggested to help the bacterium overcome the immune system of the host, although little research has been carried out to identify whether this is indeed the case. This phase variation was also exploited in the first experiments that demonstrated that flagella assemble from the distal end of the growing flagellum, not the base (Iino 1969).

Some species have flagella made up of more than one type of flagellin, for example, *S. meliloti* and *Caulobacter crescentus* have flagellar filaments composed of several related flagellins (Ely et al. 2000; Sourjik et al. 1998). The reason for this is unclear, although the *S. meliloti* filament is more rigid than other flagellar filaments and thought not to undergo polymorphic transitions. *S. meliloti* encodes genes for four flagellins, one principle flagellin and three accessory flagellins. It is proposed that the principle flagellin forms a heterodimer with one of the accessory flagellins and that these heterodimers polymerize to form protofilaments (Scharf et al. 2001; Trachtenberg et al. 1987).

The flagella of some species are sheathed in an extension of the outer membrane, for example, *Bdellovibrio bacteriovorus* and *Helicobacter* species (Seidler and Starr 1968; Thomashow and Rittenberg 1985; Geis et al. 1993; Luke et al. 1990). It has been suggested that the sheath in species such as the bipolarly flagellate gut pathogens *H. pylori* and *C. jejuni* serves to protect the flagellum from stomach acid that would depolymerize the flagella filament. The role of the sheath in free-living species such as *B. bacteriovorus* is unclear, although interestingly this is one of the fastest moving species in terms of body length per second identified to date, as befits its life-style as a bacterial predator.

Motor Structure

The semirigid helical flagellum is connected to the cell body via a short region, the hook, which has a similar structure to the filament but is composed of a different protein (Uedaira et al. 1999; Wagenknecht et al. 1982). The role of the hook is not certain; it is less rigid than the filament and may be involved in allowing the filaments to come together as a bundle and/or it may be involved in transmitting the changes in torque from the motor to the filament, causing the polymorphic transformation of the flagellum that enables bacterial reorientation (Block et al. 1991). In species with a single flagellum, such as *R. sphaeroides*, the hook is much less flexible, supporting the idea that a flexible hook helps bundle formation. There are two proteins that connect the hook and the filament; these proteins are known as hook-associated proteins (HAPs). Mutations in the gene coding for HAP3 in *E. coli* result in a filament that changes conformation far more frequently than the wild type. The HAP3 protein may be involved in holding the protofilaments in specific conformations and only allowing conformational changes when the torque transmitted through the hook from the motor changes significantly (Fahrner et al. 1994).

The hook connects the filament to the motor. In Gram-negative species a rod connecting the hook to the motor is passed through a pair of passive rings (the L- and P-rings) in the outer membrane (in Gram-negative species) and the peptidoglycan layer (*Fig. 15.3*). The rotor itself is a series of rings (MS- and C-rings) in the cytoplasmic membrane and the cytoplasm of the cell. Early work suggested that the actively rotating region was the MS-ring in the cytoplasmic membrane, but it is now clear that the MS-ring is a passive structure made of the protein product of a single gene, FliF (Ueno et al. 1992). The C-ring is attached on the cytoplasmic face of the MS-ring and is the active part of the rotor. It is estimated that about 26–30 copies of the FliG and FliM proteins and ~100 copies of the protein FliN form the C-ring (Zhao et al. 1996b; Zhao et al. 1996a; Francis et al. 1992). FliG is the rotor, while the FliM and FliN proteins interact with the cytoplasmic sensory signaling pathway to cause the motor to switch the direction of rotation (Yamaguchi et al. 1986; Garza et al. 1995; Tang et al. 1996). To allow rotation, there must be not only a rotor but also a stator. The stator is provided by a ring of up to 11 complexes consisting of four MotA plus two MotB proteins in H^+-driven motors (such as that of *E. coli* (Reid et al. 2006)). MotB has a single transmembrane domain and a large periplasmic region with a terminal peptidoglycan-binding domain (Chun and Parkinson 1988; De Mot and Vanderleyden 1994). This is thought to anchor the stator proteins to the peptidoglycan layer. MotA has four transmembrane helices, and together the $MotA_4MotB_2$ complex is believed to form two independent ion channels in the lipid bilayer (Zhou et al. 1995b; Braun et al. 2003). Protons, about 1000 per revolution, pass through the ion channels resulting in rotation of FliG (Meister et al. 1987; Blair and Berg 1990, 1991). During their passage through the channel, protons are thought to interact with the only absolutely conserved amino acid in the stator complex, Asp32 of *E. coli* MotB (Zhou et al. 1998b; Braun et al. 2003). Each of the Mot complexes has been shown to be an independent force-generating unit (Block and Berg 1984). Several amino acids essential for torque generation have been identified based on the crystal structures of MotA fragments and on analysis of second-site suppressor mutations (Lloyd and Blair 1997; Zhou and Blair 1997; Zhou et al. 1998a;

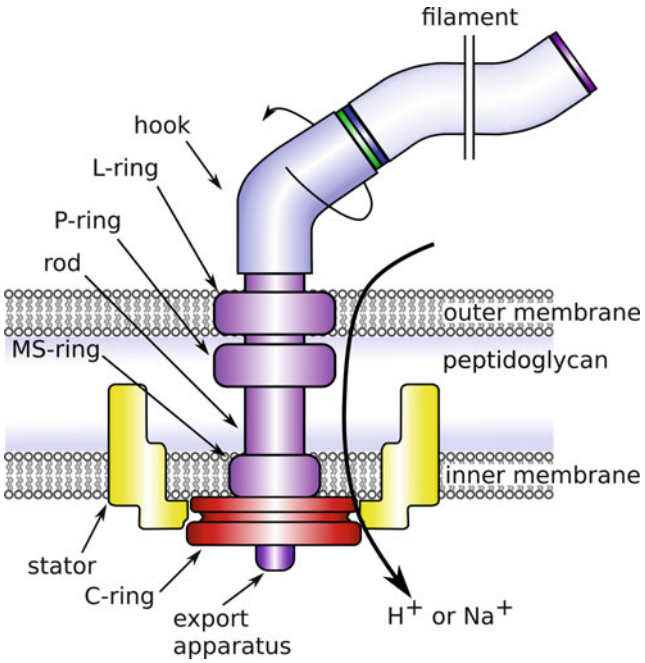


Fig. 15.3

The structure of the flagellar motor. The L- and P-rings act as a grommet through the peptidoglycan and outer membrane of Gram-negative bacteria. The MS-ring provides the scaffold to which the C-ring binds; the C-ring serves as the rotor part of the motor. The C-ring is comprised of FliG and FliM/N. FliG interacts with the stator and the electrochemical ion gradient, while FliM/N forms the switch component and interacts with the chemosensory pathway. In proton-driven motors, a ring of 8–12 MotA/B proteins make up the stator. In sodium-driven motors, MotA/B is replaced by PomA/B (After Sowa and Berry 2008)

Park et al. 2006b). All bacterial motors investigated contain homologues of these core motor proteins, suggesting that the mechanism of driving rotation is common (Chen et al. 2011). Sodium motors, such as the polar flagellar motor of *V. alginolyticus*, rotate more rapidly than proton-driven motors and employ the MotA/MotB homologues PomA/PomB. Sodium motors also utilize additional proteins MotX and MotY; the function of these proteins is not well understood, but they are thought to play a role in stabilizing the sodium stator (Asai et al. 1997; McCarter 1994a, b). Evidence for a common mechanism for diving rotation has also been provided by the generation of functional chimeric motors which include components from motors that differ in ion specificity. For example, replacement of PomA from *V. alginolyticus* with MotA from *R. sphaeroides* leads to a functional Na^+-driven motor (Asai et al. 2000). Crystal structures of FliG domains from *Thermotoga maritima* and more recently the complete structure of FliG from *Aquifex aeolicus* combined with biochemical and biophysical studies have suggested models for the organization of the FliG ring and for the mechanisms involved in interaction with the FliM and FliN rings and with the ring of stator proteins (Sarkar et al. 2010; Lee et al. 2010; Minamino et al. 2011; Paul et al. 2011).

Advances in biophysical and optical techniques in recent years have allowed the mechanics of motor rotation to be studied in some detail. Using small beads attached to short stubs of flagella at low ion-motive forces, the motor has been shown to rotate in steps, with about 26 steps per rotation, matching the number of FliG protein in the rotor ring (Sowa et al. 2005).

Perhaps one of the more surprising recent discoveries is that the flagellar motor is not a stable protein complex (reviewed in Brown et al. (2011)). In some species at least, the proteins are dynamic, exchanging with pools of proteins in the cell membrane or cytoplasm. MotA/MotB were first identified as independent force-generating units in experiments where motility was restored in a Mot-deletion strain by expression of the Mot proteins from an inducible expression vector. The rotation rate of cells tethered by a single flagellum was seen to increase in about eight equal steps as stator units became incorporated into the motor (Muramoto et al. 1994). In more recent experiments, 11 steps were observed suggesting that approximately 11 stator units bind to the *E. coli* flagellar motor (Reid et al. 2006). Using GFP fusions to MotB, the stators of *E. coli* were shown to exchange with membrane-diffusing pools of stators while the motor was rotating, a single stator only remaining around the rotor for about 30 s (Leake et al. 2006). *Shewanella oneidensis* has a single rotor and flagellum but encodes both sodium- and proton-driven stators, the genes for proton-driven stators probably having been aquired via horizontal gene transfer in very recent history (Paulick et al. 2009). This species exchanges sodium for proton stators as the sodium concentration falls. Stators in the sodium-motive-force-driven motors of *V. alginolyticus* have been shown to remain localized only when there is a sodium gradient, with the stators diffusing away when the ion gradient is exchanged for potassium and returning with the return of sodium (Fukuoka et al. 2009). These data suggest that stators localize around the rotor in response to ion flow through the stator complex. Interestingly, it has been recently shown that the FliM/N components of the rotor may also exchange with cytoplasmic pools of proteins (Fukuoka et al. 2010). Together these data suggest that the complex motor structure is not stable and that the proteins from which it is composed are in exchange with cellular protein pools even as the motor rotates.

On the cytoplasmic side of the membrane and associated with the C-ring is the export apparatus. The export apparatus, and indeed much of the rotor structure, is very similar to the toxin-exporting type III secretion pathway of bacteria such as *Yersinia pestis* and *Shigella* species, suggesting a common evolutionary origin (Kubori et al. 1998; Macnab 1999). While the proteins that make up the outer membrane L- and P-rings have classical Sec signal sequences, the rod, hook, and flagellin proteins lack these sequences and are exported through the 30 Å central channel of the forming flagellum, before polymerization at the distal end of the growing structure (Macnab 2004; Suzuki et al. 1998). The proteins are exported in an unfolded state and are thought to be held in this state by the chaperone protein, FliJ, before export which is carried out by the ATPase FliI (Minamino and Macnab 2000a, b; Minamino et al. 2000). The FliH protein binds to the N-terminal region of FliI, preventing ATPase

activity in the absence of docking to the membrane-bound export apparatus (Minamino and Macnab 2000b). The chaperone proteins guide the unfolded protein to a closed channel at the base of the flagellum MS-ring. FliI, whose molecular structure resembles that of the α- and β-subunits of the ATP synthase, forms an F1-like ring through which the protein is exported (Imada et al. 2007; Minamino et al. 2006). Protein export requires both ATP hydrolysis and a proton-motive force (Minamino and Namba 2008).

Gene Expression and Assembly

It takes about 40–50 genes to construct a functional flagellum, and as components are located in the cytoplasm, across both membranes, and extracellularly, it is extremely important that flagellar assembly is highly ordered. There are only a specific number of flagella per cell, and therefore, expression of the flagellar genes must be regulated by the growth rate (except when some species are induced to become hyperflagellate). The order in which the flagellar genes are expressed reflects the order in which the proteins assemble (reviewed in detail in Smith and Hoover 2009, and Chevance and Hughes 2008). Genes involved in the formation of the flagella of enteric bacteria such as *E. coli* are divided into three classes, I, II, and III, depending on where they appear in the transcriptional hierarchy (Kutsukake et al. 1990). In *E. coli* and *S. enterica*, a master operon, encoding FlhC and FlhD, under the control of the catabolite repression system, initiates expression of a series of operons in a highly coordinated manner. FlhC and FlhD together induce the σ70 expression of the class II flagellar genes which are required for the assembly of the basal body of the motor (Ikebe et al. 1999). A flagellar-specific σ factor, called FliA, σF, or σ28, controls the expression of class III genes, such as those encoding the structural proteins that form the flagellar filament (Ohnishi et al. 1990). Simultaneous expression of the class II and class III genes is prevented by the anti-σ factor FlgM (Chadsey et al. 1998). Expression of FlgM is induced by FlhC/FlhD; FlgM binds to σ28 until it is exported from the cell on completion of rotor assembly (Karlinsey et al. 2000). FlgM export is the signal that the motor has correctly assembled and the flagellin proteins can now be expressed and exported. Although the majority of research on the hierarchy of flagellar gene expression has been on *E. coli* and *S. enterica*, other motile species studied in detail, such as *R. sphaeroides*, also appear to have highly organized expression of the flagellar genes (Wilkinson et al. 2011). The control of flagellar gene expression in other organisms typically utilizes a σ28 homologue for control of class III genes; however, some species may have σ54- rather than σ70-dependent control of class II genes, suggesting environmental regulation of motility (Francke et al. 2011).

Control of gene expression is more complex in species such as *C. crescentus*, which is only motile during certain stages of its life cycle. A sessile stalked cell divides to produce a motile swarmer cell, which is unable to grow or divide but swims to a new location to settle and produce a stalk for attachment to a surface. The timing of flagellar gene expression in *C. crescentus* is critical, and a response regulator protein, CtrA, controls both methylation of DNA and flagella synthesis. Phosphorylated CtrA inhibits cell division and DNA replication and activates DNA methylation and flagella synthesis. Localized proteolysis of the protein in stalked cells appears to allow the cell cycle to continue in stalked cells while inhibiting division in swarmer cells (Domian et al. 1997).

Behavioral Control

Motility can be divided into at least three types: free-swimming, swarming over surfaces using flagella, and gliding or twitching. In all cases, expression of the locomotory organelle requires a large number of genes and their expression is energetically expensive. In addition, while running a proton motor may take less than 1% of the proton-motive force of a bacterium growing under rich conditions, it may take several percent under growth-limiting conditions. The observation that many species stop swimming in rich growth conditions and only start under limiting conditions strongly suggests that motility provides a major advantage under most naturally occuring conditions. To help a bacterium reach or maintain itself in its optimum environment for growth, motility must be under the control of environmental sensing systems.

In general, bacteria are far too small to be able to sense a gradient along their length; they therefore sample their environment in time, comparing the concentration or strength of a stimulus at one time with that a few seconds earlier (Segall et al. 1986; Brown and Berg 1974). All bacteria tend to move in a random pattern, whether three dimensionally, as with free-swimming cells, or two dimensionally on surfaces. This random pattern is biased in a favorable direction by the cell changing direction more often when moving away from a positive stimulus and changing direction less often when moving in a positive direction (Berg and Brown 1972; Block et al. 1982). Bacteria can sense a very wide range of stimuli, including light, oxygen and other terminal electron acceptors, extracellular chemicals, intracellular metabolic state, pH, osmolarity, and even, in some cases, the Earth's magnetic field. All of these signals must be sensed and balanced to produce an overall response. Of course different species will respond to different stimuli, and many will be repelled by the dominant attractants of other species.

To allow any kind of behavioral response, a number of sensory steps are required: (1) the stimulus must be sensed, (2) the signal must be relayed to the motor apparatus, (3) the signal must be rapidly terminated, and (4) the receptor must be reset to allow future changes to be sensed. The mechanisms involved in these stages are best understood in *E. coli.* It has become apparent that the rather straightforward chemosensory pathway identified in *E. coli* is a much simpler system than that used by many bacteria outside of the gamma subgroup. *E. coli* does, however, exemplify the core chemosensory components that are conserved across all bacterial species (Wuichet and Zhulin 2010).

Chemotaxis in *E. coli*

The best understood chemosensory system is that of *E. coli* and the closely related, *S.enterica* Typhimurium. The first step in the chemosensory response is detection of the signal. Transport and metabolism are not required for chemotaxis by *E. coli*. Transport mutants are still chemotactic, while specific chemotaxis mutants can still metabolize the chemoattractant (Adler 1969). In addition, nonmetabolizable analogues are still attractants. Four chemoreceptors, Tsr (serine receptor), Tar (aspartate and maltose), Trg (ribose and galactose), and Tap (dipeptides), have been identified in *E. coli* (Stock and Surette 1996). Tap is found in *E. coli*, but not *Salmonella* (Manson et al. 1986). *Salmonella* has a receptor, Tcp, for citrate, which is not a metabolite for *E. coli* (Yamamoto and Imae 1993). This illustrates that even the pathways of two very closely related species have adapted to the metabolic requirements of that species. In addition, a related protein has been identified, Aer, which is involved in oxygen sensing (see section on ❯ "Aerotaxis and Electron Acceptor Taxis 'Energy Taxis'").

Motile but nonchemosensing mutants in *E. coli* identified five genes responsible for encoding the proteins for general chemosensory signal transduction (Parkinson 1977). These, *cheB, cheR, cheA, cheY,* and *cheZ*, form the intracellular signaling sequence to the flagellar motor. CheA is a histidine protein kinase whose phosphorylation state is dependent upon the ligand occupancy of the chemoreceptors. CheA-P transfers phosphoryl groups to CheY; CheY-P then interacts with the flagellar motor inducing a switch in the direction of rotation. CheZ acts to dephosphorylate CheY-P thus ensuring signal termination. The remaining two proteins, CheB and CheR, are important in adaptation to persistent signaling. Bacteria employ temporal comparison of the chemoeffector concentrations encountered during swimming to bias their trajectory. This requires a mechanism for "remembering" the level of chemoeffector encountered in the recent past (Vladimirov and Sourjik 2009). This short-term memory is usually provided through variation in the methylation state of the chemoreceptors. CheB, CheR, and S-adenosyl methionine are required for resetting the signaling state of the receptors in the presence of a constant level of chemoeffector, that is, for adaptation (Stock and Koshland 1978, 1977). The extent of receptor methylation depends on whether an attractant is added or removed, resetting the receptor protein into a nonsignaling state and allowing gradient sensing. In *E. coli*, therefore, one of a limited number of attractants binds to transmembrane receptors, and as a result, a signal is generated which results in a change in swimming pattern, biasing the random swimming pattern in a favorable direction. Modification of the receptors to stop signal generation, if the concentration of stimulus remained unchanged, allows gradient sensing. Related schemes have been found in almost all other motile species, but in the majority of nonenteric species, the sensory pathways are more complex (see section on ❯ "Chemotaxis in Nonenteric Species").

Chemoreceptors

The chemoreceptors of *E. coli* are all built on the same basic design (Mowbray 1999). Proteins antigenically related to the *E. coli* chemoreceptors were idenfied in many bacterial species, and subsequent sequencing of the chemoreceptors of a wide range of species from archaea to bacteria has identified a common conserved domain (Zhulin 2001; Morgan et al. 1993). The conservation of the core of the chemosensory pathway between species has allowed the likely chemosensory genes to be identified in many species whose genomes have been sequenced. One of the major surprises has been the variation in the number of chemoreceptor genes found in different species, ranging from a single chemoreceptor in organisms such as *Mesorhizobium loti* to over 40 chemoreceptors in organisms including *Pseudomonas syringae*, *V. cholerae*, and *Magnetospirillum magnetotacticum* which have 45, 49, and 65 chemoreceptors, respectively (Alexander and Zhulin 2007). The average number of chemoreceptors appears to be in the range 15–20. This suggests that bacteria faced with complex environments have receptors to sense a wide range of chemical stimuli, while others, such as *E. coli*, use a pared down system suited to a world with limited changes.

The four *E. coli* chemoreceptors are methylated as part of the adaptation process and as such are also known as methyl-accepting chemotaxis proteins or MCPs. These MCPs are between 533 and 553 amino acids in length and form homodimers as the fundamental unit of chemoreceptor organization, illustrated schematically in ❯ *Fig. 15.4* (Milligan and Koshland 1988). They have a transmembrane region consisting of four α-helices (two contributed from each mononer) in addition to a periplasmic and cytoplasmic domain (chemoreceptor structure is reviewed in detail in Hazelbauer and Lai 2010; Hazelbauer et al. 2008). The cytoplasmic region of the protein comprises a four-helix bundle HAMP domain followed by a long antiparallel coiled:coil referred to as the kinase control element (Aravind and Ponting 1999; Kim et al. 1999). Crystal structures of periplasmic and cytoplasmic chemoreceptor domains demonstrate that the chemoreceptors are one of the longest proteins found in a bacterial cell, extending a distance of 40 nm from chemoeffector-binding domain to signaling domain (Kim et al. 1999; Milburn et al. 1991). There is very little homology between the periplasmic domains at the sequence level, nor is there obvious similarity between the transmembrane domains; however, the cytoplasmic domain is very highly conserved between transducers. It is this highly conserved cytoplasmic domain that is common to chemosensory transducers across the bacterial and archaeal world (Zhulin 2001; Morgan et al. 1993).

The periplasmic domains of the different MCPs "sense" different chemoeffector molecules. Interestingly, some receptors, for example, Tar, can sense two completely different stimuli, in this case the amino acid aspartate and, when bound to its periplasmic binding protein (PBP), the sugar maltose (Zhang et al. 1999; Gardina et al. 1998, 1997). The sensing of maltose only when bound to the PBP has interesting implications for the response of Tar to maltose and aspartate. Tar is expressed as part

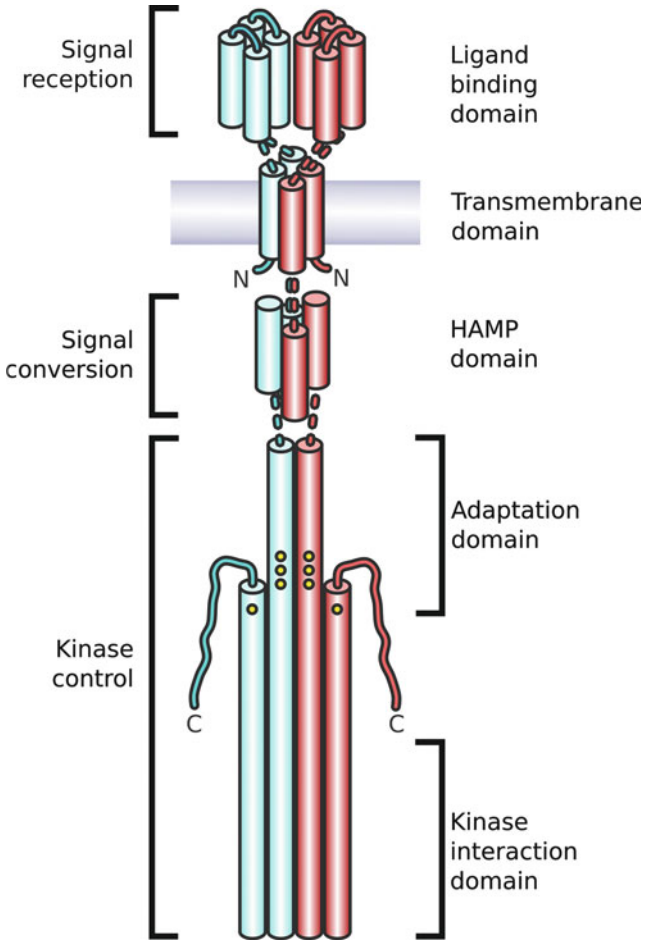


Fig. 15.4

The structure of a chemoreceptor. This cartoon illustrates the domain structure shared by many chemoreceptors. In the diagram, the helices are represented by *cylinders* and glutamate residues that are targets for methylation by open circles (After Hazelbauer et al. 2008)

of the flagellar and chemosensory regulon. As aspartate interacts directly with Tar, the response will be directly related to the extracellular concentration of the amino acid. Expression of the maltose PBP is under the control of the maltose transport system. Thus the response to maltose will depend upon the level of PBP expression. The size of the response to a given aspartate stimulus will always be the same, while that to maltose will depend on the level of induction of the PBP. Sugars can also be sensed through the phosphotransferase transport system (see section on "Phosphotransferase Sugars"). As this is also an inducible system, it is apparent that *E. coli* responds constitutively to amino acids but that the response to sugars is dependent on growth conditions.

The mechanisms through which Tar is able to respond to binding of both a small amino acid, aspartate, and a large protein, the maltose PBP, have been extensively investigated. The structure of the Tar ligand-binding domain has been determined in the presence and absence of aspartate (Milburn et al. 1991). The structures show that an aspartate ligand binds at one of two rotationally symmetric sites at the interface of the two subunits in the homodimer. Little change in secondary structure occurs on aspartate binding, but there is a difference in the relative orientation of the two subunits between the apo and ligand-bound states. A model of the Tar-maltose-PBP complex has been built based on cysteine cross-linking studies (Zhang et al. 1999). As for aspartate, the maltose-PBP complex is proposed to bind at the interface between the two MCP subunits. If both aspartate and maltose are added to *E. coli* cells, then the response is partially additive (Mowbray and Koshland 1987). Asymmetry in maltose-PBP binding to Tar may allow signaling of maltose concentration through one subunit of the MCP and asparatate concentration through the other, allowing response to one stimulus in the presence of saturating concentrations of the other (Zhang et al. 1999).

Although the basic structural unit of the MCPs is a dimer, higher-order structure plays an essential role in signaling. The MCP homodimers form mixed trimers of dimers, and these trimers of dimers form the sensory signaling protein (Amin and Hazelbauer 2010; Parkinson et al. 2005). Association of the trimers of dimers into larger arrays is important for integration of signals from different chemoeffectors and also for sensitivity (discussed further in the section "Localization of MCPs").

Transmembrane Signaling

Once the ligand has bound, this binding has to be signaled across the membrane to the cytoplasmic domain of the MCP. Extensive studies have been carried out on the possible mechanisms involved in signaling across the membrane, and current evidence suggests that only a small displacement of the transmembrane helices is necessary for signaling (Falke and Hazelbauer 2001). Cysteine mutagenesis studies have shown that it is possible to lock receptors into specific signaling states by cross-linking particular residues (Chervitz and Falke 1995; Beel and Hazelbauer 2001), while mutations that alter the position of the transmembrane helices in the lipid bilayer can also stabilize recepetor signaling states (Miller and Falke 2004; Draheim et al. 2006). Crystal structures of the periplasmic domains in apo and ligand-bound conformations are also consistent with signaling through a small vertical movement and a slight tilt in the second of the transmembrane helices (TM2) contributed by each monomer (Milburn et al. 1991; Scott et al. 1993). Recent molecular dynamics simulations investigating the effects of mutations in TM2 on its position in the bilayer enable the change in phenotype observed experimentally to be interpreted in terms of changes of the position and orientation of the TM2 helix. These simulations support the "swinging-piston" model for signaling where small piston and twisting movements of TM2 lead to transformation of the signal via the cytoplasmic HAMP domain to the kinase control element of the cytoplasmic signaling domain (Hall et al. 2011).

Cytoplasmic Domain

Changes in receptor binding are signaled through the transmembrane region to the four-helix bundle HAMP domain (Airola et al. 2010). It is currently proposed that there are two sets of conformations for the HAMP domain, a tightly packed structure and a more loosely packed structure. The register of the helices in the HAMP domain differs in these two conformations, allowing propagation of conformational changes to the kinase control region of the cytoplasmic domain (Zhou et al. 2011, 2009; Parkinson 2010).

The kinase control region of the cytoplasmic MCP domain has been further subdivided into two functional domains on the strength of structural, mutational, cross-linking, and sequence-based studies (Kim et al. 1999; LeMoual and Koshland 1996; Alexander and Zhulin 2007; Falke and Hazelbauer 2001). The adaptation domain contains the glutamate and glutamine residues which are methylated by the S-adenosylmethionine-dependent methyltransferase, CheR, during receptor adaptation (Kehry and Dahlquist 1982; Kehry et al. 1983). The kinase signaling domain located at the membrane distal end of the chemoreceptor is the site of interaction with CheW and CheA, the proteins involved in initiating cytoplasmic signaling to the motor (Liu and Parkinson 1989; Morrison and Parkinson 1997; Miller et al. 2006; Park et al. 2006a; Liu and Parkinson 1991). Genetic studies show that mutations within this domain can result in cells which are either predominantly smooth swimming or predominantly tumble, suggesting that this region controls the activity of the histidine protein kinase CheA.

Although the domain architecture of the kinase control region is shared by all chemoreceptors, the length of the domains can vary both within and between species. Chemoreceptor cytoplasmic domains are composed of multiple heptad repeats and can be divided into families according to the number of heptad repeats (Alexander and Zhulin 2007). Although chemoreceptors with different numbers of heptad repeats can be found within a bacterial species, recent work suggests that receptors must have the same number of heptad repeats in order to form functional trimers of dimers (Massazza et al. 2012).

Thousands of MCPs from different bacterial species have now been sequenced, from all the eubacterial subgroups and from archaea, and all show extensive conservation of the cytoplasmic signaling domain (Alexander and Zhulin 2007). The diversity of species with this conserved signaling domain suggests that chemotaxis has a very early evolutionary origin as while the flagellum may be different in bacteria and archaea, the chemosensory pathway is very similar. The conservation of this domain in transducers which are not only transmembrane but may be cytoplasmic, as in *R. sphaeroides* and *H. salinarium*, or may sense oxygen via bound redox groups, may argue that this domain is the early central component of the signaling pathway and the sensing domains have been added according to the niche of the species.

Cytoplasmic Signaling

A small protein, the 18-kDa CheW, links the signaling domain of the MCP to the histidine protein kinase CheA (Conley et al. 1989; Gegner et al. 1992; Liu and Parkinson 1989; Sanders et al. 1989; Schuster et al. 1993). It has no known catalytic activity, but without it signaling stops. Although it is thought to be a simple scaffolding protein, transmitting the conformational changes in the signaling domain to the kinase, its structure is believed to be fairly well conserved between species. CheW forms two 5-stranded β-sheets packed around a central hydrophobic core and is very similar in structure to the domain of CheA responsible for coupling changes in receptor structure to kinase activity (Griswold et al. 2002; Bourret et al. 1993). One role of CheW is certainly bringing the trimers of dimers into larger protein complexes allowing sensitivity in signaling. The regions of CheW that interact with CheA and the chemoreceptors have been studied using a wide range of techniques including mutagenesis, nuclear magnetic resonance, electron paramagnetic resonance spectroscopy, and mass spectometry, allowing models of the chemoreceptor-CheW-CheA complex to be generated (see, e.g., Bhatnagar et al. 2010; Griswold and Dahlquist 2002; Park et al. 2006a; Underbakke et al. 2011; Erbse et al. 2011; Boukhvalova et al. 2002).

CheA is a soluble histidine protein kinase (HPK) homodimer (Bilwes et al. 1999). It belongs to the extensive family of HPKs identified in a wide range of bacterial species. CheA functions as a dimer and ATP binding to a conserved domain allows the protein to phosphorylate a conserved histidine residue located near the N-terminus of the protein on the other monomer of the dimer (Surette et al. 1996). The region containing the conserved histidine residue, known as P1, also has a structure conserved with other HPKs (Welch et al. 1998; Zhou et al. 1995a; Zhou and Dahlquist 1997). Purified P1 has no enzymatic activity but can be phosphorylated by another kinase, after which it can function alone to transfer phosphoryl residues to its substrates, CheY and CheB (Swanson et al. 1993). The phosphoramidate bond of phospho-His is very unstable compared to, for example, phospho-Ser, as the standard free energy of phosphotransfer from ATP to His is positive. In vitro phospho-CheA can phosphorylate ADP to ATP. It is assumed that in vivo the high intracellular concentration of ATP and the rapid transfer of the phosphoryl residue to the substrate proteins keep a high rate of CheA autophosphorylation operating with little back reaction (Stewart 1997; Bilwes et al. 1999). Between the conserved histidine and the domain involved in ATP binding is a domain, P2, which binds CheY and CheB, the eventual substrates of CheA (Welch et al. 1998). The P2 domain is not essential for chemotaxis but in vivo may enhance the concentration of CheY and CheB in the region of CheA, thus increasing the rate of phosphotransfer (Jahreis et al. 2004; Stewart et al. 2000).

CheA phosphorylates conserved aspartate residues on two competing response regulators. One is the small, 14-kDa, protein CheY. CheY is a single-domain protein, having only the aspartate-containing receiver domain of archetypal response regulators (Stock et al. 2000). Mutants with *cheY* deleted or

mutated are smooth swimming, that is, they cannot switch the direction of flagellar rotation (Parkinson 1977). CheY binds to the P2 domain of CheA and removes the phosphoryl group from the conserved histidine residue in a reversible reaction to generate CheY-P (Welch et al. 1998). This is released from P2 and diffuses through the cell to the motor, binding to FliM of the flagellar switch and increasing the probability of the motor switching to CW rotation, probably by reducing the energy barrier between CCW and CW rotation (Barak and Eisenbach 1992, 1998).

The structure of CheY, a small 14-kD protein, has been studied using x-ray crystallography and by solution NMR under a range of conditions, including in a phosphorylated conformation and in complex with binding partners (Stock et al. 1989; Volz and Matsumura 1991; Halkides et al. 2000; Cho et al. 2000; Lee et al. 2001; Bren and Eisenbach 1998; Zhu et al. 1997). The structure of CheY is probably common to the phosphorylation domains of all response regulators. It has five α-helices surrounding a 5-stranded parallel β-sheet structure, with the phosphorylation site on aspartate 57. In addition, there is another group of conserved acidic, usually aspartate, residues that are located close to the active site which forms an acidic pocket. Phosphorylation depends on Mg^{2+} and involves CheY itself acting as a phosphotransferase, indeed CheY can take phosphoryl groups from several metabolic phosphodonors such as carbamoyl phosphate or acetyl phosphate, although whether this has a role under natural conditions is unclear. Mg^{2+} probably serves to stabilize the transition state (Stock et al. 1993).

Dephosphorylation of CheY-P occurs autocatalytically with a half time of approximately 10 s. This is in contrast to many other response regulators that remain phosphorylated for many tens of minutes (Stock et al. 2000) and reflects the need for both rapid signaling and signal termination in chemotaxis. The majority of HPK and response regulator systems identified are involved in controling transcription, and therefore, rapid signal termination is not required. The natural rate of autodephosphorylation of *E. coli* CheY-P is however still not fast enough for signal termination in chemotaxis, and a second protein, CheZ, increases the autodephosphorylation rate of CheY-P in enteric species (Hess et al. 1988). The strength of the binding interaction of CheZ to CheY-P is two orders of magnitude greater than that between CheZ and CheY (Blat and Eisenbach 1994). The crystal structure of the CheY-CheZ complex showed that there are two interaction regions between the CheY and the CheZ dimer which serve to clamp CheY in place in the complex (Zhao et al. 2002). The binding of CheZ to CheY is proposed to enhance the CheY-P autodephosphorylation rate by activating a water molecule for attack on the phosphoryl-aspartate residue. CheZ has been shown to localize to the same region of the cell as the MCPs, CheA, and CheW where it binds to a truncated form of CheA, CheAs, that lacks the P1 phosphotransfer domain (Wang and Matsumura 1996; O'Connor et al. 2009; Hao et al. 2009; Cantwell and Manson 2009). The localization of the phosphatase with the CheA kinases has been suggested to flatten the intracellular gradient of CheY-P to ensure all motors experience a similar concentration of CheY-P and thus respond similarly (Lipkow et al. 2004).

Although initially identified in only Betaproteobacteria and Gammaproteobacteria, CheZ homologues have been identified using genomic sequence analysis in the alpha-, beta-, gamma-, delta-, and epsilonproteobacteria (Wuichet et al. 2007). However, many species lack CheZ homologues, and different mechanisms are employed for signal termination (Silversmith 2010). Many bacteria employ members of the CheC/CheX/FliY phosphatase family (Muff and Ordal 2008). These proteins are definied by a conserved domain which is related to FliM, the protein to which CheY-P binds in the flagellar motor. The enhancement of CheY-P dephosphorylation is thought to occur through a mechanism similar to that of CheZ, with a conserved asparagine residue orienting a water molecule for attack on the phosphoryl-aspartate (Park et al. 2004). Other species employ a different strategy; *R.sphaeroides* has a bifunctional kinase/phosphatase for one of its many CheY homologues (Porter et al. 2008), while others such as *S. meliloti* have two CheYs, one of which acts as a phosphate sink for signal termination (Riepl et al. 2008; Sourjik and Schmitt 1996, 1998). In this termination mechanism, both CheY homologues can be phosphorylated by CheA-P, but only one is able to bind to the flagellar motor. In the absence of kinase stimulation, CheY-P can phosphotransfer to CheA; if the phosphotransfer kinetics favor the phosphorylation of the second non-FliM-binding CheY, then this can operate as a phosphate sink.

Adaptation

CheA not only transfers phosphoryl groups to CheY, it can also phosphorylate another response regulator, CheB. CheB is again an unusual response regulator as, in addition to the regulatory domain of a standard response regulator, it has a catalytic methylesterase domain (Stewart and Dahlquist 1988; Stock and Koshland 1978). The activity of the methylesterase is controlled by phosphorylation of a conserved aspartate residue in the regulatory domain, with phosphorylation increasing activity by more than an order of magnitude (Lupas and Stock 1989; Stewart et al. 1990). CheB plays an important role in the adaptation to persistant signaling. The second chemosensory protein involved in adaption is CheR, a constitutively active methyltransferase that employs S-adenosyl methionine as a methyl group donor (Goy et al. 1977, 1978). Adaptation is an essential part of chemotaxis. If the receptor were not reset after encountering a change in receptor occupancy, it would continue to generate a signal and the cell would be unable to respond to future changes. Mutants that are defective in CheR function show a characteristic smooth-swimming phenotype, while those defective in CheB function show constant tumbling. Although the signaling pathway is intact in these mutants, without the ability to reset the chemoreceptors, there is no chemotactic response to a chemoeffector gradient.

MCPs in *E. coli* are methylated in response to the addition of attractant and demethylated in response to removal of attractant

as part of the adaptation pathway (Toews et al. 1979). The cytoplasmic domains of most MCPs have conserved glutamate residues that serve as substrates for the two enzymes involved in receptor adaptation, CheR, the methyltransferase, and CheB, the methylesterase. These glutamate residues may be transcribed as glutamine, but CheB can posttranslationally deamidate glutamine to form glutamate. It appears that newly translated MCPs may have glutamine rather than glutamate residues at some positions to ensure that new receptors are inserted into the lipid bilayer in a neutral signaling state, thus preventing inappropriate signaling (Kehry et al. 1983). *E. coli* MCPs have four methylation sites; analysis of the methylation patterns of receptors showed that the consensus sequence for methylation sites in *E. coli* and related organisms is Glx-Glx-X-X-Ala-Ser/Thr, with methylation occurring on the second Glx residue (Kehry and Dahlquist 1982; Terwilliger and Koshland 1984). There is a specific CheR-docking motif on the C-terminal end of some, but not all, MCPs (Barnakov et al. 1998; Djordjevic and Stock 1998). In *E. coli* and *Salmonella*, Tsr, Tar, and Tcp have a CheR-binding domain, but Trg and Tap do not; nevertheless, they are still methylated in response to attractant binding. However, Trg is not methylated if neither Tsr nor Tar is present in the membrane, suggesting that the CheR bound to Tsr or Tar can methylate the glutamate residues of Trg (Feng et al. 1999). Recent experimental and molecular modeling studies suggest that a CheR molecule bound to the docking motif of one chemoreceptor is able to reach methylation sites on approximately six to nine neighboring chemoreceptors (Muppirala et al. 2009; Li and Hazelbauer 2005).

During signaling, changes in the ligand-binding domain of the chemoreceptor are transduced through the membrane and HAMP domains of the chemoreceptor to the kinase control region. A "yin-yang" model for signal propogation through the kinase control region has been proposed by Falke and coworkers (Swain et al. 2009). In this model, the packing of the kinase signaling and adaptation regions are antisymmetrically coupled. Strong interhelical interactions occur between helices in the adapation region only when the kinase signaling region is loosely packed, and vice versa. Kinase activity is stimulated, that is, the "kinase-on" state is stabilized, by tight packing of the adaptation region. Methylation of glutamate residues in the adaptation region is proposed to reduce electrostatic repulsion between helices, allowing stronger packing and stabilizing the "kinase-on" state (Starrett and Falke 2005). Conversely, demethylation of glutamate residues by CheB would be expected to destabilize the interhelical packing in the adaptation region, stabilizing the "kinase-off" state.

Localization of MCPs

Quantification of MCP copy number has shown that there are typically thousands of MCPs expressed in a cell. Early studies suggested that the MCPs were randomly located around the cell. However, immunogold electron microscopy using antibodies raised against the MCP highly conserved domain showed that MCPs are in fact localized at the poles of *C. crescentus*, *E. coli*, and *R. sphaeroides* (Alley et al. 1992; Maddock and Shapiro 1993; Harrison et al. 1999). More recently, cryoelectron microscopy showed that chemoreceptors from 13 different species are found localized to large chemoreceptor arrays, typically located at the cell pole (Briegel et al. 2008, 2009; Zhang et al. 2007; Khursigara et al. 2008). CheA and CheW associate with the membane distal end of the MCPs and are visible as a "baseplate" in cryoelectron tomography images (Briegel et al. 2009). *Figure 15.5* illustrates the localization of an MCP in *R. sphaeroides*. It has been shown that the presence of CheA and CheW is not essential for the formation of MCP clusters. However, in mutants lacking either CheA or CheW, the MCP clusters are considerably more diffuse than in wild-type cells (Kentner et al. 2006). CheA and CheW, two cytoplasmic proteins, are therefore essential for localization of MCPs into tight signaling complexes. Fluorescence studies utilizing green fluorescent protein (GPF) fused to MCPs have been used to investigate the mechanisms through which the MCP clusters assemble at the cell pole in *E. coli* (Thiem and Sourjik 2008; Greenfield et al. 2009). Large clusters, consisting of thousands of MCPs, are observed at the cell pole; however, smaller clusters of tens to hundreds of MCPs are also observed along the cell body. This distribution is consistent with a stochastic assembly process; receptors inserted into the membrane will be captured by an existing cluster if there is one nearby or will otherwise nucleate a new cluster. Over time, this will generate an arrangement with large clusters at the cell pole and smaller clusters along the cell body. The segregation of receptor clusters between daughter cells on division does, however, appear to be facilitated by the anchoring of large receptor clusters to future cell division sites, though the mechanism for this anchoring is as yet unknown (Thiem et al. 2007). Although in enterobacteria MCP cluster localization is thought to be a stochastic process, in other bacteria cluster localization can actively be promoted (Thompson et al. 2006; Ringgaard et al. 2011).

In some species, such as *R. sphaeroides*, additional receptors lacking transmembrane domains are also found in a cytoplasmic cluster. GFP fusions to *R. sphaeroides* MCPs showed that specific chemoreceptors, such as McpG, are located in clusters at the cell pole, while others, such as TlpC, are localized to a cytoplasmic cluster (Wadhams et al. 2000). Bioinformatics studies have identified MCPs that lack membrane-spanning regions in other species; however, in some species, such as *S. meliloti*, they have been shown to localize to the cell pole along with membrane-spanning MCPs (Wuichet et al. 2007; Meier and Scharf 2009).

Higher-order structures of MCP trimers of dimers are thought to be important for signal sensitivity and amplification. In this model, changes in the binding of a chemoeffector to the periplasmic domain of an MCP may alter the packing of the MCPs in the membrane, bringing together the cytoplasmic domains in a conformation that allows signaling (Duke and Bray 1999). Higher-order interaction between receptor trimer of dimers would be essential for signaling in this model. A range of theoretical models based on signaling through the coupled

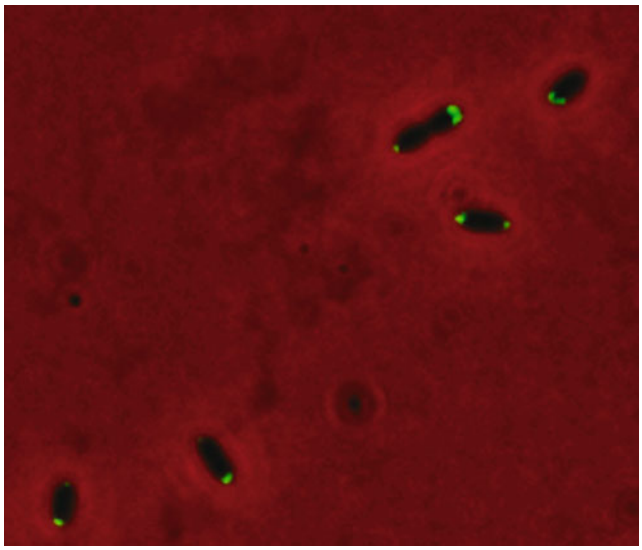


Fig. 15.5
Localization of a methyl-accepting chemotaxis protein (MCP) in *Rhodobacter sphaeroides*. The gene encoding McpG in *R. sphaeroides* was replaced behind its native promoter by a gene encoding McpG-GFP. The MCP was observed to form clusters at the poles of the cells. The formation of these clusters depends not on the MCP itself but on the chemosensory proteins CheA and CheW (From Wadhams et al. 2000)

receptors have been proposed. In these models, the dynamic range of sensitivity seen in bacterial chemosensing depends on controling the sizes of the receptor clusters, or "signaling teams." The extent of lateral packing between trimers of dimers controls the signal, allowing a bacterial cell to respond to a change of a few molecules over background concentrations of orders of magnitude (Shimizu et al. 2000, 2003; Goldman et al. 2009; Endres et al. 2008).

Thermotaxis

E. coli not only responds to changes in chemical concentration but also to changes in temperature (Maeda et al. 1976). The thermotactic and chemosensory reponses both utilize the same protein network, thus the effects of temperature and chemoeffector concentration can compete in the generation of a tactic response. Whether *E. coli* show a warm- or cold-seeking response has been shown to depend upon conditions such as the initial temperature of the cell, the presence of chemoattractants, and cell population density. Using an assay where the reponse of single cells to changes in temperature could be measured, it has been shown that below 31 °C, *E. coli* exhibit a warm-seeking response. As the temperature is increased above 31 °C, the fraction of cells showing cold-seeking behavior increases, and above 40 °C, almost all responsive cells are cold-seeking (Paster and Ryu 2008). The temperature-seeking behavior can, however, be altered by chemoeffectors, for example, incubation of cells with serine almost completely abolishes response to temperature (Maeda and Imae 1979). In a related observation, the *E. coli* thermotactic response has been shown to be dependent upon population density. Cells taken from a low population density culture and subjected to a gradient between 18 °C and 30 °C demonstrated warm-seeking behavior. In contrast, those taken from a high polulation density culture demonstrated cold-seeking behavior (Salman and Libchaber 2007). Although the precise mechanism is not yet fully understood, thermosensing is thought to be caused by temperature-dependent changes in the methylation state of the chemoreceptors (Nara et al. 1996; Nishiyama et al. 1997, 1999). The chemoreceptor Tsr plays the dominant role in generating the *E. coli* thermotactic response; Tsr is responsible for warm-seeking responses, but this response is inhibited at high concentrations of Tsr-specific chemoattractant. Under some conditions, Tar can also generate a thermotactic response, but, in contrast to Tsr, high concentrations of Tar-specific chemoattractant stimulate a Tar-dependent cold-seeking response. Thus, the change in thermotactic response with cell density can be explained by alteration of the relative activity of Tsr and Tar. At low cell density, signaling is dominated by Tsr; however, as the population density increases, glycine (a Tsr chemoattractant) is released into the growth medium inactivating Tsr and allowing Tar-mediated cold-seeking behavior to dominate (Maeda and Imae 1979; Mizuno and Imae 1984; Salman and Libchaber 2007).

Links to Metabolism

Phosphotransferase Sugars

E. coli not only responds to sugars through the periplasmic binding protein-mediated associating with MCPs but also responds to sugars transported through the phosphoenolpyruvate-dependent phosphotransferase system (PTS) independently of the MCPs (Adler and Epstein 1974; Grubl et al. 1990). A membrane-bound substrate-specific transport protein, enyzme II (EII), accepts a phosphoryl group from a nonspecific cytosolic donor enzyme, enzyme I (EI), and phosphorylates the sugar as it is transported. EI, a phosphoenolpyruvate-dependent histidine kinase, and a phosphohistidine carrier protein (HPr) are the phosphorelay to EII. As the activity of EII is substrate specific, bacteria typically contain multiple different EII proteins; *E. coli* has at least 15 PTS systems (Nikaido and Saier 1992). Although metabolism of the sugar is not required for a chemotactic response, the sugar must be transported in order to give rise to a chemotactic signal. Methylation of MCPs is not required for signaling to occur, but CheA and CheY are necessary. EI of the PTS has been shown to interact directly with CheA. It is thought that when actively involved in transporting sugars, EI suppresses CheA phosphorylation, resulting in smooth swimming (Lux et al. 1995, 1999). Interestingly, fructose is transported via a rather different PTS where phosphorylation relies on FPr, a fusion between an HPr-like protein and EI (Lux et al. 1995). This protein does not generate a chemotaxis signal, and *E. coli*

utilizing transport through this pathway are not chemotactic to fructose. *R. sphaeroides* has only an FPr PTS and its chemotactic response to fructose depends not on PTS transport but on metabolism of the sugar (Jeziore-Sassoon et al. 1998).

H-NS, Fumarate Reductase, and Acetylation

Other proteins reflecting the metabolic state of *E. coli* have been shown to interact with the bacterial flagellum, changing the speed or modifying the switching frequency, suggesting a direct interaction between metabolic state and swimming behavior. One such example is the DNA-binding protein H-NS which is a global regulator of gene expression. In addition to regulating expression of flagellar genes, H-NS can also interact directly with the flagellar motor, leading to an increase in rotational speed (Donato and Kawula 1998; Ko and Park 2000). The enzyme fumarate reductase has also been demonstrated to interact with FliG in vitro, suggesting a mechanism by which the increase in the probability of CW rotation exhibited by *E. coli* cells in the presence of fumarate may be effected (Cohen-Ben-Lulu et al. 2008). The activity of the switch-binding protein CheY also may be regulated directly by metabolism. *E. coli* CheY can be acetylated on a number of different lysine residues either enzymatically or through autoacetylation (Barak et al. 2004, 2006). The acetylation sites are located in the region of CheY that binds to the signaling partners CheA, CheZ, and FliM. Acetylation of CheY has been shown to impair binding to these proteins in vitro, while mutations that impair acetylation lead to defects in chemotaxis in vivo (Barak and Eisenbach 2001; Liarzi et al. 2010). The metabolic state of the cell is expected to influence the acetylation state of CheY and thus the frequency of flagellar motor switching.

Chemotaxis in Nonenteric Species

While the majority of research has been carried out on *E. coli* and *S. enterica* Typhimurium, there is an increasing body of literature on a wide range of other species. This combined with the increasing number of complete genome sequences suggests that chemotaxis in the majority of other species is much more complex. Other species often have multiple copies of the chemosensory genes, and expression of the different genes may be controlled by the environment in which the cells are grown, that is, they can fine-tune their chemosensory response to current growth conditions. In addition, many species have chemoreceptors that are only expressed under certain growth conditions, and some of these receptors are cytoplasmic and probably sense the metabolic state of the cell.

Alphaproteobacteria

R. sphaeroides, S. meliloti, M. magnetotacticum, and *C. crescentus* are all members of the Alphaproteobacteria. This particular subgroup includes a very large number of free-living and symbiotic soil and water microorganisms. Analysis of their chemosensory systems illustrates the common themes that are being identified outside of the much-studied Gammaproteobacteria. Sequence analysis has shown that chemosensory networks employ a common central signaling pathway, exemplified by the chemosensory system of *E. coli*, but that this is elaborated upon in other organisms (Wuichet and Zhulin 2010).

R. sphaeroides has great metabolic flexibility. It is able to grow anaerobically as a photoheterotroph or using anaerobic respiration and as an aerobic heterotroph. It also has a quorum-sensing system and can form biofilms (Puskas et al. 1997, 1997; Wilkinson et al. 2011; Kho et al. 2003; Hwang et al. 2008). It can fix nitrogen and carbon dioxide and can also ferment. *S. meliloti* is an obligate aerobe but can fix nitrogen when maintained anaerobically in a symbiotic relationship with leguminous plants. *M. magnetotacticum* is a microaerophile living in the oxic-anoxic transition zone of aquatic sediments. It uses specialized organelles known as magnetosomes to respond to the earth's magnetic field (Komeili 2012). *M. magnetotacticum* has the largest number of chemoreceptors identified to date, 65, most of which have not been characterized although it seems likely that at least three of the receptors respond to oxygen levels. *C. crescentus* on the other hand appears to have a restrictive metabolic life-style, living as an aerobic organism in oligotrophic environments. It undergoes a differentiation cycle where stalked, surface-attached, cells divide to release motile swarmer cells. After a short period of motility, swarmer cells anchor to a surface and differentiate into stalked cells to repeat the cycle (Kirkpatrick and Viollier 2012).

The genome sequences of *R. sphaeroides*, *S. meliloti*, *M. magnetotacticum*, and *C. crescentus* have provided details of the motility and chemosensory pathways. This, in combination with molecular genetic studies on the different species, suggests that their chemosensory pathways are significantly more complex than those of *E. coli* and *Salmonella*. Obviously, if chemotaxis is to be of any advantage to the survival of a species, bacteria must respond to metabolites that they have evolved to use. For example, while amino acids are the dominant attractants for *E. coli*, they are minor attractants for *R.sphaeroides*, which shows the strongest responses to organic acids, its favored carbon source. Chemosensory receptors have almost certainly evolved in parallel with the metabolic pathways of the species, and the number of chemoreceptors encoded by a particular species is likely to reflect both metabolic and environmental diversity. Sequence analysis has identified a larger number of MCPs in *R. sphaeroides*, *S. meliloti*, *M. magnetotacticum*, and *C. crescentus* than found in *E. coli* (12, 10, 65, and 18 MCPs, respectively) (Alexander and Zhulin 2007). Deletion of these putative receptor genes does not typically result in the complete loss of chemosensory response to a specific attractant but instead results in a reduction in the magnitude of the reponse. In many cases, multiple MCPs participate in the response to a single attractant, for example, McpU, McpX, and McpV are all important for response to the attractant proline by *S. meliloti* (Meier et al. 2007). Although all of the receptor genes identified

include the highly conserved signaling domains characteristic of MCPs, not all of them encode membrane-spanning proteins. These cytoplasmic MCPs most probably sense metabolites inside the cell rather than in the extracellular environment. Chemosensory responses are also often dependent upon growth conditions, for example, *R. sphaeroides* will respond to some carbon and nitrogen sources only when they are limiting for growth (Poole and Armitage 1989). A large number of studies have shown that transport and metabolism are required for some responses to compounds as diverse as ammonia, sugars, and amino acids, again suggesting a link to growth conditions.

The chemosensory networks of *R. sphaeroides, C. crescentus, and S. meliloti* are also more complex than is seen in *E. coli*. Genetic analysis and gene sequencing has shown that *R. sphaeroides* has three, and *C. crescentus* and *S. meliloti* have two operons coding for multiple homologues of the *E. coli* chemosensory genes. In *S. meliloti*, one of the *che* operons is located on the chromosome and the other on the plasmid pSymA (Galibert et al. 2001). Deletions of chromosomally located *che* genes have been shown to impair or abrogate chemotaxis, while the second *che* operon is most likely to regulate a nonchemosensory trait (Greek et al. 1995; Wuichet and Zhulin 2010). All three species differ from the *E. coli* paradigm in the lack of a CheZ homologue but instead show two or more CheY homologues; in the case of *R. sphaeroides*, six *cheY* genes have been identified and characterized (Porter et al. 2006). Rapid signal termination is an essential component of chemotaxis, and CheZ is thought to increase the rate of CheY-P dephosphorylation in *E. coli*. In vitro studies examining the phosphorylation and dephosphorylation kinetics of the purified CheA and two CheY homologues of *S. meliloti* suggest that one of the CheY homologues functions as a phosphate sink to terminate the chemosensory signal. Only one of the CheYs has been shown to bind to the flagellar motor when phosphorylated. In the absence of kinase stimulation, the motor-binding CheY may rephosphorylate CheA; the second (non-motor-binding) CheY could then act as a phosphate sink and cause rapid signal termination (Sourjik and Schmitt 1996, 1998; Riepl et al. 2008). It has also been proposed that the role of one of the *R. sphaeroides* CheYs may be as a phosphate sink, although the proposed mechanism of action is quite different (Tindall et al. 2010a). The role of multiple CheYs, particularly in *R. sphaeroides*, remains uncertain. Mutagenesis suggests that in *R. sphaeroides* only one CheY stops motor rotation, while two others regulate the behavior of the motor-stopping CheY (Porter et al. 2006). Experimental data from *H. salinarium* and *B. subtilis* suggests that the indirect interaction of CheY-P with MCPs, via two chemosensory proteins not found in *E. coli*, CheC and CheD, can play an important role in adaptation (Kirby et al. 1999; Kristich and Ordal 2002; Muff and Ordal 2007). Although no CheC homologue has been reported in these organisms, *S. meliloti* and *R. sphaeroides* do have *cheD* genes, and it is therefore possible that one or more of the CheY homologues in these organisms may play a role in adaptation.

Detailed analysis of the *R. sphaeroides* chemosensory system has shown that the proteins encoded by one chemosensory operon localize to the cell poles with the membrane-spanning MCPs, while those of the second operon form a discrete cluster in the cytoplasm with the soluble receptors (Wadhams et al. 2003). Both pathways are essential for chemotaxis, and the proteins of each pathway need to be localized to the correct cluster in order to function (Porter et al. 2002). On division, the cytoplasmic cluster segregates, with each daughter cell inheriting a complete cytoplasmic cluster. This ensures that both daughter cells are chemotactic immediately following division. Segregation depends on a ParA/B system analogous to that used to segregate plasmids (Thompson et al. 2006). Mathematical models suggest that signals from the cytoplasmic cluster regulate the activity of the polar cluster to tune the chemosensory response relative to current growth conditions (Tindall et al. 2010a).

Interestingly, in *R. sphaeroides* the expression of the operon encoding the components of the membrane-bound chemosensory cluster is under the control of the Reg/Prr histidine protein kinase system. This system also controls expression of the genes for CO_2 and N_2 fixation and for photosynthesis in response to changes in electron flow through the terminal cbb_3-type cytochrome oxidase (Eraso and Kaplan 2000; Oh and Kaplan 2000; Dubbs et al. 2000). This indicates a tight interconnection between metabolic capability and chemosensory behavior under these conditions. Many more species have now been found to have multiple copies of the chemosensory genes, and hopefully research on other species will identify whether the sensory pathways operate under different growth conditions or are linked to different sensory receptors.

There is evidence that some transport proteins may be able to cause chemotactic signaling independently of the MCPs. One example is that of PcaK from *Pseudomonas putida. P. putida* grows on the aromatic acid 4-hydroxybenzoate and shows chemotaxis toward this compound. The permease for 4-hydroxybenzoate, PcaK, has been identified as a member of the major facilitator superfamily of transporters. PcaK does not resemble an MCP in structure, being a classical transporter protein with 12 transmembrane helices, yet it is required for the chemotactic response to 4-hydroxybenzoate and several other aromatic acids (Harwood et al. 1994). Mutations in PcaK result in the loss of taxis to the 4-hydroxybenzoate aromatic acids, even under conditions where aromatic acids are able to diffuse into the cell and allow normal growth, suggesting a real signaling role for the transport proteins in chemotaxis (Ditty and Harwood 1999). Analysis of sequenced genomes indicates that several more species may have transporters that could also be involved in sensory signaling.

Bacillus subtilis

Few species have had the same attention lavished on them as *E. coli*, and therefore, their sensory pathways are less well understood. The chemosensory network of *B. subtilis* has, however, been studied in detail (Rao et al. 2008). *B. subtilis* responds to a very wide range of amino acids and sugars, and a large number

of chemically diverse repellents have also been identified. The chemotactic response to sugars may involve the PTS, as in *E. coli*, but in the case of *B. subtilis*, MCPs are also required for a response to be elicited. It is possible, however, that the requirement for MCPs in PTS signaling is indirect rather than direct, with the MCPs providing the scaffold for the CheA kinase (Garrity et al. 1998).

B. subtilis does not have multiple homologues of *che* genes as seen in many of the Alphaproteobacteria. Instead, the chemosensory network of *B. subtilis* has all of the conserved chemosensory components, MCPs, CheA, CheW, CheY, CheB, and CheR but also includes the proteins CheV, CheC, CheD, and FliY. A major difference compared with the *E. coli*–sensing paradigm is that an increase in CheY-P concentration occurs in response to an increase, rather than decrease, in attractant (Bischoff and Ordal 1992). The outcome of an increase in attractant concentration is smooth swimming in both cases; however, as in *B. subtilis*, CheY-P binding to the flagellar motor induces CCW rotation (in *E. coli* CheY-P binding to the motor leads to CW rotation and tumbling).

What is the function of other proteins? One possible role would be in signal termination. CheV consists of a CheW-like domain fused to a C-terminal response regulator receiver domain that can be phosphorylated by CheA-P, while CheC and FliY both show phosphatase activity toward CheY-P (Fredrick and Helmann 1994; Szurmant et al. 2004). There are therefore several possible mechanisms through which signal termination could occur. The presence of two CheY-like RRs in the chemosensory network could allow signal termination to occur through the use of CheV as a phosphate sink. Alternatively one or both of CheC and FliY may dephosphorylate CheY-P. Experimental evidence suggests that FliY plays the dominant role in signal termination; deletion of FliY leads to a completely smooth-swimming phenotype characteristic of a high level of CheY-P in the cell (Szurmant et al. 2003). Deletion of CheV, CheC, and CheD suggests that their primary roles are in adaptation to persistent signaling (Rosario et al. 1994, 1995).

The adaptation process of *B. subtilis* is more complex than that of *E. coli*, with three adaptation systems utilizing CheV, CheC/CheD, and CheB/CheR (Rao et al. 2008). CheV acts as a negative regulator of CheA kinase activity. The receiver domain can be phosphorylated on a conserved aspartate residue, in competition with CheY and CheB (Rosario et al. 1994). It is thought that phosphorylation of CheV can decouple CheA kinase activity from the chemoreceptors, resulting in a decrease in the concentration of CheA-P and hence adaptation (Karatan et al. 2001). CheD catalyzes the deamidation of specific glutamine residues to glutamate residues that can then act as substrates for CheR-/CheB-dependent methylation/demethylation (Kristich and Ordal 2002). CheD also plays an additional role in adaptation in conjunction with CheC and CheY-P (Muff and Ordal 2007). CheD has been shown to bind to specific MCPs, and this binding is important for the stimulation of CheA kinase activity by the MCPs. CheC can compete with the MCPs for binding with CheD, with the formation of a CheC/CheY-P complex enhancing the interaction of CheC with CheD (Chao et al. 2006; Muff and Ordal 2007). Thus in the absense of a chemosensory stimulus, there will be a high concentration of CheD bound to the chemoreceptors. On stimulation of CheA kinase activity, the concentration of CheY-P and hence that of the CheC/CheY-P complex will increase. Some of the CheC/CheY-P complexes will bind to CheD, reducing the concentration of CheD bound to the chemoreceptors and hence decreasing CheA kinase activity.

In *E. coli*, methyl groups are released as methanol following demethylation of MCPs only after the addition of a repellant or removal of an attractant. In both *B. subtilis* and the archaeon *H. salinarium*, methanol is released when either attractants or repellents are added, rather than on attractant removal or addition of a repellent (Kirby et al. 1999). Detailed analysis of *B. subtilis* McpB showed that methylation at some glutamate residues increased receptor activity, while methylation of others decreased receptor activity suggesting differing roles for specific glutamate residues in adaptation (Zimmer et al. 2000). The pattern of methylation and methanol release is very similar to that seen in archaea, suggesting an early origin for this mechanism. Interestingly, sequence analysis of chemotaxis operons of *S. meliloti* and *R. sphaeroides* suggests that a *cheD* homologue is present in these Gram-negative bacteria. Mutations in this gene result in abnormal behavior, with a change in the pattern of methanol release, but its role has not been fully characterized (Sourjik et al. 1998). It may be relevant that patterns and the extent of methanol release in *S. meliloti* and *R. sphaeroides* are not as simple as in either *E. coli* or *B. subtilis*.

Phototaxis

Most motile bacterial species exhibit a tactic response to the electron acceptors required for generation of an electrochemical proton gradient, and many that are capable of photosynthetic growth also respond to light. Light sensing in photosynthetic species is often linked to photosynthetic electron transfer. Nonphotosynthetic bacteria will, however, respond to blue light by tumbling, and prolonged exposure can lead to a complete loss of motility (Macnab and Koshland 1974; Taylor et al. 1979). This behavior is most likely the result of the photooxidation of porphyrins, which then act as repellent, signaling through the classical MCP system (Yang et al. 1995, 1996).

Some photosynthetic eubacteria, for example, *Ectothiorhodospira halophila, Chromatium salexigens*, and some strains of *R. sphaeroides*, have been found to contain a 4-hydroxycinnamic-acid-dependent soluble protein, the photoactive yellow protein (PYP), which undergoes a photocycle in blue light. Many of these species respond to flashes of blue light by stopping or reversing their direction of movement. PYP was proposed to be responsible for the negative photoresponse of *E. halophilia*, and the absorption spectrum of PYP matches the response spectrum of the phototactic response (Sprenger et al. 1993). However, the role of PYP in phototactic response remains uncertain. For example, *Rhodocista centenaria* (originally described as *Rhodospirillum centenum*) encodes a PYP fusion

protein, Ppr, which includes the PYP domain, a putative bilin-binding domain and a histidine kinase domain. The kinase domain of Ppr was shown to form a complex with CheW both in vivo and in vitro, and expression of Ppr in *E. coli* inhibits chemotaxis, suggesting a role for this protein in a tactic response (Kreutel et al. 2010). In contrast, although the absorption spectrum of PYP fits the response spectrum of *R. sphaeroides*, deletion of the gene does not result in the loss of the blue-light response (Jiang et al. 1999; Kort et al. 2000).

Most photosynthetic bacteria respond to light by reversing after swimming over a light/dark boundary. In fact, reversing or stopping seems to be the most common mechanism for changing direction in photosynthetic species, few species have been described that tumble in a similar manner to *E. coli*. Observations suggest there is little change in swimming pattern when photosynthetic bacteria swim over a dark/light boundary (Armitage 1997). They therefore appear to sense and respond primarily to a reduction in light intensity, rather than an increase. However, if *R. sphaeroides* is tethered by its flagellum and given subsaturating increases and decreases in light, the cells respond both the increase and decrease in intensity (Romagnoli and Armitage 1999). They respond to a decrease in light intensity by stopping, and then adapting to the change, and to an increase in light intensity by reducing the stopping frequency, followed by adaptation. The response to the increase in light is less obvious than to the decrease because the natural bias (stopping frequency) of the *R. sphaeroides* flagellar motor is 0.8, which means that an *R. sphaeroides* cell will be spending more time swimming than being stopped in the absence of stimulus. In *E. coli*, where the bias is closer to 0.5, the relative change in stopping frequency when moving up a gradient is greater than that seen in *R. sphaeroides* (Berry and Armitage 2000). The motor biases of other species have not been measured, but it would be interesting to identify whether a high bias is linked to a specific environment or mechanism of changing direction.

Whether or not bacteria can respond to a gradient of light, rather than to crossing a light/dark boundary, is arguable and may depend on their environment and the type of gradient formed in that environment. Free-swimming cells are unlikely to be able to move far enough in a given time to experience the 1% drop in light intensity required to cause an increase in the motor-stopping frequency. However, in environments where light intensities fall rapidly, such as in microbial mats or dense colonies, the response could be different. Indeed, *R. centenum* has been shown to respond as a moving colony to a light gradient, with the whole colony moving across an agar plate toward infrared light and away from white light. If presented with light from two directions, the colony will move along the averaged path (Ragatz et al. 1995; Jiang et al. 1997, 1998). When cells from a moving colony were resuspended in liquid medium, they did not respond to light gradients, suggesting the response is dependent on the cell density within the colony (Sackett et al. 1997). Oxygen electrode measurements showed a large oxygen gradient within the colony (Romagnoli et al. 1997). It is therefore possible that the colony movement is a combination of tactic response away from oxygen and toward light.

Photosynthetic electron transport has been shown to be essential in all cases of positive responses to light by photosynthetic bacteria. Inhibitors of photosynthetic electron transport inhibit photoresponses, as do mutations within the reaction centers which leave pigments intact but block electron transport (Grishanin et al. 1997; Packer et al. 1996). The sensory signal is probably a change in electron transport rate rather than a change in the transmembrane proton-motive force, ρp; low concentrations of uncouplers which cause a step-down in ρp do not alter the phototactic response, but electron transport inhibitors which alter the rate of electron flow, but not the size of ρp, do elicit a response. The sensory receptor protein signaling the change in electron transfer in the *R. sphaeroides* phototactic response has not yet been identified. The cbb_3 cytochrome oxidase PrrB, which is involved both in reponse to oxygen and in the expression of one of the chemosensory operons, was considered a potential candidate for the sensory receptor protein. However, Δ*prrB* mutants still respond to changes in light level indicating the presence of another light-sensing system (Romagnoli et al. 2002). The photoresponse in *R. sphaeroides* requires both the CheA and CheW components of the membrane-bound chemosensory cluster, suggesting the involvement of an MCP-like receptor in signaling (Romagnoli and Armitage 1999). This is supported by the observation of methanol release following the reduction in light intensity, which indicates that changes in receptor methylation occur during adaptation to the new light intensity.

Aerotaxis and Electron Acceptor Taxis "Energy Taxis"

Responses to oxygen were among the earliest observed in bacteria (Engelmann 1883). As would be expected, the responses to oxygen vary from species to species. Obligate aerobes will swim toward oxygen, while obligate anaerobes are repelled. Microaerophiles respond positively to low concentrations of oxygen but are repelled by atmospheric levels. In some cases, the responses are different under different growth conditions, for example, *R. sphaeroides* is attracted to oxygen when growing as a heterotroph, but oxygen is a repellent for cells grown under photoheterotrophic conditions. *S. enterica* Typhimurium shows a tactic response to oxygen concentrations that correspond to the *K*m values of its cytochrome oxidases. As for the photoresponse in other bacteria, inhibition of electron transport results in the loss of the response to oxygen (Laszlo and Taylor 1981; Laszlo et al. 1984). This is indicative of the presence of a redox sensor which responds to changes in the rate of respiratory electron flow.

A redox sensor, Aer, has been identified in *E. coli*. Aer is a flavin adenine dinucleotide–binding protein with a cytoplasmic domain homologous to the highly conserved domain of an MCP (Bibikov et al. 1997, 2000; Rebbapragada 1997). Despite the homology to the MCPs, however, Aer does not appear to be methylated during the sensory response (Bibikov et al. 2004). The FAD cofactor is bound to a PAS

domain located in the cytoplasm of the cell at the N-terminus of Aer, and a membrane-anchored linker joins the PAS domain to the cytoplasmic MCP-like region (Amin et al. 2006). The oxidation and reduction of the FAD cofactor in response to changes in the redox state of the cell is believed to induce conformational changes in the PAS domain that are transmitted, through direct interaction with the HAMP domain, to the the C-terminal signaling domain (Watts et al. 2004, 2011; Campbell et al. 2010). As for the MCPs, this conformational change is propogated through CheW to CheA, ultimately resulting in the modulation in the cellular concentration of CheY-P. The tactic response to changes in oxygen concentration is reduced, but not abolished, in mutant *E. coli* strains lacking Aer, indicating the presence of an alternative mechanism for detecting changes in oxygen levels. It was shown that *E. coli* strains in which both Aer and Tsr were deleted do not show a tactic response to changes in oxygen levels (Rebbapragada 1997). Tsr has been shown to function as a pH sensor and is thought to mediate oxygen-dependent taxis by detecting changes in the proton-motive force across the lipid bilayer (Edwards et al. 2006; Umemura et al. 2002). It has been suggested that a balancing of the response to changes in electron transport and the proton-motive force could account for the positive responses toward optimum oxygen concentrations and the negative responses shown to potentially toxic high concentrations. Whether this would have a role under physiological conditions is not known.

Several independent mechanisms for sensing oxygen levels appear to have evolved. Sequencing of the *B. subtilis* genome allowed the identification of an MCP-like protein, HemAT-Bs, whose N-terminal domain showed homology to myoglobin. The near-ultraviolet and visible absorption spectra of purificated HemAT-Bs were characteristic of oxygen-bound Fe-heme proteins, and in a *B. subtilis* strain expressing HemAT-Bs in the absence of other MCP-like proteins, taxis toward increasing oxygen concentration was observed. This demonstrates that *B. subtilis* can sense molecular oxygen directly, that is, that it shows aerotaxis (Hou et al. 2000). A heme cofactor is also found in the sensory protein DcrA encoded by the strictly anaerobic bacterium *Desulfovibrio vulgaris*. DcrA is composed of an N-terminal, c-type heme binding, periplasmic domain connected to the highly conserved cytoplasmic signaling domain of an MCP. The mechanism through which oxygen is sensed is not known. DcrA could sense oxygen directly through oxygen binding in the c-type heme, or indirectly through changes in the oxidation state of the heme iron. Unlike Aer, DcrA has been shown to be methylated in response to changing oxygen concentrations. Although there is variation in the sensory modules used to detect changes in oxygen level, in each case the sensor is linked to the highly conserved signaling domain of MCPs (Fu et al. 1994). This suggests that this highly conserved domain appeared early in the evolution of chemosensory pathways and that different sensory domains have been grafted onto this common element by different species to better serve their specific niche.

Electron transfer is involved in both light and oxygen sensing, and in many species electron transfer components are shared between the different pathways, which could result in competition between the sensory signals. This competition does appear to take place. Most bacteria that grow using respiratory electron transfer can also use other electron acceptors when oxygen is absent (anaerobic respiration). Thus *E. coli* will grow on nitrate in the absence of oxygen and under these condition will show tactic responses toward increased concentrations of nitrate ions. *R. sphaeroides* will grow on dimethyl sulfoxide (DMSO) in the absence of oxygen and again shows taxis toward DMSO under these conditions (Gauden and Armitage 1995). In both cases, upon exposure to oxygen, electron transfer is diverted from the alternative acceptor to oxygen and the response to nitrate or DMSO is either reduced or lost completely. The cells recover a tactic response toward oxygen. In *R. sphaeroides*, light inhibits or reduces responses to either DMSO or oxygen, and oxygen reduces the size of the photoresponse in photosynthetically growing cells. Under all of these conditions, electron transfer components are shared; photosynthetic electron transfer has been shown to be more rapid than respiratory electron transfer, while electron flow to a terminal cytochrome oxidase will be dominant over flow to DMSO reductase (Grishanin et al. 1997). These experimental data suggest that *R. sphaeroides* and *E. coli* are not responding to light, oxygen, or nitrate under these conditions. Instead, they are responding to the change in electron flow through a common receptor which then signals through the chemosensory pathway. As in the Aer- and Tsr-mediated sensing of oxygen levels, there may be more than one receptor proteins contributing to the sensory response. In *R. sphaeroides*, deletion of the CheA localized to the membrane-bound chemosensory cluster results in a reduction in the magnitude of the response to oxygen. In addition, deletion of PrrB, the redox sensor of electron flow through the terminal cbb_3 oxidase, also results in a loss of oxygen-dependent taxis. These data suggest that the histidine protein kinase PrrB not only controls the activity of the transcriptional activator PrrA but can directly control the chemosensory pathway. No research has yet been carried out into competition between electron-transport-dependent signals and the chemosensory signals.

Pathogenicity

There is a great deal of clear evidence that chemotactically directed swimming is one of the sensory systems used by many species to help colonization, either pathogenic or symbiotic, of plant, fungal, insect, amoebae, and animal hosts. Indeed, the species where it has now been shown to be important is too extensive to list. *Agrobacterium tumefaciens* shows chemotaxis to plant wounds (Harighi 2009), as do *Erwinia* and *Pseudomonas* species (Antunez-Lamas et al. 2009). *Vibrio* fish pathogens show chemotaxis to fish exudates (O'Toole et al. 2004), while *Helicobacter pylori* shows strong negative pH taxis (a form of energy taxis) that allows it to rapidly colonize the gut mucosa. Deletion of the chemoreceptor for pH results in a nonpathogenic strain, emphasizing the role of chemotaxis in invasion (Croxen et al. 2006).

Other human pathogens, from *Listeria* monocytogenes to *Campylobacter* use motility in the early phases of colonization, as with *V. cholerae* which ceases to express motility genes when attached to a surface. Many noninvasive species, however, rather than switching off flagella synthesis may become hyperflagellate. *Proteus mirabilis* becomes filamentous and hyperflagellate on surfaces, rowing across surfaces using bundles of flagella (Jones et al. 2004). A number of other species have been shown to become hyperflagellate on surfaces, from marine species such as *V. alginolyticus*, a fish pathogen, and *Clostridium septicum* (Kearns 2010). The role of hyperflagellation in colonization is unclear, but it has been suggested that this allows noninvasive species to colonize areas such as the urethra (Allison et al. 1992; Mobley and Belas 1995).

Aquatic Environments

Until recently, very little was known about bacterial motility in open waters, whether fresh or marine, as many species of aquatic bacteria have proven hard to grow in the laboratory and studying them in situ is difficult. Many species of aquatic bacteria show large diurnal migrations, but typically these depend on the development and collapse of gas vacuoles (Walsby 1994). Several phototrophic consortia have been identified in eutrophic fresh water, "*Chlorochromatium aggregatum*" and "*Pelochromatium roseum*" being two whose behavior have been investigated (Overmann and Schubert 2002; Overmann 2010). They are both consortia formed between a large central, motile nonphotosynthetic cell and nonmotile photosyntheic cells that surround it. These consortia are not rare and can make up as much as two-thirds of the biomass of the chemocline of a lake and may therefore be important in the general physiology of these ecosystems. Analysis of the vertical distribution of specific consortia shows that they are found at specific regions with maximum light intensity but very low oxygen levels. The behavioral response must balance the signals from oxygen, light, sulfide, and iron to keep the consortia in these regions. When the behavior of these bacteria was analyzed in the laboratory, it was found that although the motile member of the group was not photosynthetic, the consortia responded to changes in light intensity and accumulated in wavelengths that corresponded to bacteriochlorophyll c and d, the pigments found in the nonmotile members of the group. The nonmotile species therefore must signal the motile bacterium when the light intensity changes.

The view of marine environments and motile behavior changed with the Global Ocean Survey, which revealed a much larger marine population of bacteria than previously thought (Shaw et al. 2008). Many of the species have flagella and chemosensory genes, but the majority seem nonmotile. There are intriguing findings however, for example, closely related *Silicibacter* species while motile have very different complements of chemosensory genes, one having none and one having at least two chemosensory pathways. Recent studies of some marine species also suggest that they may not undergo chemotaxis using the well-studied patterns of model species. Clouds of very fast swimming bacteria are often found close to, but not associated with, surfaces. These unclassified bacteria show strong aerotactic and chemotactic behavior, which may maintain them close to surfaces where nutrient levels are higher than in the open waters (Mitchell et al. 1995). Some marine bacteria are also chemotactic to dimethylsulfoniopropionate secreted by marine algae, and its metabolism to dimethylsulfonate may be a major source of greenhouse gases. As nutrient supplies in ocean water are typically low and transient, aquatic bacteria that can rapidly respond to and move toward transient nutrient supplies can gain significant advantage. *Pseudoalteromonas haloplanktis* is known to accumulate in response to a transient pulse of nutrient within tens of seconds; the chemotactic response of *P. haloplanktis* is more than ten times faster than that of *E. coli* (Stocker et al. 2008; Seymour et al. 2010).

Conclusion

Sequencing of bacterial genomes suggests that the majority of bacterial species move. What has become apparent over the past few years is that the motility and chemosensory pathways of the majority of bacterial species are built from the same basic skeleton as is found in *E. coli*. However, different species have expanded and added to the basic system to tune it to their requirements. Therefore, different species not only sense different stimuli, but also the number of sensory signals varies greatly. Many species also seem to have several different chemosensory pathways, some expressed under different conditions. The reason for this is unclear and may reflect the kinetic requirements of responses to different signals. What is really apparent however is that motility and chemotaxis do not stand alone as physiological traits but are part of a large, interconnected sensory network that makes a particular species robust for life in their current specific niche.

Acknowledgments

Research on *R. sphaeroides* in the Armitage lab is funded by the UK BBSRC.

References

Adler J (1969) Chemoreceptors in bacteria. Science 166:1588

Adler J, Epstein W (1974) Phosphotransferase-system enzymes as chemoreceptors for certain sugars in *Escherichia coli* chemotaxis. Proc Natl Acad Sci USA 71:2895–2899

Airola MV, Watts KJ, Bilwes AM, Crane BR (2010) Structure of concatenated HAMP domains provides a mechanism for signal transduction. Structure 18:436–448

Alexander RP, Zhulin IB (2007) Evolutionary genomics reveals conserved structural determinants of signaling and adaptation in microbial chemoreceptors. Proc Natl Acad Sci USA 104:2885–2890

Alley MRK, Maddock JR, Shapiro L (1992) Polar localization of a bacterial chemoreceptor. Genes Dev 6:825–836

Allison C, Coleman N, Jones PL, Hughes C (1992) Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. Infect Immun 60:4740–4746

Amin DN, Hazelbauer GL (2010) The chemoreceptor dimer is the unit of conformational coupling and transmembrane signaling. J Bacteriol 192:1193–1200

Amin DN, Taylor BL, Johnson MS (2006) Topology and boundaries of the aerotaxis receptor Aer in the membrane of *Escherichia coli*. J Bacteriol 188:894–901

Antunez-Lamas M, Cabrera-Ordonez E, Lopez-Solanilla E, Raposo R, Trelles-Salazar O, Rodriguez-Moreno A, Rodriguez-Palenzuela P (2009) Role of motility and chemotaxis in the pathogenesis of *Dickeya dadantii* 3937 (ex *Erwinia chrysanthemi* 3937). Microbiology-Sgm 155:434–442

Aravind L, Ponting CP (1999) The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signalling proteins. FEMS Microbiol Lett 176:111–116

Arkhipov A, Freddolino PL, Imada K, Namba K, Schulten K (2006) Coarse-grained molecular dynamics simulations of a rotating bacterial flagellum. Biophys J 91:4589–4597

Armitage JP (1997) Behavioural responses of bacteria to light and oxygen. Arch Microbiol 168:249–261

Armitage J (2006) Bacterial behavior. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 102–139

Armitage JP, Macnab RM (1987) Unidirectional, intermittent rotation of the flagellum of *Rhodobacter sphaeroides*. J Bacteriol 169:514–518

Armitage JP, Pitta TP, Vigeant MA, Packer HL, Ford RM (1999) Transformations in flagellar structure of *Rhodobacter sphaeroides* and possible relationship to changes in swimming speed. J Bacteriol 181:4825–4833

Asai Y, Kojima S, Kato H, Nishioka N, Kawagishi I, Homma M (1997) Putative channel components for the fast-rotating sodium-driven flagellar motor of a marine bacterium. J Bacteriol 179:5104–5110

Asai Y, Kawagishi I, Sockett ER, Homma M (2000) Hybrid motor with the H+ and Na+-driven components can rotate of vibrio polar flagella using sodium ions. J Bacteriol 181:6332–6338

Atsumi T, McCartert L, Imae Y (1992) Polar and lateral flagellar motors of marine vibrio are driven by different ion-motive forces. Nature 355:182–184

Barak R, Eisenbach M (1992) Correlation between phosphorylation of the chemotaxis protein-chey and its activity at the flagellar motor. Biochemistry 31:1821–1826

Barak R, Eisenbach M (2001) Acetylation of the response regulator, CheY, is involved in bacterial chemotaxis. Mol Microbiol 40:731–743

Barak R, Prasad K, Shainskaya A, Wolfe AJ, Eisenbach M (2004) Acetylation of the chemotaxis response regulator CheY by acetyl-CoA synthetase purified from *Escherichia coli*. J Mol Biol 342:383–401

Barak R, Yan J, Shainskaya A, Eisenbach M (2006) The chemotaxis response regulator CheY can catalyze its own acetylation. J Mol Biol 359:251–265

Bardy S, Ng SYM, Jarrell KF (2004) Recent advances in the structure and assembly of the archaeal flagellum. J Mol Microbiol Biotechnol 7:41–51

Barnakov AN, Barnakova LA, Hazelbauer GL (1998) Comparison in vitro of a high- and a low-abundance chemoreceptor of *Escherichia coli*: similar kinase activation but different methyl-accepting activities. J Bacteriol 180:6713–6718

Beel BD, Hazelbauer GL (2001) Signalling substitutions in the periplasmic domain of chemoreceptor Trg induce or reduce helical sliding in the transmembrane domain. Mol Microbiol 40:824–834

Berg HC (1976) How spirochetes may swim. J Theor Biol 56:269–273

Berg HC (1993) Random walks in biology. Princeton University Press, Princeton

Berg HC, Anderson RA (1973) Bacteria swim by rotating their flagellar filaments. Nature 245:380–382

Berg HC, Brown DA (1972) Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. Nature 239:500–504

Berg HC, Turner L (1995) Cells of *Escherichia coli* swim either end forward. Proc Natl Acad Sci USA 92:477–479

Berry RM, Armitage JP (2000) Response kinetics of tethered *Rhodobacter sphaeroides* to changes in light intensity. Biophys J 78:1207–1215

Bhatnagar J, Borbat PP, Pollard AM, Bilwes AM, Freed JH, Crane BR (2010) Structure of the ternary complex formed by a chemotaxis receptor signaling domain, the CheA histidine kinase, and the coupling protein CheW as determined by pulsed dipolar ESR spectroscopy. Biochemistry 49:3824–3841

Bibikov SI, Biran R, Rudd KE, Parkinson JS (1997) A signal transducer for aerotaxis in *Escherichia coli*. J Bacteriol 179:4075–4079

Bibikov SI, Barnes LA, Gitin Y, Parkinson JS (2000) Domain organization and flavin adenine dinucleotide-binding determinants in the aerotaxis signal transducer Aer of *Escherichia coli*. Proc Natl Acad Sci USA 97:5830–5835

Bibikov SI, Miller AC, Gosink KK, Parkinson JS (2004) Methylation-independent aerotaxis mediated by the *Escherichia coli* aer protein. J Bacteriol 186:3730–3737

Bilwes AM, Alex LA, Crane BR, Simon MI (1999) Structure of CheA, a signal-transducing histidine kinase. Cell 96:131–141

Bischoff DS, Ordal GW (1992) *Bacillus subtilis* chemotaxis: a deviation from the *Escherichia coli* paradigm. Mol Microbiol 6:23–28

Blair DF, Berg HC (1990) The Mota protein of *Escherichia coli* Is a proton-conducting component of the flagellar motor. Cell 60:439–449

Blair DF, Berg HC (1991) Mutations in the Mota protein of *Escherichia coli* reveal domains critical for proton conduction. J Mol Bio 221:1433–1442

Blat Y, Eisenbach M (1994) Phosphorylation-dependent binding of the chemotaxis signal molecule CheY to its phosphatase, CheZ. Biochemistry 33:902–906

Block SM, Berg HC (1984) Successive incorporation of force-generating units in the bacterial rotary motor. Nature 309:470–472

Block SM, Segall JE, Berg HC (1982) Impulse responses in bacterial chemotaxis. Cell 31:215–226

Block SM, Blair DF, Berg HC (1989) Compliance of bacterial flagella measured with optical tweezers. Nature 338:514–518

Block SM, Blair DF, Berg HC (1991) Compliance of bacterial polyhooks measured with optical tweezers. Cytometry 12:492–496

Boukhvalova M, VanBruggen R, Stewart RC (2002) CheA kinase and chemoreceptor interaction surfaces on CheW. J Biol Chem 277:23596–23603

Bourret RB, Davagnino J, Simon MI (1993) The carboxy-terminal portion of the CheA kinase mediates regulation of autophosphorylation by transducer and CheW. J Bacteriol 175:2097–2101

Braun TF, Al-Mawsawi LQ, Kojima S, Blair DF (2003) Arrangement of core membrane segments in the MotA/MotB proton-channel complex of *Escherichia coli*. Biochemistry 43:35–45

Bren A, Eisenbach M (1998) The N-terminus of the flagellar switch protein, FliM, is the binding domain for the chemotactic response regulator CheY. J Mol Biol 278:507–514

Briegel A, Ding HJ, Li Z, Werner J, Gitai Z, Dias DP, Jensen RB, Jensen GJ (2008) Location and architecture of the *Caulobacter crescentus* chemoreceptor array. Mol Microbiol 69:30–41

Briegel A, Ortega DR, Tocheva EI, Wuichet K, Li Z, Chen S, Muller A, Iancu CV, Murphy GE, Dobro MJ, Zhulin IB, Jensen GJ (2009) Universal architecture of bacterial chemoreceptor arrays. Proc Natl Acad Sci USA 106:17181–17186

Brown DA, Berg HC (1974) Temporal stimulation of chemotaxis in *Escherichia coli*. Proc Natl Acad Sci USA 71:1388–1392

Brown MT, Delalez NJ, Armitage JP (2011) Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor. Curr Opin Microbiol 14:734–740

Campbell AJ, Watts KJ, Johnson MS, Taylor BL (2010) Gain-of-function mutations cluster in distinct regions associated with the signalling pathway in the PAS domain of the aerotaxis receptor. Aer Mol Microiol 77:575–586

Cantwell BJ, Manson MD (2009) Protein domains and residues involved in the CheZ/CheAS interaction. J Bacteriol 191:5838–5841

Chadsey MS, Karlinsey JE, Hughes KT (1998) The flagellar anti-σ factor FlgM actively dissociates *Salmonella typhimurium* σ28 RNA polymerase holoenzyme. Genes Dev 12:3123–3136

Chao X, Muff TJ, Park SY, Zhang S, Pollard AM, Ordal GW, Bilwes AM, Crane BR (2006) A receptor-modifying deamidase in complex with a signaling phosphatase reveals reciprocal regulation. Cell 124:561–571

Charon NW, Goldstein SF (2002) Genetics of motility and chemotaxis of a fascinating group of bacteria: the spirochetes. Annu Rev Genet 36:47–73

Chen S, Beeby M, Murphy GE, Leadbetter JR, Hendrixson DR, Briegel A, Li Z, Shi J, Tocheva EI, Muller A, Dobro MJ, Jensen GJ (2011) Structural diversity of bacterial flagellar motors. EMBO J 30:2972–2981

Chervitz SA, Falke JJ (1995) Lock on/off disulfides identify the transmembrane signaling helix of the aspartate receptor. J Biol Chem 270:24043–24053

Chevance FFV, Hughes KT (2008) Coordinating assembly of a bacterial macromolecular machine. Nat Rev Micro 6:455–465

Cho HS, Lee SY, Yan D, Pan X, Parkinson JS, Kustu S, Wemmer DE, Pelton JG (2000) NMR structure of activated CheY. J Mol Biol 297:543–551

Choudhary M, Fu YX, Mackenzie C, Kaplan S (2004) DNA sequence duplication in *Rhodobacter sphaeroides* 2.4.1: evidence of an ancient partnership between chromosomes I and II. J Bacteriol 186:2019–2027

Chun SY, Parkinson JS (1988) Bacterial motility: membrane topology of the *Escherichia coli* MotB protein. Science 239:276–278

Cohen-Ben-Lulu GN, Francis NR, Shimoni E, Noy D, Davidov Y, Prasad K, Sagi Y, Cecchini G, Johnstone RM, Eisenbach M (2008) The bacterial flagellar switch complex is getting more complex. EMBO J 27:1134–1144

Conley MP, Wolfe AJ, Blair DF, Berg HC (1989) Both chea and chew are required for reconstitution of chemotactic signaling in *Escherichia coli*. J Bacteriol 171:5190–5193

Croxen MA, Sisson G, Melano R, Hoffman PS (2006) The *Helicobacter pylori* chemotaxis receptor TlpB (HP0103) is required for pH taxis and for colonization of the gastric mucosa. J Bacteriol 188:2656–2665

De Mot R, Vanderleyden J (1994) The C-terminal sequence conservation between OmpA-related outer membrane proteins and MotB suggests a common function in both Gram-positive and Gram-negative bacteria, possibly in the interaction of these domains with peptidoglycan. Mol Microiol 12:333–334

Ditty JL, Harwood CS (1999) Conserved cytoplasmic loops are important for both the transport and chemotaxis functions of PcaK, a protein from *Pseudomonas putida* with 12 membrane-spanning regions. J Bacteriol 181:5068–5074

Djordjevic S, Stock AM (1998) Chemotaxis receptor recognition by protein methyltransferase CheR. Nat Struct Biol 5:446–450

Domian IJ, Quon KC, Shapiro L (1997) Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle. Cell 90:415–424

Donato GM, Kawula TH (1998) Enhanced binding of altered H-NS protein to flagellar rotor protein FliG causes increased flagellar rotational speed and hypermotility in *Escherichia coli*. J Biol Chem 273:24030–24036

Draheim RR, Bormans AF, Lai RZ, Manson MD (2006) Tuning a bacterial chemoreceptor with protein−membrane interactions. Biochemistry 45:14655–14664

Dubbs JM, Bird TH, Bauer CE, Tabita FR (2000) Interaction of CbbR and RegA* transcription regulators with the *Rhodobacter sphaeroide*s cbb(I) promoter-operator region. J Biol Chem 275:19224–19230

Duke TAJ, Bray D (1999) Heightened sensitivity of a lattice of membrane receptors. Proc Natl Acad Sci USA 96:10104–10108

Edwards JC, Johnson MS, Taylor BL (2006) Differentiation between electron transport sensing and proton motive force sensing by the Aer and Tsr receptors for aerotaxis. Mol Microbiol 62:823–837

Ely B, Ely TW, Crymes WB, Minnich SA (2000) A family of six flagellin genes contributes to the caulobacter crescentus flagellar filament. J Bacteriol 182:5001–5004

Endres RG, Oleksiuk O, Hansen CH, Meir Y, Sourjik V, Wingreen NS (2008) Variable sizes of *Escherichia coli* chemoreceptor signaling teams. Mol Syst Biol 4:211

Engelmann TW (1883) Bacterium photometricum. Ein beitrag zur vergleichenden physiologie des Licht- und Farensinnes. Pfluegers Arch. Gesamte Physiol. Menschen Tiere 95–124

Eraso JM, Kaplan S (2000) From redox flow to gene regulation: role of the PrrC protein of *Rhodobacter sphaeroides* 2.4.1. Biochemistry 39:2052–2062

Erbse AH, Berlinberg AJ, Cheung CY, Leung WY, Falke JJ (2011) OS-FRET: a new one-sample method for improved FRET measurements. Biochemistry 50:451–457

Fahrner KA, Block SM, Krishnaswamy S, Parkinson JS, Berg HC (1994) A mutant hook-associated protein (Hap3) facilitates torsionally induced transformations of the flagellar filament of *Escherichia coli*. J Mol Biol 238:173–186

Falke JJ, Hazelbauer GL (2001) Transmembrane signaling in bacterial chemoreceptors. Trends Biochem Sci 26:257–265

Fedorov OV, Khechinashvili NN, Kamiya R, Asakura S (1984) Multidomain of flagellin. J Mol Biol 175:83–87

Feng XH, Lilly AA, Hazelbauer GL (1999) Enhanced function conferred on low-abundance chemoreceptor Trg by a methyltransferase-docking site. J Bacteriol 181:3164–3171

Francis NR, Irikura VM, Yamaguchi S, DeRosier DJ, Macnab RM (1992) Localization of the *Salmonella*-Typhimurium flagellar switch protein flig to the cytoplasmic M-ring face of the basal body. Proc Natl Acad Sci USA 89:6304–6308

Francke C, Kormelink TG, Hagemeijer Y, Overmars L, Sluijter V, Moezelaar R, Siezen RJ (2011) Comparative analyses imply that the enigmatic sigma factor 54 is a central controller of the bacterial exterior. Bmc Genomics 12:385

Fredrick KL, Helmann JD (1994) Dual chemotaxis signaling pathways in *Bacillus subtilis*: a sigma D-dependent gene encodes a novel protein with both CheW and CheY homologous domains. J Bacteriol 176:2727–2735

Fu R, Wall JD, Voordouw G (1994) DcrA, a c-type heme-containing methyl-accepting protein from *Desulfovibrio vulgaris* Hildenborough, senses the oxygen concentration or redox potential of the environment. J Bacteriol 176:344–350

Fukuoka H, Wada T, Kojima S, Ishijima A, Homma M (2009) Sodium-dependent dynamic assembly of membrane complexes in sodium-driven flagellar motors. Mol Microiol 71:825–835

Fukuoka H, Inoue Y, Terasawa S, Takahashi H, Ishijima A (2010) Exchange of rotor components in functioning bacterial flagellar motor. Biochem Biophys Res Comm 394:130–135

Galibert F, Finan TM, Long SR, Phler A, Abola P, Ampe DRF, Barloy-Hubler DRF, Barnett MJ, Becker A, Boistard P, Bothe G, Boutry M, Bowser L, Buhrmester J, Cadieu E, Capela D, Chain P, Cowie A, Davis RW, Drano SP, Federspiel NA, Fisher RF, Gloux SP, Godrie TRS, Goffeau A, Golding B, Gouzy J, Gurjal M, Hernandez-Lucas I, Hong A, Huizar L, Hyman RW, Jones T, Kahn D, Kahn ML, Kalman S, Keating DH, Kiss E, Komp C, Lelaure VR, Masuy D, Palm C, Peck MC, Pohl TM, Portetelle D, Purnelle B, Ramsperger U, Surzycki R, Thebault P, Vandenbol M, VorÂlter FJ, Weidner S, Wells DH, Wong K, Yeh KC, Batut J (2001) The composite genome of the legume symbiont *Sinorhizobium meliloti*. Science 293:668–672

Galkin VE, Yu X, Bielnicki J, Heuser J, Ewing CP, Guerry P, Egelman EH (2008) Divergence of quaternary structures among bacterial flagellar filaments. Science 320:382–385

Gardina PJ, Bormans AF, Hawkins MA, Meeker JW, Manson MD (1997) Maltose-binding protein interacts simultaneously and asymmetrically with both subunits of the Tar chemoreceptor. Mol Microiol 23:1181–1191

Gardina PJ, Bormans AF, Manson MD (1998) A mechanism for simultaneous sensing of aspartate and maltose by the Tar chemoreceptor of *Escherichia coli*. Mol Microiol 29:1147–1154

Garrity LF, Schiel SL, Merrill R, Reizer J, Saier MH, Ordal GW (1998) Unique regulation of carbohydrate chemotaxis in *Bacillus subtilis* by the phosphoenolpyruvate-dependent phosphotransferase system and the methyl-accepting chemotaxis protein McpC. J Bacteriol 180:4475–4480

Garza AG, Harrishaller LW, Stoebner RA, Manson MD (1995) Motility protein interactions in the bacterial flagellar motor. Proc Natl Acad Sci USA 92:1970–1974

Gauden DE, Armitage JP (1995) Electron transport-dependent taxis in *Rhodobacter sphaeroides*. J Bacteriol 177:5853–5859

Gegner JA, Graham DR, Roth AF, Dahlquist FW (1992) Assembly of an MCP receptor, CheW, and kinase CheA complex in the bacterial chemotaxis signal transduction pathway. Cell 70:975–982

Geis G, Suerbaum S, Forsthoff B, Leying H, Opferkuch W (1993) Ultrastructure and biochemical-studies of the flagellar sheath of *Helicobacter-pylori*. J Med Micro 38:371–377

Goldman JP, Levin MD, Bray D (2009) Signal amplification in a lattice of coupled protein kinases. Mol Biosyst 5:1853–1859

Goy MF, Springer MS, Adler J (1977) Sensory transduction in *Escherichia coli*: role of a protein methylation reaction in sensory adaptation. Proc Natl Acad Sci USA 74:4964–4968

Goy MF, Springer MS, Adler J (1978) Failure of sensory adaptation in bacterial mutants that are defective in a protein methylation reaction. Cell 15:1231–1240

Greek M, Platzer J, Sourjik V, Schmitt R (1995) Analysis of a chemotaxis operon in *Rhizobium meliloti*. Mol Microbiol 15:989–1000

Greenfield D, McEvoy AL, Shroff H, Crooks GE, Wingreen NS, Betzig E, Liphardt J (2009) Self-organization of the *Escherichia coli* chemotaxis network imaged with super-resolution light microscopy. PLoS Biol 7:e1000137

Grishanin RN, Gauden DE, Armitage JP (1997) Photoresponses in *Rhodobacter sphaeroides*: role of photosynthetic electron transport. J Bacteriol 179:24–30

Griswold IJ, Dahlquist FW (2002) The dynamic behavior of CheW from *Thermotoga maritima* in solution, as determined by nuclear magnetic resonance: implications for potential protein-protein interaction sites. Biophys Chem 101:359–373

Griswold IJ, Zhou HJ, Matison M, Swanson RV, McIntosh LP, Simon MI, Dahlquist FW (2002) The solution structure and interactions of CheW from *Thermotoga maritima*. Nat Struct Biol 9:121–125

Grubl G, Vogler AP, Lengeler JW (1990) Involvement of the histidine protein (Hpr) of the phosphotransferase system in chemotactic signaling of *Escherichia coli* K-12. J Bacteriol 172:5871–5876

Halkides CJ, McEvoy MM, Casper E, Matsumura P, Volz K, Dahlquist FW (2000) The 1.9 Å resolution crystal structure of phosphono-CheY, an analogue of the active form of the response regulator, CheY. Biochemistry 39:5280–5286

Hall BA, Armitage JP, Sansom MSP (2011) Transmembrane helix dynamics of bacterial chemoreceptors supports a piston model of signalling. Plos Comput Biol 7:e1002204

Hao S, Hamel D, Zhou H, Dahlquist FW (2009) Structural basis for the localization of the chemotaxis phosphatase CheZ by $CheA_S$. J Bacteriol 191:5842–5844

Harighi B (2009) Genetic evidence for CheB- and CheR-dependent chemotaxis system in *A. tumefaciens* toward *acetosyringone*. Microbiol Res 164:634–641

Harrison DM, Skidmore J, Armitage JP, Maddock JR (1999) Localization and environmental regulation of MCP-like proteins in *Rhodobacter sphaeroides*. Mol Microbiol 31:885–892

Harshey RM (1994) Bees aren't the only ones – swarming in gram-negative bacteria. Mol Microiol 13:389–394

Harshey RM (2003) Bacterial motility on a surface: many ways to a common goal. Annu Rev Microbiol 57:249–273

Harshey RM, Matsuyama T (1994) Dimorphic transition in *Escherichia coli* and *Salmonella*-Typhimurium– Surface-induced differentiation into hyperflagellate swarmer cells. Proc Natl Acad Sci USA 91:8631–8635

Harwood CS, Nichols NN, Kim MK, Ditty JL, Parales RE (1994) Identification of the Pcarkf gene-cluster from *Pseudomonas-Putida* – involvement in chemotaxis, biodegradation, and transport of 4-Hydroxybenzoate. J Bacteriol 176:6479–6488

Hazelbauer GL, Lai WC (2010) Bacterial chemoreceptors: providing enhanced features to two-component signaling. Curr Opin Microbiol 13:124–132

Hazelbauer GL, Falke JJ, Parkinson JS (2008) Bacterial chemoreceptors: high-performance signaling in networked arrays. Trends Biochem Sci 33:9–19

Hess JF, Oosawa K, Kaplan N, Simon MI (1988) Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. Cell 53:79–87

Holt SC (1978) Anatomy and chemistry of spirochetes. Microbiol Rev 42:114–160

Hou SB, Larsen RW, Boudko D, Riley CW, Karatan E, Zimmer M, Ordal GW, Alam M (2000) Myoglobin-like aerotaxis transducers in Archaea and Bacteria. Nature 403:540–544

Hwang W, Lee KE, Lee JK, Park BC, Kim KS (2008) Genes of *Rhodobacter sphaeroides* 2.4.1 regulated by innate quorum-sensing signal, 7,8-cis-N-(tetradecenoyl) homoserine lactone. J Microbiol Biotech 18:219–227

Iino T (1969) Polarity of flagellar growth in *Salmonella*. J Gen Microbiol 56:227

Ikebe T, Iyoda S, Kutsukake K (1999) Promoter analysis of the class 2 flagellar operons of *Salmonella*. Genes Genet Syst 74:179–183

Imada K, Minamino T, Tahara A, Namba K (2007) Structural similarity between the flagellar type III ATPase FliI and F1-ATPase subunits. Proc Natl Acad Sci USA 104:485–490

Jahreis K, Morrison TB, Garzon A, Parkinson JS (2004) Chemotactic signaling by an *Escherichia coli* CheA mutant that lacks the binding domain for phosphoacceptor partners. J Bacteriol 186:2664–2672

Jeziore-Sassoon Y, Hamblin PA, Bootle WC, Poole PS, Armitage JP (1998) Metabolism is required for chemotaxis to sugars in *Rhodobacter sphaeroides*. Microbiology 144:229–239

Jiang ZY, Gest H, Bauer CE (1997) Chemosensory and photosensory perception in purple photosynthetic bacteria utilize common signal transduction components. J Bacteriol 179:5720–5727

Jiang Z, Rushing BG, Bai Y, Gest H, Bauer CE (1998) Isolation of *Rhodospirillum centenum* mutants defective in phototactic colony motility by transposon mutagenesis. J Bacteriol 180:1248–1255

Jiang ZY, Swem LR, Rushing BG, Devanathan S, Tollin G, Bauer CE (1999) Bacterial photoreceptor with similarity to photoactive yellow protein and plant phytochromes. Science 285:406–409

Jones BV, Young R, Mahenthiralingam E, Stickler DJ (2004) Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. Infect Immun 72:3941–3950

Kaiser GE, Doetsch RN (1975) Enhanced translational motion of Leptospira in viscous environments. Nature 255:656–657

Karatan E, Saulmon MM, Bunn MW, Ordal GW (2001) Phosphorylation of the response regulator CheV is required for adaptation to attractants during *Bacillus subtilis* chemotaxis. J Biol Chem 276:43618–43626

Karlinsey JE, Tanaka S, Bettenworth V, Yamaguchi S, Boos W, Aizawa SI, Hughes KT (2000) Completion of the hook-basal body complex of the *Salmonella* typhimurium flagellum is coupled to FlgM secretion and fliC transcription. Mol Microiol 37:1220–1231

Kawagishi I, Imagawa M, Imae Y, McCarter L, Homma M (1996) The sodium-driven polar flagellar motor of marine vibrio as the mechanosensor that regulates lateral flagellar expression. Mol Microiol 20:693–699

Kearns DB (2010) A field guide to bacterial swarming motility. Nat Rev Microbiol 8:634–644

Kehry MR, Dahlquist FW (1982) The methyl-accepting chemotaxis proteins of *Escherichia coli*. Identification of the multiple methylation sites on methyl-accepting chemotaxis protein I. J Biol Chem 257:10378–10386

Kehry MR, Bond MW, Hunkapiller MW, Dahlquist FW (1983) Enzymatic deamidation of methyl-accepting chemotaxis proteins in *Escherichia coli* catalyzed by the cheB gene product. Proc Natl Acad Sci USA 80:3599–3603

Kentner D, Thiem S, Hildenbeutel M, Sourjik V (2006) Determinants of chemoreceptor cluster formation in *Escherichia coli*. Mol Microbiol 61:407–417

Khan S, Macnab RM (1980) The steady-state counterclockwise-clockwise ratio of bacterial flagellar motors is regulated by protonmotive force. J Mol Biol 138:563–597

Kho DH, Jang JH, Kim HS, Kim KS, Lee JK (2003) Quorum sensing of *Rhodobacter sphaeroides* negatively regulates cellular poly-beta-hydroxybutyrate content under aerobic growth conditions. J Microbiol Biotech 13:477–481

Khursigara CM, Wu X, Subramaniam S (2008) Chemoreceptors in *Caulobacter crescentus*: trimers of receptor dimers in a partially ordered hexagonally packed array. J Bacteriol 190:6805–6810

Kim KK, Yokota H, Kim SH (1999) Four-helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor. Nature 400:787–792

Kirby JR, Saulmon MM, Kristich CJ, Ordal GW (1999) CheY-dependent methylation of the asparagine receptor, McpB, during chemotaxis in *Bacillus subtilis*. J Biol Chem 274:11092–11100

Kirkpatrick CL, Viollier PH (2012) Decoding Caulobacter development. FEMS Microbiol Rev 36:193–205

Kitao A, Yonekura K, Maki-Yonekura S, Samatey FA, Imada K, Namba K, Go N (2006) Switch interactions control energy frustration and multiple flagellar filament structures. Proc Natl Acad Sci USA 103:4894–4899

Ko M, Park C (2000) Two novel flagellar components and H-NS are involved in the motor function of *Escherichia coli*. J Mol Biol 303:371–382

Komeili A (2012) Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria. FEMS Microbiol Rev 36:232–255

Kort R, Crielaard W, Spudich JL, Hellingwerf KJ (2000) Color-sensitive motility and methanol release responses in *Rhodobacter sphaeroides*. J Bacteriol 182:3017–3021

Kreutel S, Kuhn A, Kiefer D (2010) The photosensor protein Ppr of *Rhodocista centenaria* is linked to the chemotaxis signalling pathway. Bmc Microbiol 10:281

Kristich CJ, Ordal GW (2002) *Bacillus subtilis* CheD is a chemoreceptor modification enzyme required for chemotaxis. J Biol Chem 277:25356–25362

Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galan JE, Aizawa S (1998) Supramolecular structure of the *Salmonella* typhimurium type III protein secretion system. Science 280:602–605

Kutsukake K, Ohya Y, Iino T (1990) Transcriptional analysis of the flagellar regulon of *Salmonella typhimurium*. J Bacteriol 172:741–747

Kutsukake K, Nakashima H, Tominaga A, Abo T (2006) Two DNA invertases contribute to flagellar phase variation in *Salmonella enterica* Serovar Typhimurium Strain LT2. J Bacteriol 188:950–957

Laszlo DJ, Taylor BL (1981) Aerotaxis in *Salmonella-Typhimurium* – role of electron-transport. J Bacteriol 145:990–1001

Laszlo DJ, Fandrich BL, Sivaram A, Chance B, Taylor BL (1984) Cytochrome-O as a terminal oxidase and receptor for aerotaxis in *Salmonella-Typhimurium*. J Bacteriol 159:663–667

Leake MC, Chandler JH, Wadhams GH, Bai F, Berry RM, Armitage JP (2006) Stoichiometry and turnover in single, functioning membrane protein complexes. Nature 443:355–358

Lee AG, Fitzsimons JTR (1976) Motility in normal and filamentous forms of *Rhodospirillum-rubrum*. J Gen Microbiol 93:346–354

Lee SY, Cho H, Pelton JG, Yan D, Berry EA, Wemmer DE (2001) Crystal structure of activated CheY. J Biol Chem 276:16425–16431

Lee LK, Ginsburg MA, Crovace C, Donohoe M, Stock D (2010) Structure of the torque ring of the flagellar motor and the molecular basis for rotational switching. Nature 466:996–1000

LeMoual H, Koshland DE (1996) Molecular evolution of the C-terminal cytoplasmic domain of a superfamily of bacterial receptors involved in taxis. J Mol Biol 261:568–585

Li M, Hazelbauer GL (2005) Adaptational assistance in clusters of bacterial chemoreceptors. Mol Microbiol 56:1617–1626

Liarzi O, Barak R, Bronner V, Dines M, Sagi Y, Shainskaya A, Eisenbach M (2010) Acetylation represses the binding of CheY to its target proteins. Mol Microbiol 77:1606

Lipkow K, Andrews SS, Bray D (2004) Simulated diffusion of phosphorylated CheY through the cytoplasm of *E. coli*. J Bacteriol 187:45–53

Liu JD, Parkinson JS (1989) Role of Chew protein in coupling membrane-receptors to the intracellular signaling system of bacterial chemotaxis. Proc Natl Acad Sci USA 86:8703–8707

Liu JD, Parkinson JS (1991) Genetic-evidence for interaction between the Chew and Tsr proteins during chemoreceptor signaling by *Escherichia coli*. J Bacteriol 173:4941–4951

Lloyd SA, Blair DF (1997) Charged residues of the rotor protein FliG essential for torque generation in the flagellar motor of *Escherichia coli*. J Mol Biol 266:733–744

Luke CJ, Kubiak E, Cockayne A, Elliott TS, Penn CW (1990) Identification of flagellar and associated polypeptides of *Helicobacter* (formerly *Campylobacter*) *pylori*. FEMS Microbiol Lett 59:225–230

Lupas A, Stock J (1989) Phosphorylation of an N-terminal regulatory domain activates the CheB methylesterase in bacterial chemotaxis. J Biol Chem 264:17337–17342

Lux R, Jahreis K, Bettenbrock K, Parkinson JS, Lengeler JW (1995) Coupling the phosphotransferase system and the methyl-accepting chemotaxis protein-dependent chemotaxis signaling pathways of *Escherichia coli*. Proc Natl Acad Sci USA 92:11583–11587

Lux R, Munasinghe VRN, Castellano F, Lengeler JW, Corrie JET, Khan S (1999) Elucidation of a PTS-carbohydrate chemotactic signal pathway in *Escherichia coli* using a time-resolved behavioral assay. Mol Biol Cell 10:1133–1146

Mackenzie C (2001) The home stretch, a first analysis of the nearly completed genome of *Rhodobacter sphaeroides* 2.4.1. Photosynth Res 70:19–41

Macnab RM (1976) Examination of bacterial flagellation by dark-field microscopy. J Clin Microbiol 4:258–265

Macnab RM (1977) Bacterial flagella rotating in bundles – study in helical geometry. Proc Natl Acad Sci USA 74:221–225

Macnab RM (1999) The bacterial flagellum: reversible rotary propeller and type III export apparatus. J Bacteriol 181:7149–7153

Macnab RM (2004) Type III flagellar protein export and flagellar assembly. BBA-Mol Cell Res 1694:207–217

Macnab RM, DeRosier DJ (1988) Bacterial flagellar structure and function. Can J Microbiol 34:442–451

Macnab R, Koshland DE (1974) Bacterial motility and chemotaxis - light-induced tumbling response and visualization of individual flagella. J Mol Biol 84:399–406

Maddock JR, Shapiro L (1993) Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science 259:1717–1723

Maeda K, Imae Y (1979) Thermosensory transduction in *Escherichia coli* – inhibition of the thermoresponse by L-serine. Proc Natl Acad Sci USA 76:91–95

Maeda K, Imae Y, Shioi JI, Oosawa F (1976) Effect of temperature on motility and chemotaxis of *Escherichia coli*. J Bacteriol 127:1039–1046

Magariyama Y, Ichiba M, Nakata K, Baba K, Ohtani T, Kudo S, Goto T (2005) Difference in bacterial motion between forward and backward swimming caused by the wall effect. Biophys J 88:3648–3658

Manson MD, Blank V, Brade G, Higgins CF (1986) Peptide chemotaxis in *escherichia coli* involves the Tap signal transducer and the dipeptide permease. Nature 321:253–256

Massazza DA, Izzo SA, Gasperotti AF, Herrera Seitz MK, Studdert CA (2012) Functional and structural effects of seven-residue deletions on the coiled-coil cytoplasmic domain of a chemoreceptor. Mol Microiol 83:224–239

McCarter LL (1994a) MotX, the channel component of the sodium-type flagellar motor. J Bacteriol 176:5988–5998

McCarter LL (1994b) Mot Y, A component of the sodium-type flagellar motor. J Bacteriol 176:4219–4225

McCarter LL (2004) Dual flagellar systems enable motility under different circumstances. J Mol Microbiol Biotechnol 7:18–29

Meister M, Lowe G, Berg HC (1987) The proton flux through the bacterial flagellar motor. Cell 49:643–650

Meier VM, Muschler P, Scharf BE (2007) Functional analysis of nine putative chemoreceptor proteins in *Sinorhizobium meliloti*. J Bacteriol 189:1816–1826

Meier VM, Scharf BE (2009) Cellular localization of predicted transmembrane and soluble chemoreceptors in *Sinorhizobium meliloti*. J Bacteriol 191:5724–5733

Milburn MV, Prive GG, Milligan DL, Scott WG, Yeh J, Jancarik J, Koshland DE, Kim SH (1991) Three-dimensional structures of the ligand-binding domain of the bacterial aspartate receptor with and without a ligand. Science 254:1342–1347

Miller AS, Falke JJ (2004) Side chains at the membrane-water interface modulate the signaling state of a transmembrane receptor. Biochemistry 43:1763–1770

Miller AS, Kohout SC, Gilman KA, Falke JJ (2006) CheA kinase of bacterial chemotaxis: chemical mapping of four essential docking sites. Biochemistry 45:8699–8711

Milligan DL, Koshland DE (1988) Site-directed cross-linking. Establishing the dimeric structure of the aspartate receptor of bacterial chemotaxis J Biol Chem 263:6268–6275

MimoriKiyosue Y, Vonderviszt F, Namba K (1997) Locations of terminal segments of flagellin in the filament structure and their roles in polymerization and polymorphism. J Mol Biol 270:222–237

Minamino T, Macnab RM (2000a) Interactions among components of the *Salmonella* flagellar export apparatus and its substrates. Mol Microiol 35:1052–1064

Minamino T, Macnab RM (2000b) FliH, a soluble component of the type III flagellar export apparatus of *Salmonella*, forms a complex with FliI and inhibits its ATPase activity. Mol Microiol 37:1494–1503

Minamino T, Namba K (2008) Distinct roles of the FliI ATPase and proton motive force in bacterial flagellar protein export. Nature 451:485–488

Minamino T, Chu R, Yamaguchi S, Macnab RM (2000) Role of FliJ in flagellar protein export in *Salmonella*. J Bacteriol 182:4207–4215

Minamino T, Kazetani KI, Tahara A, Suzuki H, Furukawa Y, Kihara M, Namba K (2006) Oligomerization of the bacterial flagellar ATPase FliI is controlled by its extreme N-terminal region. J Mol Biol 360:510–519

Minamino T, Imada K, Kinoshita M, Nakamura S, Morimoto YV, Namba K (2011) Structural insight into the rotational switching mechanism of the bacterial flagellar motor. Plos Biol 9:e1000616

Mitchell JG, Pearson L, Bonazinga A, Dillon S, Khouri H, Paxinos R (1995) Long lag times and high velocities in the motility of natural assemblages of marine-bacteria. Appl Environ Microb 61:877–882

Mizuno T, Imae Y (1984) Conditional inversion of the thermoresponse in *Escherichia coli*. J Bacteriol 159:360–367

Mobley HLT, Belas R (1995) Swarming and pathogenicity of *Proteus-Mirabilis* in the urinary-tract. Trends Microbiol 3:280–284

Morgan DG, Baumgartner JW, Hazelbauer GL (1993) Proteins antigenically related to methyl-accepting chemotaxis proteins of *Escherichia coli* detected in a wide-range of bacterial species. J Bacteriol 175:133–140

Morgan DG, Owen C, Melanson LA, DeRosier DJ (1995) Structure of bacterial flagellar filaments at 11 Ångstrom resolution – packing of the alpha-helices. J Mol Biol 249:88–110

Morrison TB, Parkinson JS (1997) A fragment liberated from the *Escherichia coli* CheA kinase that blocks stimulatory, but not inhibitory, chemoreceptor signaling. J Bacteriol 179:5543–5550

Mowbray SL (1999) Bacterial chemoreceptors: recent progress in structure and function. Mol Cells 9:115–118

Mowbray SL, Koshland DE (1987) Additive and independent responses in a single receptor – aspartate and maltose stimuli on the Tar Protein. Cell 50:171–180

Muff TJ, Ordal GW (2007) The CheC phosphatase regulates chemotactic adaptation through CheD. J Biol Chem 282:34120–34128

Muff TJ, Ordal GW (2008) The diverse CheC-type phosphatases: chemotaxis and beyond. Mol Microbiol 70:1054–1061

Muppirala UK, Desensi S, Lybrand TP, Hazelbauer GL, Li ZJ (2009) Molecular modeling of flexible arm-mediated interactions between bacterial chemoreceptors and their modification enzyme. Protein Sci 18:1702–1714

Muramoto K, Sugiyama S, Cragoe EJ, Imae Y (1994) Successive inactivation of the force-generating units of sodium-driven bacterial flagellar motors by a photoreactive amiloride analog. J Biol Chem 269:3374–3380

Namba K, Vondervisztt F (1997) Molecular architecture of bacterial flagellum. Q Rev Biophys 30:1–65

Namba K, Yamashita I, Vonderviszt F (1989) Structure of the core and central channel of bacterial flagella. Nature 342:648–654

Nara T, Kawagishi I, Nishiyama S, Homma M, Imae Y (1996) Modulation of the thermosensing profile of the *Escherichia coli* aspartate receptor Tar by covalent modification of its methyl-accepting sites. J Biol Chem 271:17932–17936

Ng SYM, Chaban B, Jarrell KF (2006) Archaeal flagella, bacterial flagella and type IV pili: a comparison of genes and posttranslational modifications. J Mol Microbiol Biotech 11:167–191

Nikaido H, Saier MH (1992) Transport proteins in bacteria - common themes in their design. Science 258:936–942

Nishiyama SI, Nara T, Homma M, Imae Y, Kawagishi I (1997) Thermosensing properties of mutant aspartate chemoreceptors with methyl-accepting sites replaced singly or multiply by alanine. J Bacteriol 179:6573–6580

Nishiyama S, Umemura T, Nara T, Homma M, Kawagishi I (1999) Conversion of a bacterial warm sensor to a cold sensor by methylation of a single residue in the presence of an attractant. Mol Microiol 32:357–365

O'Connor C, Matsumura P, Campos A (2009) The CheZ binding interface of CheAS is located in alpha-helix E. J Bacteriol 191:5845–5848

Oh JI, Kaplan S (2000) Redox signaling: globalization of gene expression. EMBO J 19:4237–4247

Ohnishi K, Kutsukake K, Suzuki H, Iino T (1990) Gene flia encodes an alternative sigma factor specific for flagellar operons in *Salmonella-typhimurium*. Mol Gen Genet 221:139–147

O'Toole R, von Hofsten J, Rosqvist R, Olsson PE, Wolf-Watz H (2004) Visualisation of zebrafish infection by GFP-labelled *Vibrio anguillarum*. Microb Pathogenesis 37:41–46

Overmann J (2010) The phototrophic consortium "*Chlorochromatium aggregatum*" – A model for bacterial heterologous multicellularity. Adv Exp Med Biol 675:15–29

Overmann J, Schubert K (2002) Phototrophic consortia: model systems for symbiotic interrelations between prokaryotes. Arch Microbiol 177:201–208

Packer HL, Gauden DE, Armitage JP (1996) The behavioural response of anaerobic *Rhodobacter sphaeroides* to temporal stimuli. Microbiology 142:593–599

Park SY, Chao X, Gonzalez-Bonet G, Beel BD, Bilwes AM, Crane BR (2004) Structure and function of an unusual family of protein phosphatases: the bacterial chemotaxis proteins CheC and CheX. Mol Cell 16:563–574

Park SY, Borbat PP, Gonzalez-Bonet G, Bhatnagar J, Pollard AM, Freed JH, Bilwes AM, Crane BR (2006a) Reconstruction of the chemotaxis receptor-kinase assembly. Nat Struct Mol Biol 13:400–407

Park SY, Lowder B, Bilwes AM, Blair DF, Crane BR (2006b) Structure of FliM provides insight into assembly of the switch complex in the bacterial flagella motor. Proc Natl Acad Sci USA 103:11886–11891

Parkinson JS (1977) Behavioral genetics in bacteria. Annu Rev Genet 11:397–414

Parkinson JS (2010) Signaling mechanisms of HAMP domains in chemoreceptors and sensor kinases. Annu Rev Microbiol 64:101–122

Parkinson JS, Ames P, Studdert CA (2005) Collaborative signaling by bacterial chemoreceptors. Curr Opin Microbiol 8:116–121

Paster E, Ryu WS (2008) The thermal impulse response of *Escherichia coli*. Proc Natl Acad Sci USA 105:5373–5377

Patrick JE, Kearns DB (2012) Swarming motility and the control of master regulators of flagellar biosynthesis. Mol Microiol 83:14–23

Paul K, Gonzalez-Bonet G, Bilwes AM, Crane BR, Blair D (2011) Architecture of the flagellar rotor. EMBO J 30:2962–2971

Paulick A, Koerdt A, Lassak J, Huntley S, Wilms I, Narberhaus F, Thormann KM (2009) Two different stator systems drive a single polar flagellum in *Shewanella oneidensis* MR-1. Mol Microiol 71:836–850

Platzer J, Sterr W, Hausmann M, Schmitt R (1997) Three genes of a motility operon and their role in flagellar rotary speed variation in *Rhizobium meliloti*. J Bacteriol 179:6391–6399

Poggio S, Abreu-Goodger C, Fabela S, Osorio A, Dreyfus G, Vinuesa P, Camarena L (2007) A complete set of flagellar genes acquired by horizontal transfer coexists with the endogenous flagellar system in *Rhodobacter sphaeroides*. J Bacteriol 189:3208–3216

Poole PS, Armitage JP (1989) Role of metabolism in the chemotactic response of *Rhodobacter sphaeroides* to ammonia. J Bacteriol 171:2900–2902

Porter SL, Warren AV, Martin AC, Armitage JP (2002) The third chemotaxis locus of *Rhodobacter sphaeroides* is essential for chemotaxis. Mol Microbiol 46:1081–1094

Porter SL, Wadhams GH, Martin AC, Byles ED, Lancaster DE, Armitage JP (2006) The CheYs of *Rhodobacter sphaeroides*. J Biol Chem 281:32694–32704

Porter SL, Roberts MAJ, Manning CS, Armitage JP (2008) A bifunctional kinase-phosphatase in bacterial chemotaxis. Proc Natl Acad SciUSA 105:18531–18536

Puskas A, Greenberg EP, Kaplan S, Schaeffer AL (1997) A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. J Bacteriol 179:7530–7537

Ragatz L, Jiang ZY, Bauer CE, Gest H (1995) Macroscopic phototactic behavior of the purple photosynthetic bacterium *Rhodospirillum-Centenum*. Arch Microbiol 163:1–6

Rao CV, Glekas GD, Ordal GW (2008) The three adaptation systems of *Bacillus subtilis* chemotaxis. Trends Microbiol 16:480–487

Rebbapragada A (1997) The Aer protein and the serine chemoreceptor Tsr independently sense intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli* behavior. Proc Natl Acad Sci USA 94:10541–10546

Reid SW, Leake MC, Chandler JH, Lo CJ, Armitage JP, Berry RM (2006) The maximum number of torque-generating units in the flagellar motor of *Escherichia coli* is at least 11. Proc Natl Acad Sci USA 103:8066–8071

Riepl H, Maurer T, Kalbitzer HR, Meier VM, Haslbeck M, Schmitt R, Scharf B (2008) Interaction of CheY2 and CheY2-P with the cognate CheA kinase in the chemosensory-signalling chain of *Sinorhizobium meliloti*. Mol Microbiol 69:1373–1384

Ringgaard S, Schirner K, Davis BM, Waldor MK (2011) A family of ParA-like ATPases promotes cell pole maturation by facilitating polar localization of chemotaxis proteins. Gene Dev 25:1544–1555

Romagnoli S, Armitage JP (1999) Roles of chemosensory pathways in transient changes in swimming speed of *Rhodobacter sphaeroides* induced by changes in photosynthetic electron transport. J Bacteriol 181:34–39

Romagnoli S, Hochkoeppler A, Damgaard L, Zannoni D (1997) The effect of respiration on the phototactic behavior of the purple nonsulfur bacterium *Rhodospirillum centenum*. Arch Microb 167:99–105

Romagnoli S, Packer HL, Armitage JP (2002) Tactic responses to oxygen in the phototrophic bacterium *Rhodobacter sphaeroides* WS8N. J Bacteriol 184:5590–5598

Rosario MM, Fredrick KL, Ordal GW, Helmann JD (1994) Chemotaxis in *Bacillus subtilis* requires either of two functionally redundant CheW homologs. J Bacteriol 176:2736–2739

Rosario MM, Kirby JR, Bochar DA, Ordal GW (1995) Chemotactic methylation and behavior in *Bacillus subtilis*: role of two unique proteins, CheC and CheD. Biochemistry 34:3823–3831

Sackett MJ, Armitage JP, Sherwood EE, Pitta TP (1997) Photoresponses of the purple nonsulfur bacteria Rhodospirillum centenum and *Rhodobacter sphaeroides*. J Bacteriol 179:6764–6768

Salman H, Libchaber A (2007) A concentration-dependent switch in the bacterial response to temperature. Nat Cell Biol 9:1098–1100

Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Namba K (2001) Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. Nature 410:331–337

Sanders DA, Mendez B, Koshland DE (1989) Role of the Chew protein in bacterial chemotaxis – overexpression is equivalent to absence. J Bacteriol 171:6271–6278

Sarkar MK, Paul K, Blair DF (2010) Subunit organization and reversal-associated movements in the flagellar switch of *Escherichia coli*. J Biol Chem 285:675–684

Scharf BE, Fahrner KA, Turner L, Berg HC (1998) Control of direction of flagellar rotation in bacterial chemotaxis. Proc Natl Acad Sci USA 95:201–206

Scharf B, Schuster-Wolff-Buhring H, Rachel R, Schmitt R (2001) Mutational analysis of the *Rhizobium lupini* H13-3 and *Sinorhizobium meliloti* flagellin genes: Importance of flagellin a for flagellar filament structure and transcriptional regulation. J Bacteriol 183:5334–5342

Schuster SC, Swanson RV, Alex LA, Bourret RB, Simon MI (1993) Assembly and function of a quaternary signal-transduction complex monitored by surface-plasmon resonance. Nature 365:343–347

Scott WG, Milligan DL, Milburn MV, Prive GG, Yeh J, Koshland DE, Kim SH (1993) Refined structures of the ligand-binding domain of the aspartate receptor from *Salmonella-typhimurium*. J Mol Biol 232:555–573

Segall JE, Block SM, Berg HC (1986) Temporal comparisons in bacterial chemotaxis. Proc Natl Acad Sci USA 83:9486–9493

Seidler RJ, Starr MP (1968) Structure of flagellum of *Bdellovibrio bacteriovorus*. J Bacteriol 95:1952

Seymour JR, Simo R, Ahmed T, Stocker R (2010) Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. Science 329:342–345

Shaw AK, Halpern AL, Beeson K, Tran B, Venter JC, Martiny JBH (2008) It's all relative: ranking the diversity of aquatic bacterial communities. Environ Microbiol 10:2200–2210

Shimizu TS, Le Novere N, Levin MD, Beavil AJ, Sutton BJ, Bray D (2000) Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. Nat Cell Biol 2:792–796

Shimizu TS, Aksenov SV, Bray D (2003) A spatially extended stochastic model of the bacterial chemotaxis signalling pathway. J Mol Biol 329:291–309

Shinoda S, Okamoto K (1977) Formation and function of *Vibrio-parahaemolyticus* lateral flagella. J Bacteriol 129:1266–1271

Silversmith RE (2010) Auxiliary phosphatases in two-component signal transduction. Curr Opin Microbiol 13:177–183

Smith TG, Hoover TR (2009) Deciphering bacterial flagellar gene regulatory networks in the genomic era. In: Allen IL (ed) Advances in applied microbiology. Academic, New York, pp 257–295, Chapter 8

Sourjik V, Schmitt R (1996) Different roles of CheY1 and CheY2 in the chemotaxis of *Rhizobium meliloti*. Mol Microbiol 22:427–436

Sourjik V, Schmitt R (1998) Phosphotransfer between CheA, CheY1, and CheY2 in the chemotaxis signal transduction chain of *Rhizobium meliloti*. Biochemistry 37:2327–2335

Sourjik V, Sterr W, Platzer J, Bos I, Haslbeck M, Schmitt R (1998) Mapping of 41 chemotaxis, flagellar and motility genes to a single region of the *Sinorhizobium meliloti* chromosome. Gene 223:283–290

Sowa Y, Berry RM (2008) Bacterial flagellar motor. Q Rev Biophys 41:103–132

Sowa Y, Rowe AD, Leake MC, Yakushi T, Homma M, Ishijima A, Berry RM (2005) Direct observation of steps in rotation of the bacterial flagellar motor. Nature 437:916–919

Sprenger WW, Hoff WD, Armitage JP, Hellingwerf KJ (1993) The eubacterium *Ectothiorhodospira-halophila* is negatively phototactic, with a wavelength dependence that fits the absorption-spectrum of the photoactive yellow protein. J Bacteriol 175:3096–3104

Springer WR, Koshland DE (1977) Identification of a protein methyltransferase as the cheR gene product in the bacterial sensing system. Proc Natl Acad Sci USA 74:533–537

Starrett DJ, Falke JJ (2005) Adaptation mechanism of the aspartate receptor: electrostatics of the adaptation subdomain play a key role in modulating kinase activity. Biochemistry 44:1550–1560

Stewart RC (1997) Kinetic characterization of phosphotransfer between CheA and CheY in the bacterial chemotaxis signal transduction pathway. Biochemistry 36:2030–2040

Stewart RC, Dahlquist FW (1988) N-terminal half of Cheb is involved in methylesterase response to negative chemotactic stimuli in *Escherichia coli*. J Bacteriol 170:5728–5738

Stewart RC, Roth AF, Dahlquist FW (1990) Mutations that affect control of the methylesterase activity of Cheb, a component of the chemotaxis adaptation system in *Escherichia coli*. J Bacteriol 172:3388–3399

Stewart RC, Jahreis K, Parkinson JS (2000) Rapid phosphotransfer to CheY from a CheA protein lacking the CheY-binding domain. Biochemistry 39:13157–13165

Stock JB, Koshland DE (1978) A protein methylesterase involved in bacterial sensing. Proc Natl Acad SciUSA 75:3659–3663

Stock JB, Surette MG (1996) Chemotaxis. In: Neidhardt FC, Curtiss RI, Ingraham JL, Lin EEC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC, pp 1103–1129

Stock AM, Mottonen JM, Stock JB, Schutt CE (1989) Three-dimensional structure of CheY, the response regulator of bacterial chemotaxis. Nature 337:745–749

Stock AM, Martinezhackert E, Rasmussen BF, West AH, Stock JB, Ringe D, Petsko GA (1993) Structure of the Mg^{2+} bound form of CheY and mechanism of phosphoryl transfer in bacterial chemotaxis. Biochemistry 32:13375–13380

Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. Annu Rev Biochem 69:183–215

Stocker R, Seymour JR, Samadani A, Hunt DE, Polz MF (2008) Rapid chemotactic response enables marine bacteria to exploit ephemeral microscale nutrient patches. Proc Natl Acad Sci USA 105:4209–4214

Streif S, Staudinger WF, Marwan W, Oesterhelt D (2008) Flagellar rotation in the archaeon *Halobacterium salinarum* depends on ATP. J Mol Biol 384:1–8

Surette MG, Levit M, Liu Y, Lukat G, Ninfa EG, Ninfa A, Stock JB (1996) Dimerization is required for the activity of the protein histidine kinase CheA that mediates signal transduction in bacterial chemotaxis. J Biol Chem 271:939–945

Suzuki H, Yonekura K, Murata K, Hirai T, Oosawa K, Namba K (1998) A structural feature in the central channel of the bacterial flagellar FliF ring complex is implicated in type III protein export. J Struct Biol 124:104–114

Swain KE, Gonzalez MA, Falke JJ (2009) Engineered socket study of signaling through a four-helix bundle: evidence for a yin−yang mechanism in the kinase control module of the aspartate receptor. Biochemistry 48:9266–9277

Swaney KF, Huang CH, Devreotes PN (2010) Eukaryotic chemotaxis: a network of signaling pathways controls motility, directional sensing, and polarity. Annu Rev Biophys 39:265–289

Swanson RV, Schuster SC, Simon MI (1993) Expression of CheA fragments which define domains encoding kinase, phosphotransfer, and CheY binding activities. Biochemistry 32:7623–7629

Szurmant H, Bunn MW, Cannistraro VJ, Ordal GW (2003) *Bacillus subtilis* hydrolyzes CheY-P at the location of its action: the flagellar switch. J Biol Chem 278:48611–48616

Szurmant H, Muff TJ, Ordal GW (2004) *Bacillus subtilis* CheC and FliY are members of a novel class of CheY-P-hydrolyzing proteins in the chemotactic signal transduction cascade. J Biol Chem 279:21787–21792

Tang H, Braun TF, Blair DF (1996) Motility protein complexes in the bacterial flagellar motor. J Mol Biol 261:209–221

Taylor BL, Koshland DE (1974) Reversal of flagellar rotation in monotrichous and peritrichous bacteria – Generation of changes in direction. J Bacteriol 119:640–642

Taylor BL, Miller JB, Warrick HM, Koshland DE (1979) Electron-acceptor taxis and blue-light effect on bacterial chemotaxis. J Bacteriol 140:567–573

Taylor BL, Zhulin IB, Johnson MS (1999) Aerotaxis and other energy-sensing behavior in bacteria. Annu Rev Microbiol 53:103–128

Terwilliger TC, Koshland DE (1984) Sites of methyl esterification and deamination on the aspartate receptor involved in chemotaxis. J Biol Chem 259:7719–7725

Thiem S, Sourjik V (2008) Stochastic assembly of chemoreceptor clusters in *Escherichia coli*. Mol Microbiol 68:1228–1236

Thiem S, Kentner D, Sourjik V (2007) Positioning of chemosensory clusters in E. coli and its relation to cell division. EMBO J 26:1615–1623

Thomas NA, Bardy SL, Jarrell KF (2001) The archaeal flagellum: a different kind of prokaryotic motility structure. FEMS Microbiol Rev 25:147–174

Thomashow LS, Rittenberg SC (1985) Isolation and composition of sheathed flagella from *Bdellovibrio-bacteriovorus*-109 J. J Bacteriol 163:1047–1054

Thompson SR, Wadhams GH, Armitage JP (2006) The positioning of cytoplasmic protein clusters in bacteria. Proc Natl Acad Sci USA 103:8209–8214

Tindall MJ, Porter SL, Maini PK, Armitage JP (2010a) Modeling chemotaxis reveals the role of reversed phosphotransfer and a bi-functional kinase-phosphatase. PLoS Comput Biol 6:e1000896

Toews ML, Goy MF, Springer MS, Adler J (1979) Attractants and repellents control demethylation of methylated chemotaxis proteins in *Escherichia coli*. Proc Natl Acad Sci USA 76:5544–5548

Trachtenberg S, DeRosier DJ (1987) 3-Dimensional structure of the frozen hydrated flagellar filament – the left-handed filament of *Salmonella typhimurium*. J Mol Biol 195:581–601

Trachtenberg S, DeRosier DJ (1991) A molecular switch – subunit rotations involved in the right-handed to left-handed transitions of *Salmonella typhimurium* flagellar filaments. J Mol Biol 220:67–77

Trachtenberg S, DeRosier DJ (1992) Conformational switching in the flagellar filament of *Salmonella typhimurium*. J Mol Biol 226:447–454

Trachtenberg S, DeRosier DJ, Macnab RM (1987) Three-dimensional structure of the complex flagellar filament of *Rhizobium lupini* and its relation to the structure of the plain filament. J Mol Biol 195:603–620

Turner L, Ryu WS, Berg HC (2000) Real-time imaging of fluorescent flagellar filaments. J Bacteriol 182:2793–2801

Uedaira H, Morii H, Ishimura M, Taniguchi H, Namba K, Vonderviszt F (1999) Domain organization of flagellar hook protein from *Salmonella* typhimurium. FEBS Lett 445:126–130

Ueno T, Oosawa K, Aizawa SI (1992) M ring, S ring and proximal rod of the flagellar basal body of *Salmonella* typhimurium are composed of subunits of a single protein FliF. J Biol Chem 227:672–677

Umemura T, Matsumoto Y, Ohnishi K, Homma M, Kawagishi I (2002) Sensing of cytoplasmic pH by bacterial chemoreceptors involves the linker region that connects the membrane-spanning and the signal-modulating helices. J Biol Chem 277:1593–1598

Underbakke ES, Zhu YM, Kiessling LL (2011) Protein footprinting in a complex milieu: identifying the interaction surfaces of the chemotaxis adaptor protein CheW. J Mol Biol 409:483–495

Vladimirov N, Sourjik V (2009) Chemotaxis: how bacteria use memory. Biol Chem 390:1097–1104

Volz K, Matsumura P (1991) Crystal structure of *Escherichia coli* CheY refined at 1.7-A resolution. J Biol Chem 266:15511–15519

Vonderviszt F, Aizawa SI, Namba K (1991) Role of the disordered terminal regions of flagellin in filament formation and stability. J Mol Biol 221:1461–1474

Wadhams GH, Martin AC, Armitage JP (2000) Identification and localization of a methyl-accepting chemotaxis protein in *Rhodobacter sphaeroides*. Mol Microbiol 36:1222–1233

Wadhams GH, Warren AV, Martin AC, Armitage JP (2003) Targeting of two signal transduction pathways to different regions of the bacterial cell. Mol Microbiol 50:763–770

Wagenknecht T, DeRosier DJ, Aizawa S, Macnab RM (1982) Flagellar hook structures of *Caulobacter* and *Salmonella* and their relationship to filament structure. J Mol Biol 162:69–87

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Wang H, Matsumura P (1996) Characterization of the CheA(S)/CheZ complex: a specific interaction resulting in enhanced dephosphorylating activity on CheY-phosphate. Mol Microiol 19:695–703

Watts KJ, Ma QH, Johnson MS, Taylor BL (2004) Interactions between the PAS and HAMP domains of the *Escherichia coli* aerotaxis receptor Aer. J Bacteriol 186:7440–7449

Watts KJ, Johnson MS, Taylor BL (2011) Different conformations of the kinase-on and kinase-off signaling states in the Aer HAMP domain. J Bacteriol 193:4095–4103

Welch M, Chinardet N, Mourey L, Birck C, Samara JP (1998) Structure of the CheY-binding domain of histidine kinase CheA in complex with CheY. Nat Struct Biol 5:25–29

Wilkinson DA, Chacko SJ, Vénien-Bryan C, Wadhams GH, Armitage JP (2011) Regulation of flagellum number by FliA and FlgM and role in biofilm formation by *Rhodobacter sphaeroides*. J Bacteriol 193:4010–4014

Wolgemuth CW, Charon NW (2005) The kinky propulsion of spiroplasma. Cell 122:827–828

Wolgemuth CW, Charon NW, Goldstein SF, Goldstein RE (2006) The flagellar cytoskeleton of the spirochetes. J Mol Microbiol Biotechnol 11:221–227

Wuichet K, Alexander RP, Zhulin IB (2007) Comparative genomic and protein sequence analyses of a complex system controlling bacterial chemotaxis. Methods Enzymol 422:1–31

Wuichet K, Zhulin IB (2010) Origins and diversification of a complex signal transduction system in prokaryotes. Sci Signal 3:ra50

Yamaguchi S, Aizawa SI, Kihara M, Isomura M, Jones CJ, Macnab RM (1986) Genetic-evidence for a switching and energy-transducing complex in the flagellar motor of *Salmonella Typhimurium*. J Bacteriol 168:1172–1179

Yamamoto K, Imae Y (1993) Cloning and characterization of the *Salmonella typhimurium*-specific chemoreceptor Tcp for taxis to citrate and from phenol. Proc Natl Acad Sci USA 90:217–221

Yang HJ, Inokuchi H, Adler J (1995) Phototaxis away from blue-light by An *Escherichia coli* mutant accumulating protoporphyrin-Ix. Proc Natl Acad Sci USA 92:7332–7336

Yang HJ, Sasarman A, Inokuchi H, Adler J (1996) Non-iron porphyrins cause tumbling to blue light by an *Escherichia coli* mutant defective in hemG. Proc Natl Acad Sci USA 93:2459–2463

Young GM, Smith MJ, Minnich SA, Miller VL (1999) The *Yersinia enterocolitica* motility master regulatory operon, flhDC, is required for flagellin production, swimming motility, and swarming motility. J Bacteriol 181:2823–2833

Zhang YH, Gardina PJ, Kuebler AS, Kang HS, Christopher JA, Manson MD (1999) Model of maltose-binding protein/chemoreceptor complex supports intrasubunit signaling mechanism. Proc Natl Acad Sci USA 96:939–944

Zhang P, Khursigara CM, Hartnell LM, Subramaniam S (2007) Direct visualization of *Escherichia coli* chemotaxis receptor arrays using cryo-electron microscopy. Proc Natl Acad Sci USA 104:3777–3781

Zhao RB, Amsler CD, Matsumura P, Khan S (1996a) FliG and FliM distribution in the *Salmonella* typhimurium cell and flagellar basal bodies. J Bacteriol 178:258–265

Zhao RH, Pathak N, Jaffe H, Reese TS, Khan S (1996b) FliN is a major structural protein of the C-ring in the *Salmonella* typhimurium flagellar basal body. J Mol Biol 261:195–208

Zhao R, Collins EJ, Bourret RB, Silversmith RE (2002) Structure and catalytic mechanism of the *E. coli* chemotaxis phosphatase CheZ. Nat Struct Biol 9:570–575

Zhou JD, Blair DF (1997) Residues of the cytoplasmic domain of MotA essential for torque generation in the bacterial flagellar motor. J Mol Biol 273:428–439

Zhou HJ, Dahlquist FW (1997) Phosphotransfer site of the chemotaxis-specific protein kinase CheA as revealed by NMR. Biochemistry 36:699–710

Zhou HJ, Lowry DF, Swanson RV, Simon MI, Dahlquist FW (1995a) Nmr-studies of the phosphotransfer domain of the histidine kinase Chea from *Escherichia coli* – assignments, secondary structure, general fold, and backbone dynamics. Biochemistry 34:13858–13870

Zhou J, Fazzio RT, Blair DF (1995b) Membrane topology of the MotA protein of *Escherichia coli*. J Mol Biol 251:237–242

Zhou JD, Lloyd SA, Blair DF (1998a) Electrostatic interactions between rotor and stator in the bacterial flagellar motor. Proc Natl Acad Sci USA 95:6436–6441

Zhou JD, Sharp LL, Tang HL, Lloyd SA, Billings S, Braun TF, Blair DF (1998b) Function of protonatable residues in the flagellar motor of *Escherichia coli*: a critical role for Asp 32 of MotB. J Bacteriol 180:2729–2735

Zhou Q, Ames P, Parkinson JS (2009) Mutational analyses of HAMP helices suggest a dynamic bundle model of input-output signalling in chemoreceptors. Mol Microbiol 73:801–814

Zhou Q, Ames P, Parkinson JS (2011) Biphasic control logic of HAMP domain signalling in the *Escherichia coli* serine chemoreceptor. Mol Microbiol 80:596–611

Zhu XY, Rebello J, Matsumura P, Volz K (1997) Crystal structures of CheY mutants Y106W and T871/Y106W – CheY activation correlates with movement of residue 106. J Biol Chem 272:5000–5006

Zhulin IB (2001) The superfamily of chemotaxis transducers: from physiology to genomics and back. Adv Microb Physiol 45(45):157–198

Zimmer MA, Tiu J, Collins MA, Ordal GW (2000) Selective methylation changes on the *Bacillus subtilis* chemotaxis receptor McpB promote adaptation. J Biol Chem 275:24264–24272

16 Prokaryotic Life Cycles

Lawrence J. Shimkets
Department of Microbiology, The University of Georgia, Athens, GA, USA

Introduction

Prokaryotic development generates a cell with a different purpose from the parent particularly (1) dormancy, (2) nutrient acquisition, or (3) dispersal. Some species produce specialized cells that simultaneously perform two of these functions, such as baeocytes and zoospores, which function in both dormancy and dispersal. Some organisms have complex life cycles that produce several different types of specialized cells, such as *Anabaena* and *Rhodomicrobium*, and are best viewed with a more holistic bend that focuses on the entire life cycle. This chapter compares the structure, function, and, where known, the mechanism of differentiation of the three classes of specialized cell types. This approach has the advantage of illustrating and emphasizing similarities and differences in origin, means, and mechanics.

Resting cells are usually generated in response to nutrient limitation and provide a metabolically quiescent state that permits survival during famine or drought. The *Bacillus* endospore is the most carefully studied resting cell. However, dormant cells are formed by many mechanisms, each with a unique evolutionary origin, attesting at once to the efficacy of this survival approach (Table 16.1). An interesting twist to endospore formation is found in the closely related genera *Metabacterium*, which forms two or more internal endospores, and *Epulopiscium*, which produces two live offspring internally. In *Metabacterium*, binary fission is no longer the sole means of reproduction. Instead, endospore formation has become hardwired into the cell division cycle and may be the primary means of proliferation (Angert and Losick 1998). Endospore formation was modified further in *Epulopiscium* to produce live offspring internally (Angert et al. 1996). With a few modifications to the endospore developmental program, the purpose of the life cycle seems to have shifted from dormancy to reproduction.

Some cyanobacteria produce specialized cells for fixing nitrogen. All living cells require organic nitrogen, but only a few bacteria can reduce atmospheric nitrogen (N_2) to ammonia (NH_3), an ATP-dependent process that utilizes the enzyme nitrogenase. Nitrogenase is oxygen labile. While some bacteria deal with the O_2 sensitivity of nitrogenase by growing in anaerobic or microaerophilic environments, the problem is compounded in organisms where oxygenic photosynthesis is the primary means of growth. There are a wide variety of symbioses involving nitrogen-fixing bacteria, for example, the *Rhizobium*-legume plant symbiosis. In this symbiosis, the legume produces an O_2 binding protein known as leghemoglobin to protect nitrogenase. The most well studied example of a single species producing a specialized cell type for nitrogen fixation is the *Anabaena* heterocyst. Nitrogen-fixing cyanobacteria have devised two strategies to deal with nitrogenase sensitivity to O_2. Some species use a circadian clock to separate the two processes temporally. Other filamentous species, like *Anabaena*, use a heterocyst to fix nitrogen thus separating the two processes spatially. In *Anabaena*, the fixed nitrogen is shuttled from the heterocysts into the photosynthetic cells.

Nongrowing, motile cells are differentiated in some species as a mechanism for dispersal (Table 16.1). The most well studied example is the swarmer cell of *Caulobacter crescentus*, which is produced by a sessile stalked cell as an obligatory part of the cell division cycle. Dispersal mechanisms are found in many different phyla suggesting their utility in long-term survival of the species. Among them are zoospores that are both dormant and flagellated. Zoospores are produced by certain Actinobacteria like *Kineococcus* and the baeocytes of *Pleurocapsa*. The hormogonia of *Nostoc* are multicellular filaments that move by gliding on surfaces and serve as the infective units during symbiosis with certain plants.

Prokaryotic development is strictly asexual unlike eukaryotic development, which generates progeny with genotypes that differ from either parent. Prokaryotic development is often a direct or indirect response to nutrient limitation particularly carbon, nitrogen, and/or phosphorus. In *Caulobacter crescentus*, growth in oligotrophic (nutrient-limited) environments led to a life cycle whereby a sessile parent produces motile progeny that disperse to reduce competition with the parent. Here, the life cycle is hardwired and not directly induced by nutritional cues, but long-term growth in oligotrophic environments may have shaped the developmental program to reduce competition with the stalked parent.

This chapter is meant to serve as an introduction to the fascinating diversity of life cycles among prokaryotes. Precious

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_54,

Table 16.1
Types of prokaryotic differentiated cells

Function	Cell	Representative genus	Phylum
Dormancy	Aerial spore	*Streptomyces*	Actinobacteria
	Akinete	*Anabaena*	Cyanobacteria
	Baeocyte	*Pleurocapsa*	Cyanobacteria
	Cyst	*Azotobacter*	γ-Proteobacteria
	Cyst	*Rhodospirillum*	α-Proteobacteria
	Cyst (myxospore)	*Myxococcus*	δ-Proteobacteria
	Elementary body	*Chlamydia*	Chlamydia
	Endospore	*Bacillus*	Firmicutes
	Endospore	*Metabacterium*	Firmicutes
	Exospore	*Methylosinus*	α-Proteobacteria
	Exospore	*Rhodomicrobium*	α-Proteobacteria
	Zoospore	*Kineococcus*	Actinobacteria
Nutrient acquisition	Heterocyst	*Anabaena*	Cyanobacteria
Dispersal	Attack phase cells	*Bdellovibrio*	δ-Proteobacteria
	Baeocyte	*Pleurocapsa*	Cyanobacteria
	Hormogonium	*Nostoc*	Cyanobacteria
	Swarmer cell	*Caulobacter*	α-Proteobacteria
	Swarmer cell	*Rhodomicrobium*	α-Proteobacteria
	Swarmer cell	*V. parahaemolyticus*	γ-Proteobacteria
	Zoospore	*Kineococcus*	Actinobacteria

little is known about the details of most prokaryotic developmental cycles. Nevertheless insights into certain genetic and biochemical strategies are evident from those few organisms that have been studied.

Differentiation Leading to Dormancy

Resting cells are generated in response to nutritional stress and can maintain dormancy for long periods of time until conditions favor growth. Various types of resting cells include aerial spores, akinetes, baeocytes, cysts, elementary bodies, endospores, exospores, and zoospores, which are formed by different physical processes (◉ *Table 16.1*). Nevertheless, they have some structural and functional similarities: (1) dormancy is achieved by dehydrating the cytoplasm, (2) additional layers around the cell enhance resistance to environmental stresses, and (3) storage molecules are produced to aid in germination.

Aerial Spores. Streptomyces, one of the genera comprising the Actinobacteria, is distinguished by hyphal growth. Following spore germination, vegetative hyphae are produced that form a thick network on the substrate surface. Cell division is rare in the vegetative hyphae and not essential for growth or viability (McCormick 2009). Next, aerial hyphae are extended vertically. The multinucleate aerial hyphae coil and then synchronously septate many times to produce a chain of spores (◉ *Fig. 16.1*). The production of spores on aerial hyphae gives the colonies a powdery appearance. Many genes that mediate development have been identified. The *bld* (bald) mutants cannot form aerial hyphae, and the *whi* (white) mutants cannot form the white aerial spores.

The *bld*-signaling cascade acts as a checkpoint between growth of vegetative hyphae and production of aerial hyphae (Willey et al. 2006). The *bld*-signaling cascade includes 5 extracellular oligopeptides and 'A'-factor, a γ-butyrolactone, whose role appears to be activation of the *ram* (rapid aerial mycelium) and chaplin operons. The *ramCSAB* genes synthesize and secrete surface-active peptide SapB, product of the *ramS* gene. SapB is initially synthesized as a 42 kDa protein then processed and modified to form a double cyclic structure similar to lantibiotics (◉ *Fig. 16.2*). The *bld* genes also stimulate production of the surface-active chaplin proteins that bind to the cell walls of aerial hyphae. The chaplins and SapB initiate escape of the aerial hyphae from the colony surface by reducing surface tension.

The *whi* genes mediate spore differentiation of the multinucleate aerial hyphae. WhiH activates expression of the cell division gene *ftsZ* to initiate septation of the aerial hyphae. This is a critical and specific developmental step since FtsZ is not essential for hyphal growth due to the lack of cross-walls. The spores are metabolically quiescent resting cells and are resistant to desiccation and to slightly elevated temperatures (i.e., 55 °C).

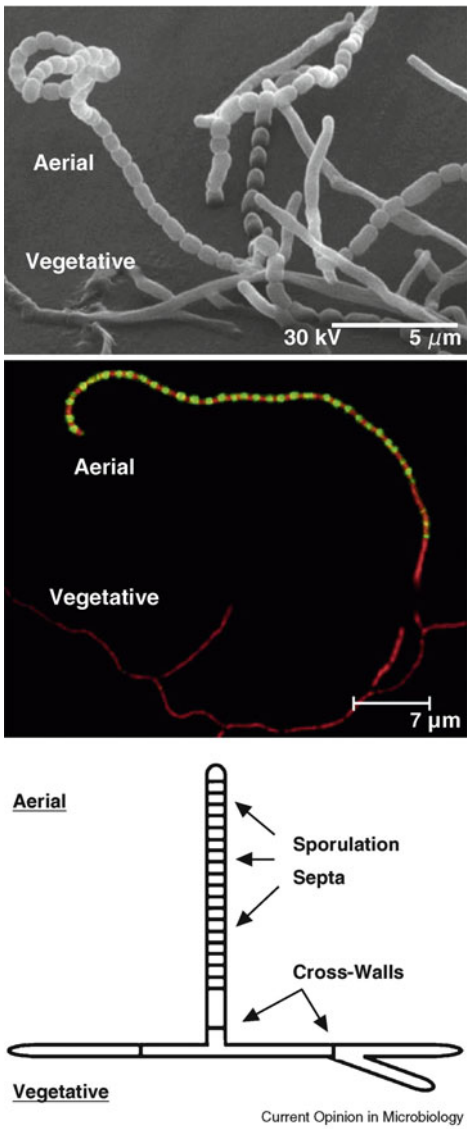


Fig. 16.1
Microscopic images of *S. coelicolor* sporulation and synchronous developmentally regulated cytokinesis. (*Top*) A scanning electron micrograph illustrates the culmination of development, the production of a string of spores from a long apical cell of an aerial hypha (aerial). Branching syncytial vegetative hyphae are located near the bottom (vegetative). (*Middle*) A laser confocal scanning micrograph of a wild-type strain expressing FtsZ–EGFP (*green*) is shown. Nucleic acid is stained with propidium iodide (*red*). Evenly spaced FtsZ rings are spatially restricted to the differentiating aerial hypha (aerial). Branching syncytial vegetative hyphae (vegetative) are devoid of FtsZ rings, and the genomic material is not segregated into well-defined nucleoids. (*Bottom*) Diagram shows the locations of widely spaced vegetative cross-walls and evenly spaced sporulation septa (From McCormick 2009)

Akinetes. Resting cells produced by some cyanobacteria, akinetes are formed from a vegetative cell that has become enlarged by cell wall thickening. *Anabaena* akinetes often form next to heterocysts (Fig. 16.3). *Nostoc punctiforme* and *Anabaena* strain CA convert to akinetes at the end of exponential growth suggesting that the conversion is caused by nutritional shift down. A DNA microarray consisting of 6,893 *N. punctiforme* genes suggested that akinete formation is accompanied by downregulation of genes involved in primary and secondary metabolism (Campbell et al. 2007). Akinetes often possess storage materials such as glycogen, a polymer of glucose, and cyanophycin, a polymer of arginine and aspartate. *Anabaena cylindrica* (PCC 6309) akinetes exhibit resistant to desiccation and subfreezing temperatures but sensitivity to ultraviolet light and temperatures above 60 °C (Olsson-Francis et al. 2009).

When the akinetes germinate, the spore coat usually ruptures, and the germline begins to grow and divide. When this happens to a chain of akinetes, an unusual pattern forms (Fig. 16.4). If germination takes place in the absence of fixed nitrogen, heterocysts appear at approximately every seventh cell. The process of heterocyst production is discussed in section "Nutrient Acquisition."

Cysts. A single vegetative cell rounds up and accumulates a thick coat to produce a cyst. Cyst formation has been observed in many bacterial groups including the myxobacteria, *Azospirillum, Azotobacter*, and the purple photosynthetic bacterium *Rhodospirillum*. Technically speaking, the akinete is a cyst, though for historical reasons, it is given a separate name. Given the phylogenetic diversity of organisms that undergo encystment and the diversity of cyst wall structures, cysts are likely to have several evolutionary origins.

Azotobacter vinelandii is an aerobic soil bacterium that fixes nitrogen while simultaneously protecting nitrogenase from oxygen damage (Fig. 16.5). When carbon is limiting, *Azotobacter* forms cysts that are resistant to desiccation (Setubal et al. 2009). The cells shed their flagella, cease nitrogen fixation, gradually become round, and finally become optically refractile. The cyst is surrounded by a multilayered outer coat called the exine, consisting of lipoprotein, polysaccharide, and phenolic lipids (Fig. 16.6). Alginate, a polysaccharide composed of 1-4-linked beta-D-mannuronic acid and alpha-L-guluronic acid, protects nitrogenase from oxygen damage but is also a major component of the cyst coat. Germination, which occurs when the cysts are placed in the presence of a carbon source such as glucose, immediately induces respiration, macromolecular synthesis, and the conversion of the cyst to the vegetative cell. Alginate can be degraded by alginate lyases, which hydrolyze the polysaccharide using a beta-elimination reaction. While *Azotobacter vinelandii* strains produce several different alginate lyases, mutants lacking alginate lyase AlyA3 germinate poorly compared to wild-type cells suggesting that this enzyme is responsible for degrading the exine (Gimmestad et al. 2009).

In *Rhodospirillum centenum*, development of a cyst involves accumulation of polyhydroxybutyrate (PHB) storage granules, loss of flagella, and change in cell shape. An exine protective outer coat typically surrounds four to eight cells and provides resistance to desiccation. One of the three chemotaxis-like signal transduction cascades initiates cyst development. Another

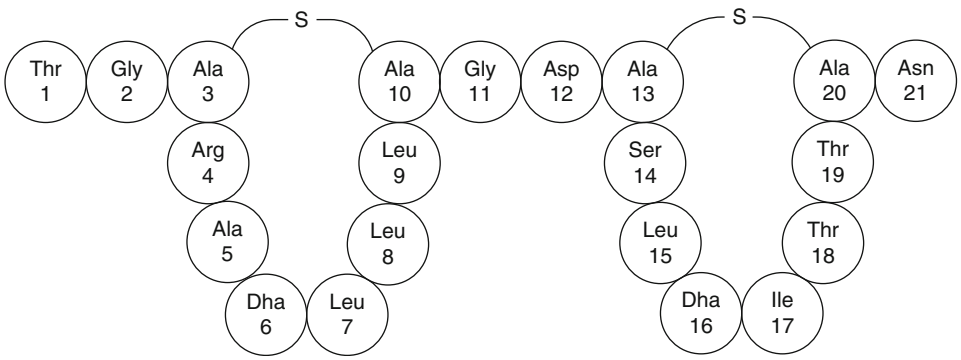


Fig. 16.2
SapB primary sequence. SapB maturation is believed to occur in three stages. First, serine residues are dehydrated giving rise to 2,3 didehydroalanine (Dha) residues. Next, nucleophilic attack by the cysteine sulfhydryl residues at positions 31 and 41 (10 and 20 in the mature protein) on the β-carbon of Dha at positions 24 and 34 (3 and 13 in the mature protein) gives rise to intramolecular cross-links, each of which consists of two alanine residues connected by a thioether linkage. Finally, the leader is removed to generate mature SapB (Adapted from Willey et al. 2006)

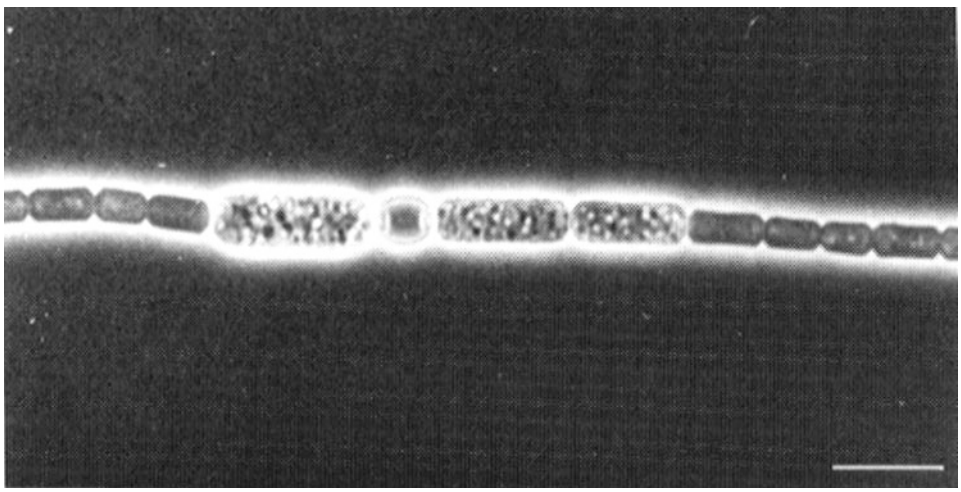


Fig. 16.3
Phase contrast photomicrograph of part of a filament of *Anabaena cylindrica*, showing a heterocyst with akinetes on either side and vegetative cells to the far *left* and *right* (From Nichols and Adams 1982)

controls chemotactic and phototactic responses, and the third controls flagellation (Berleman and Bauer 2005).

The myxobacteria also produce cysts known as myxospores as part of a more complicated developmental cycle involving formation of a multicellular fruiting body. Fruiting body development will be discussed in section ❯ "Fruiting Body Development in *Myxococcus xanthus*."

Elementary Bodies. Chlamydia sp. is an obligate intracellular parasite that causes several prevalent human diseases including trachoma, the leading cause of human blindness, and a sexually transmitted disease. *Chlamydia* alternates between extracellular and intracellular states using the elementary body (EB) and the reticulate body (RB), respectively (Abdelrahman and Belland 2005). The EB is metabolically inactive, about 300 nm in diameter, resistant to desiccation, and highly infectious. The DNA inside EBs is compacted by histone-like proteins HctA and HctB. Although EBs lack peptidoglycan, proteins in the outer membrane are extensively cross-linked by disulfide bonds. EBs also produce a type III secretion system that injects toxins into host cells.

The life cycle of *Chlamydia* begins when an EB binds to an epithelial cell and delivers the actin-recruiting protein TARP into the host with the type III secretion system. Actin filaments in the vicinity of the EB induce phagocytosis that engulfs the EB, internalizing it and surrounding it with a membrane to form a phagosome. The EB begins to enlarge and differentiate into an RB. Differentiation requires disruption of DNA-histone interactions by the small metabolite 2-C-methylerythritol 2, 4-cyclodiphosphate and the *euo* gene product, which may be a histone-specific protease. Chromosome unpacking leads to

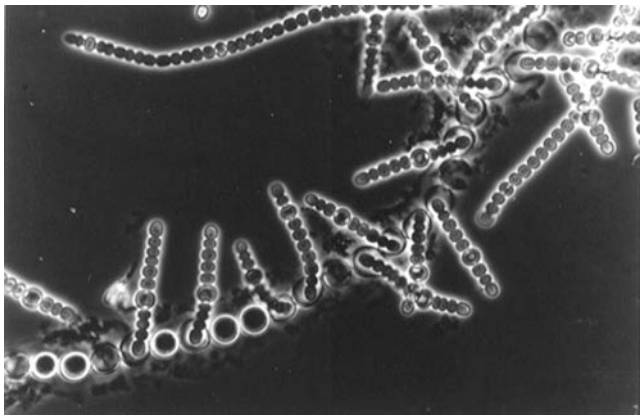


Fig. 16.4
Phase contrast photomicrograph of a filament of germinated akinetes of *Anabaena*. Germlings emerge from the akinete then produce chains of cells, each containing a heterocyst (From Nichols and Adams 1982)

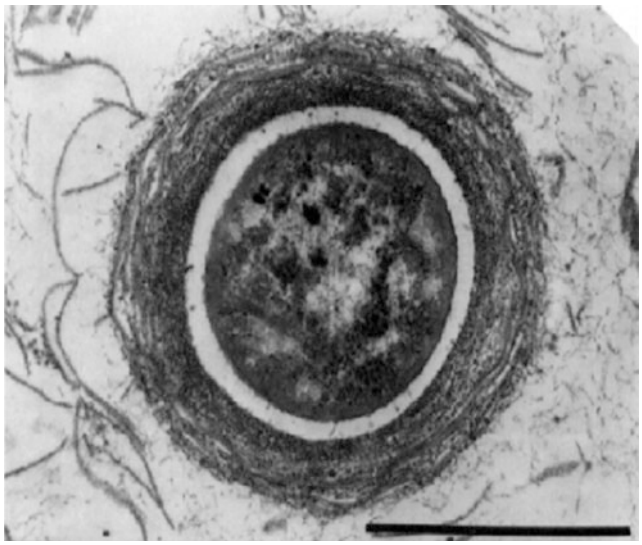


Fig. 16.6
Thin section of a mature cyst of *Azotobacter vinelandii*. Bar = 1 μm (From Hitchins and Sadoff 1970)

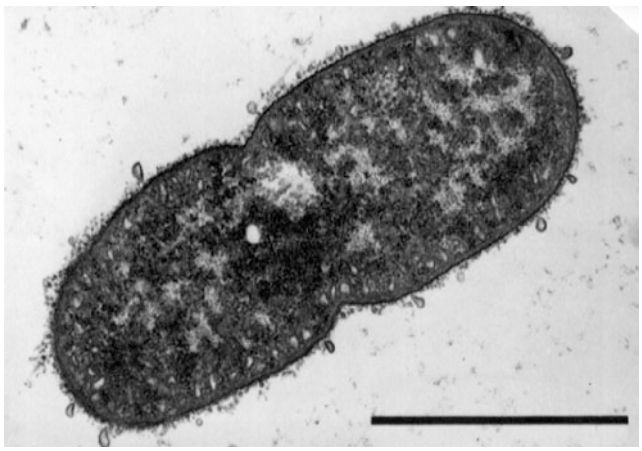


Fig. 16.5
Thin section of a dividing vegetative cell of *Azotobacter vinelandii*. Bar = 1 μm (From Hitchins and Sadoff 1970)

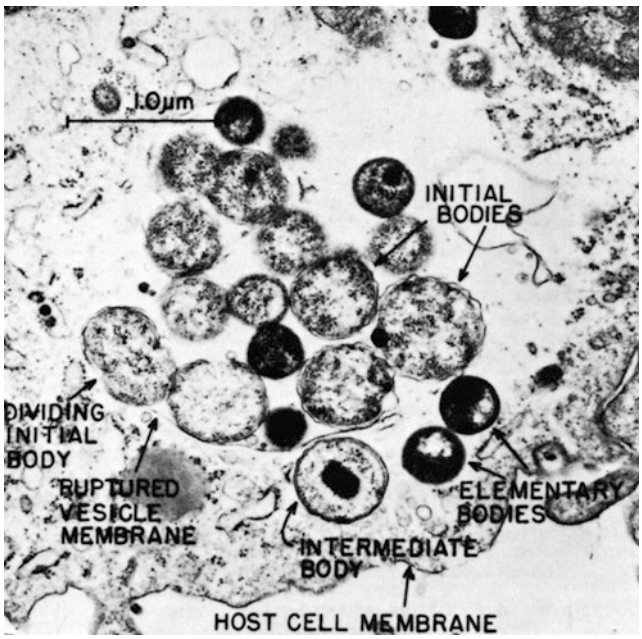


Fig. 16.7
Thin section of an animal cell infected with *Chlamydia psittaci*. The "initial bodies" are the reticulate bodies (From Cutlip 1970)

transcription of genes involved in nutrient assimilation including ABC transporters, oligopeptide permeases, and ATP transporters. Within 10–15 h, the RB synthesizes ribosomes, reorganizes the DNA, acquires a different cell wall, and begins to divide by binary fission. After a period of rapid growth and division, the RBs begin to differentiate into EBs. The genes expressed during this stage include those involved in making the cross-linked outer membrane complex and those involved in condensing the chromosome. The EBs eventually occupy the entire host cytoplasm. EBs are released by two pathways, one involving extrusion through the host membrane and the other involving lysis of the host cell. A phagosome filled with both types of cells and some intermediary forms is shown in *Fig. 16.7*.

Endospores. Endospores are produced inside a mother cell and released when the mother cell lyses. In addition to *Bacillus*, a variety of other Firmicute genera also form endospores. The morphological events leading to endospore formation in other Firmicutes seem relatively similar except for the fact that some genera like *Metabacterium* produce multiple endospores.

Endospores are the most durable cells known. Endospore-forming bacteria have been isolated from bees trapped in

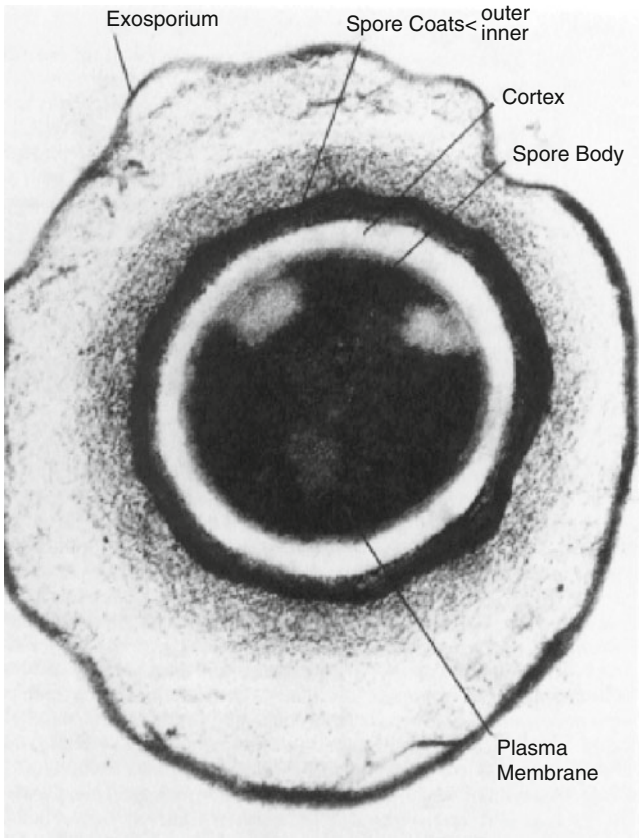


Fig. 16.8
Thin section of a *Bacillus sphaericus* endospore (Courtesy of Dr. S. Holt)

25–40-million-year-old amber (Cano and Borucki 1995) and from 250-million-year-old salt crystals (Vreeland et al. 2000). The durability of the endospore is due in part to the many thick layers surrounding it, which surpass those of any other type of spore. Starting from the outside and proceeding inward, the spore layers include the exosporium, inner and outer coats, outer membrane, cortex, germ cell wall, inner membrane, and central core (*Fig. 16.8*). The unique structure of the endospore is due to the unique manner of its synthesis. The outermost layers including the exosporium, coats, outer membrane, and cortex are contributed by the mother cell using a genetic program different from that of the spore interior. The mechanism of endospore formation is known in extraordinary detail for *Bacillus subtilis* and will be discussed in section "Endospore Formation in *Bacillus subtilis*."

The composition of the endospore layers and the contribution of each layer to the resistance properties of the endospore are known in some detail (*Fig. 16.8*). Beginning with the outermost layer and working inward, the exosporium is a loose-fitting structure made of protein found on endospores of some but not all species. The inner and outer spore coats contain about 70 different proteins in *B. subtilis*. The coat confers resistance to some chemicals and to external lytic enzymes but has little or no role in resistance to heat and radiation. The precise function of the outer membrane is unclear because its removal has no affect on spore resistance. The cortex is composed of peptidoglycan that is similar to vegetative peptidoglycan but with a few spore-specific modifications (Popham 2002). The cortex is essential for dormancy and dehydration. The germ cell wall is also composed of peptidoglycan, probably identical to vegetative cell peptidoglycan, and becomes the cell wall during germination. The inner membrane composition is similar to that of the plasma membrane of growing cells. However, the inner membrane is compressed and the lipid molecules largely immobile until germination. The core contains most spore enzymes and the molecular machinery for germination and growth.

Endospores exhibit extraordinary resistance to heat, desiccation, and radiation (Setlow 2006). Resistance to wet heat is due to dehydration of the spore core. While water comprises 75–80% of the wet weight of the vegetative cell cytoplasm, it comprises only 27–55% of the spore core wet weight. The core also contains dipicolinic acid at concentrations well above solubility, which further contributes to the dehydrated state of the core. Dehydration restricts macromolecular movement and reduces modification of macromolecules by soluble toxic agents. Saturation of the spore DNA with α/β-type small, acid-soluble spore proteins (SASP) alters DNA structure and increases its resistance properties.

The mechanism of chemical resistance varies with the chemical. There are many mechanisms including interaction with one of the spore coat proteins, the impermeability of the spore membrane that restricts access to the spore core, protection of the spore DNA by α/β-type SASP, and DNA repair systems that become active upon germination. The mechanism of spore resistance to γ-radiation is not understood but does not involve α/β-type SASP. Protection to ultraviolet (UV) irradiation is due, in part, to binding of α/β-type SASP to the DNA, the presence of dipicolinic acid, and the use of DNA repair pathways during germination.

Very little is known about germination with any spore type; perhaps the most is known about endospore germination (Moir 2006). The endospore is an exquisite biosensor that germinates in response to specific germinants. The types of germinants vary with the species and include specific amino acids, sugars, or nucleosides. Mutants with defects in germination have been examined genetically and biochemically. A composite of results from several different species have enabled construction of a rough draft of the germination process.

The first step in germination involves passage of the germinant through the outer coat and cortex before coming in contact with the germinant receptors. The *gerP* mutants seem to have a defect in germinant passage through the outer layers and germinate poorly unless the coat is first removed (Moir 2006). The germination receptors appear to be contained within the *gerA* and *gerB* operons which are widespread among *Bacillus* species. Though the Ger proteins are somewhat variable between species, probably due to differences in germinants, they tend to be integral membrane proteins and lipoproteins that reside in the inner membrane. Point mutations in these proteins result in strains that require higher concentrations of germinant for

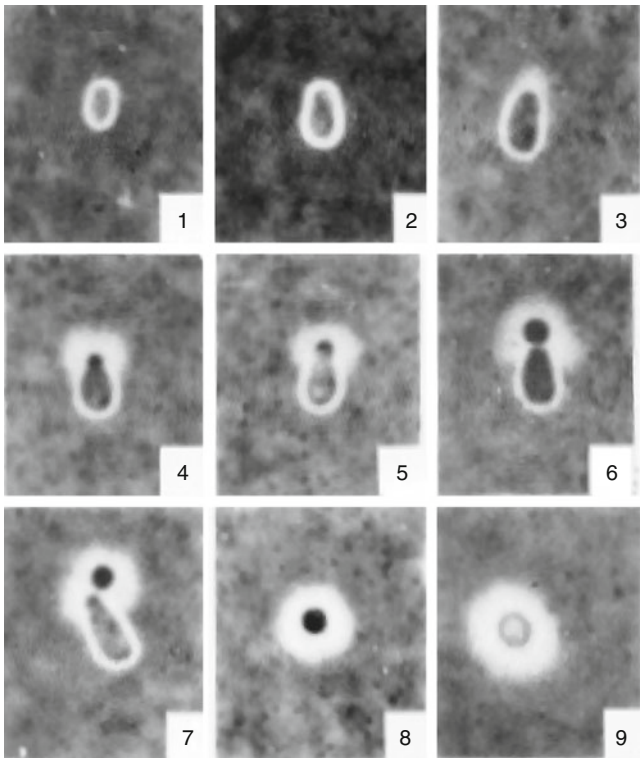


Fig. 16.9
Phase contrast micrographs of exospore formation by *M. trichosporium*. The capsulated, rod-shaped, vegetative cell becomes pear-shaped, and the tapered end buds off the cell that is eventually released as the exospore (From Whittenbury et al. 1970)

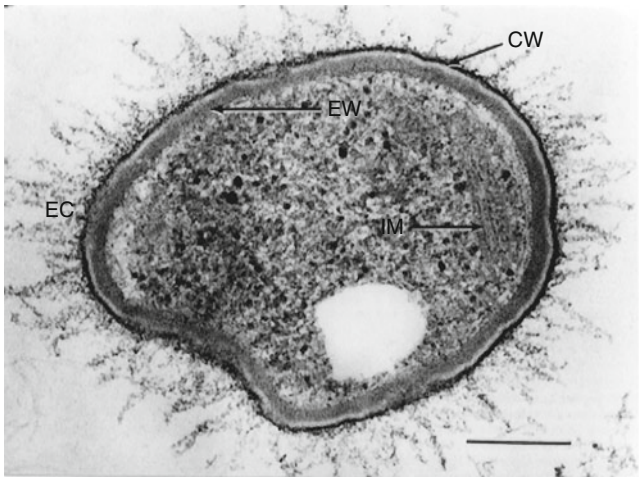


Fig. 16.10
Thin section of *Methylosinus trichosporium* exospore. Bar = 0.2 μm. *CW* cell wall, *EC* exospore capsule, *EW* exospore wall, and *IM* intracytoplasmic membranes (From Reed et al. 1980)

germination. None of these proteins resemble transport proteins, and there appears to be little or no transport of the germinant. Rather, these proteins appear to be receptors that work in a manner that is, as yet, unknown to initiate germination.

Many types of ion fluxes are observed early in germination. H^+, K^+, Na^+, Ca^{+2}, and dipicolinic acid are released from the spore core. The release of Ca-dipicolinic acid is significant since it comprises 10% of the spore dry weight. The SpoVA proteins, implicated in dipicolinic acid uptake during spore formation, may also mediate dipicolinic acid release during germination. The Na^+/H^+K^+ antiporter protein GerN seems to play a role in inosine-stimulated germination in *B. cereus*. It is possible that this germinant receptor moves ions as part of the mechanism initiating germination.

One of the major steps in germination is degradation of the spore cortex with lytic enzymes CwlJ and SleB which are embedded in the outer layers of the spore. SleB is a muramidase and is somehow activated during germination.

Exospores. Exospores are produced by a process resembling budding in several α-proteobacteria, including the methylotroph *Methylosinus trichosporium* and the phototroph *Rhodomicrobium vannielii*. When *M. trichosporium* cells reach stationary phase, some of the cells elongate, become tapered, and bud off rounded bodies that gradually become optically refractile (*Fig. 16.9*). Exospores are resistant to desiccation and to elevated temperatures of at least 78 °C but, in general, are not as durable as endospores. Electron micrographs of thin sections through exospores demonstrate a much simpler and thinner spore coat than the endospore (*Fig. 16.10*). Germination occurs slowly when the cells are placed under conditions conducive to growth and resembles budding (*Fig. 16.11*).

Rhodomicrobium vannielii is a phototrophic, budding bacterium that produces both exospores and motile swarmer cells. A batch culture of *R. vannielii* may simultaneously contain flagellated rods, ovoid cells linked together by branched, mycelial connections, and angular exospores. Swarmer cells can be separated from the budding mycelial forms (*Fig. 16.12*) by passing the culture through glass wool. Thus, homogeneous populations of each cell type can be obtained and the sequence of developmental events connecting them determined (Whittenbury and Dow 1977). *Figure 16.13* illustrates spore formation and *Fig. 16.14* diagrams events following germination. The swarmer cells will be discussed in section "Dispersal Strategies."

Endospore Formation in *Bacillus subtilis*

The events leading to endospore formation are customarily divided into seven stages diagrammatically represented in *Fig. 16.15*. The definition of these stages is based on morphological changes discerned through transmission electron microscopy and mutants that are blocked in a particular stage. The developmental program is driven in large part by stage and compartment-specific sigma factors, also shown in *Fig. 16.15*. A recent review describing the transcription network in detail may be found in de Hoon et al. and is summarized in *Fig. 16.16* (de Hoon et al. 2010).

Endospore formation begins only after a round of DNA replication has been completed to ensure that two chromosomes

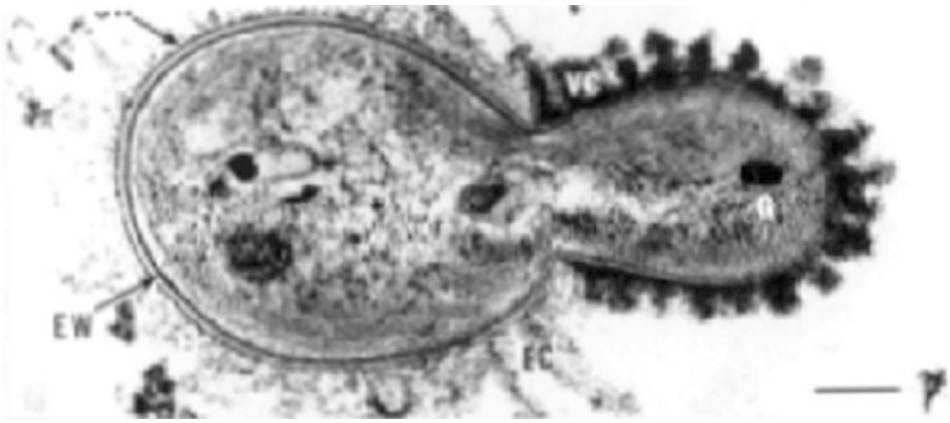


Fig. 16.11
Thin section of a germinating exospore of *Methylosinus trichosporium*. Bar = 0.2 μm (From Reed et al. 1980)

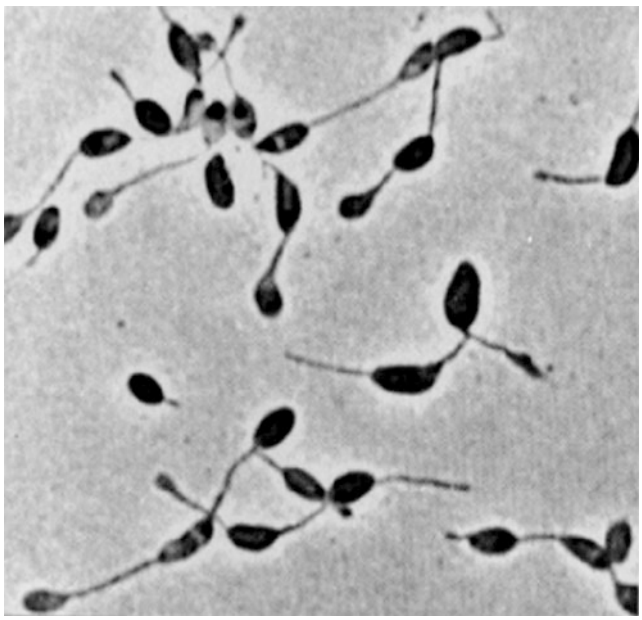


Fig. 16.12
Electron micrograph of growing *Rhodomicrobium* cells. Ovoid cells are linked together by mycelial connections (Courtesy of Dr. P. Hirsch.)

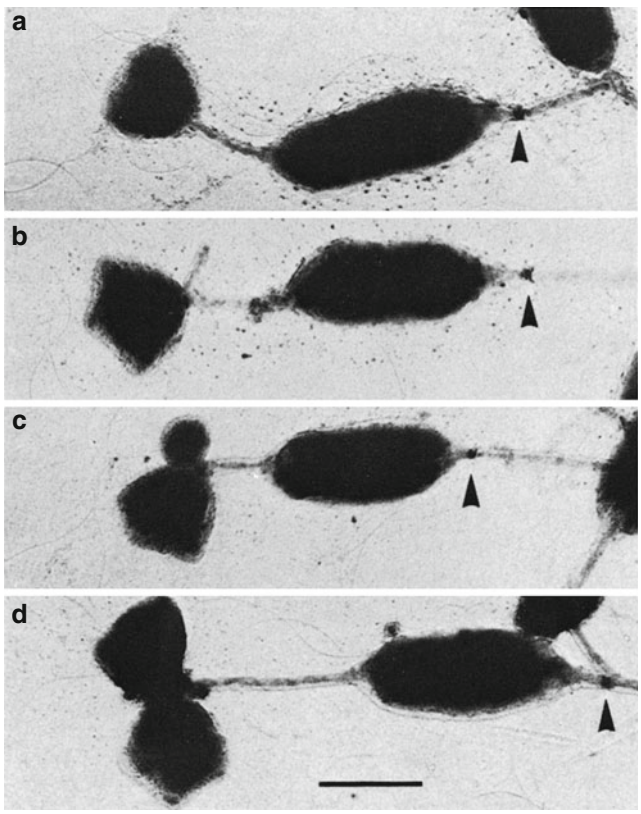


Fig. 16.13
Electron micrographs of *Rhodomicrobium* cells forming exospores. (a) First exospore formed. (b) Filament for second exospore is extended. (c) Beginning of second exospore. (d) Completion of second exospore. *Arrowheads* indicate filament plug separating the mother cell from the rest of the vegetative cells. Bar = 1 μm (From Whittenbury and Dow 1977)

are available in the predivisional cell. The chromosomes are anchored with their origin of replication at a pole (*Fig. 16.15*, red lines). Endospore formation commences with asymmetric cell division. The smaller compartment, the forespore, eventually becomes the endospore. The larger compartment becomes the mother cell. Shortly after asymmetric cell division, different programs of gene expression are established in each compartment and coordinated through intercompartmental signaling to maintain spatial and temporal checkpoints for endospore formation. The forespore is engulfed by the mother cell by a process analogous to phagocytosis resulting in the deposition of another membrane around the forespore. The cortex is synthesized between the two forespore membranes. At least 70 different coat proteins are synthesized by the mother cell and deposited in layers on top of the cortex. Finally, the mother cell lyses to release the dormant endospore.

Endospore formation is regulated by a cascade of compartment-specific sigma factors. The master regulator of

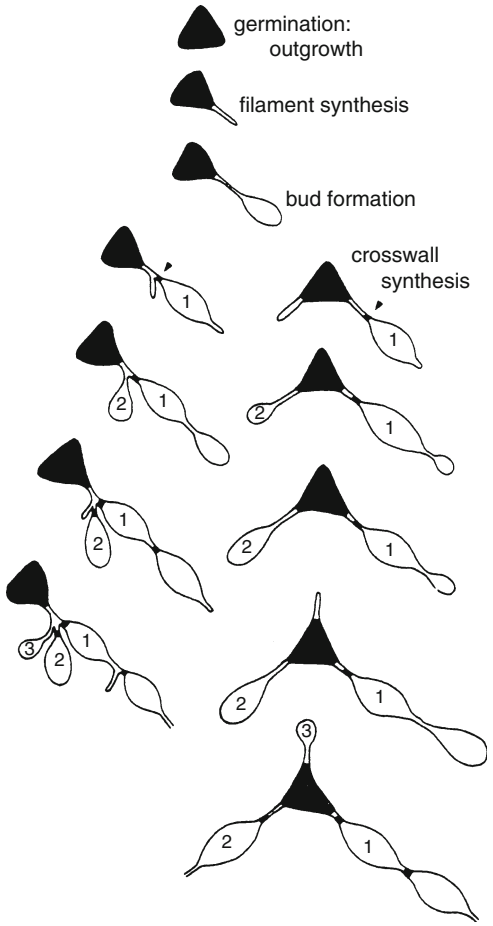


Fig. 16.14
Diagrammatic representation of exospore germination in *Rhodomicrobium*. The *left* and *right* sides show two different exospores (From Whittenbury and Dow 1977)

sporulation, SpoOA∼P, is not a sigma factor but a response regulator whose concentration and phosphorylation state determine the initiation of sporulation. Phosphorylation of SpoOA alters the transcription of more than 500 genes, about 120 directly. SpoOA∼P is an indirect activator of *sigH* to produce σ^H, the first sigma factor in this cascade. σ^H directs transcription of *sigF* to produce the major sigma factor in the forespore following asymmetric cell division, σ^F. σ^F directs transcription of about 50 genes including σ^G. σ^G, the final forespore-specific transcription factor, regulates about 100 genes. In the mother cell, σ^E is produced by a σ^A-specific promoter that is activated by SpoOA∼P. σ^E directs transcription of about 270 genes. σ^E directs expression of σ^K, the last sigma factor in the mother cell.

The forespore and mother cell lines of expression are connected by intercompartmental signaling to ensure coordinated regulation. These points of coordination are known as developmental checkpoints. Three such checkpoints have been identified. The first checkpoint occurs in the predivisional cell which accumulates both the forespore sigma factor σ^F and the mother cell sigma factor σ^E, as both are induced by SpoOA∼P (◉ *Fig. 16.16*). However, these sigma factors are held in an inactive state until asymmetric division is completed. σ^F is sequestered by two molecules of the anti-sigma factor SpoIIAB, while σ^E is produced with a 27-amino acid leader sequence that renders it inactive (pro-σ^E). The anti-anti-sigma factor SpoIIAA binds to the SpoIIAB$_2$-σ^F complex to stimulate σ^F release. SpoIIAB is a kinase that phosphorylates SpoIIAA. To effect σ^F release, SpoIIAA needs to be in an unphosphorylated state. After completion of asymmetric cell division, the septum-associated phosphatase SpoIIE dephosphorylates SpoIIAA, facilitating release of σ^F and initiating the forespore-specific genetic program. In the other compartment, the processing of pro-σ^E is mediated by the membrane-associated protease SpoIIGA. SpoIIGA is stimulated by contact with SpoIIR, produced by the forespore and one of the first proteins expressed in response to σ^F activation. Timing is critical. A delay in SpoIIR production is catastrophic and results in forespores at both poles and a mother cell devoid of DNA.

The second checkpoint involves the activation of σ^G in the forespore by a mechanism that remains unknown. SpoIIIAH in the mother cell interacts with SpoIIQ in the forespore to form a channel. One thought is the channel imports a regulatory factor that activates σ^G. Another idea is that the channel functions as a feeding tube to shuttle small metabolites into the forespore.

The third checkpoint involves the activation of the mother cell sigma factor pro-σ^K by the SpoIVFB protease. Proteolysis of pro-σ^K is stimulated by SpoIVB, a forespore-specific protein produced by σ^G.

Endospore formation involves five feed-forward motifs, each consisting of a sigma factor and a downstream transcription factor: σ^F, RsfA; σ^G, SpoVT; σ^E, GerR; σ^E, SpoIIID; and σ^K, GerE (◉ *Fig. 16.16*). In each of these motifs, a sigma factor induces production of a transcription factor and, along with the transcription factor, jointly regulates expression of downstream sporulation genes. The regulatory modules of each sigma factor are conserved among 14 different genera of endospore-forming bacteria (de Hoon et al. 2010). The feed-forward motifs are less conserved than the sigma factors but more conserved than the downstream sporulation genes they regulate. Overall, the level of conservation is remarkable, given more than a billion years of evolution. These results stand in marked contrast to the developmental cycle of the myxobacteria which shows little conservation over a comparable period of evolution (see below).

Fruiting Body Development in *Myxococcus xanthus*

Myxobacteria are δ-proteobacteria that move on surfaces without the use of flagella. During the vegetative phase of their life cycle, they grow as carnivores or scavengers swarming across surfaces in search of food (◉ *Fig. 16.17*). When faced with starvation, they use their motility to form large, spore-filled fruiting bodies. The spores germinate in the presence of nutrients to resume the life cycle. Multicellularity maximizes the ability of a myxobacterial community to prey on other bacteria

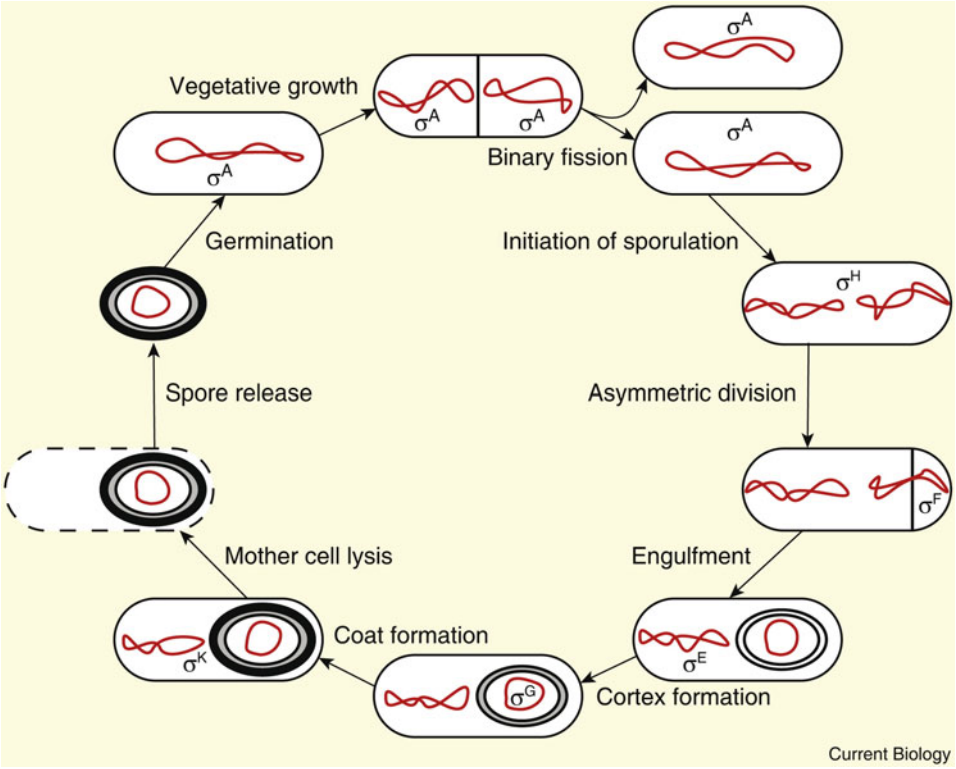


Fig. 16.15

Morphological stages of the *B. subtilis* life cycle. The temporal and compartment-specific activity of each sporulation sigma (σ) factor is indicated. During vegetative growth, cells divide by binary fission to generate two identical daughter cells. Sporulation is initiated in response to starvation. In the predivisional sporulating cell, the chromosomes (*red*) are oriented with their origin-proximal region anchored at the cell poles. During asymmetric division, two membrane-bounded compartments are generated: a small forespore and a large mother cell. After asymmetric division, the remainder of the forespore chromosome (i.e., the origin-distal region) is pulled into the forespore by translocation. Engulfment of the forespore by the mother cell results in the release of the forespore as a free protoplast in the mother cell. The cortex (composed of modified peptidoglycan, *gray*) is synthesized between the two membranes surrounding the forespore. The coat (*black*) is a complex structure made of at least 70 distinct proteins that assemble around the forespore surface. Following mother cell lysis, the mature spore is released into the environment. *B. subtilis* cells can remain in a dormant spore state for an extended period of time, but spores will germinate in response to the presence of particular small molecules that act as germinants and resume vegetative growth (From de Hoon et al. 2010)

and hydrolyze macromolecular substrates such as cell walls, proteins, lipids, polysaccharides, and nucleic acids. Fruiting body formation ensures that a population of cells optimized for feeding will emerge following germination. A recent book edited by D. C. Whitworth (2008) describes many aspects of the life cycle of this fascinating group of bacteria.

Fruiting bodies are 10–1,000 μm in size (*Fig. 16.18*). The fruiting bodies generated by *Myxococcus* species are spherical. The *Chondromyces* and *Stigmatella* species form numerous sporangia-containing spores. The sporangia are held by stalks that are branched in certain *Chondromyces* species or consist of tubules in *Stigmatella aurantiaca*. The fruiting bodies contain thousands of myxospores that are metabolically quiescent and resistant to heat and UV radiation. *M. xanthus* myxospores are protected by a thick electron-dense coat composed of carbohydrates and proteins.

Fruiting body development of *M. xanthus* is induced by amino acid limitation using the stringent response (*Fig. 16.19*). Inhibition of protein synthesis stimulates pppGpp and ppGpp synthesis by RelA. ppGpp activates genes involved in the production of the extracellular A-signal, consisting of a mixture of amino acids (Trp, Pro, Phe, Tyr, Leu, and Ile). A-signal is sensed by histidine protein kinase SasS, which activates the response regulator SasR. SasR along with σ^{54} activates a set of developmental genes. During the period of A-signaling between 0 h and 8 h, no specific changes in cell morphology or multicellular arrangement can be discerned (*Fig. 16.19*). The second phase is accompanied by accumulation of intracellular lipid droplets and a sudden burst of motility. Swarms of cells come together and form numerous small aggregates three or four layers thicker than the cell mat. These move across the surface of the biofilm sometimes disappearing into

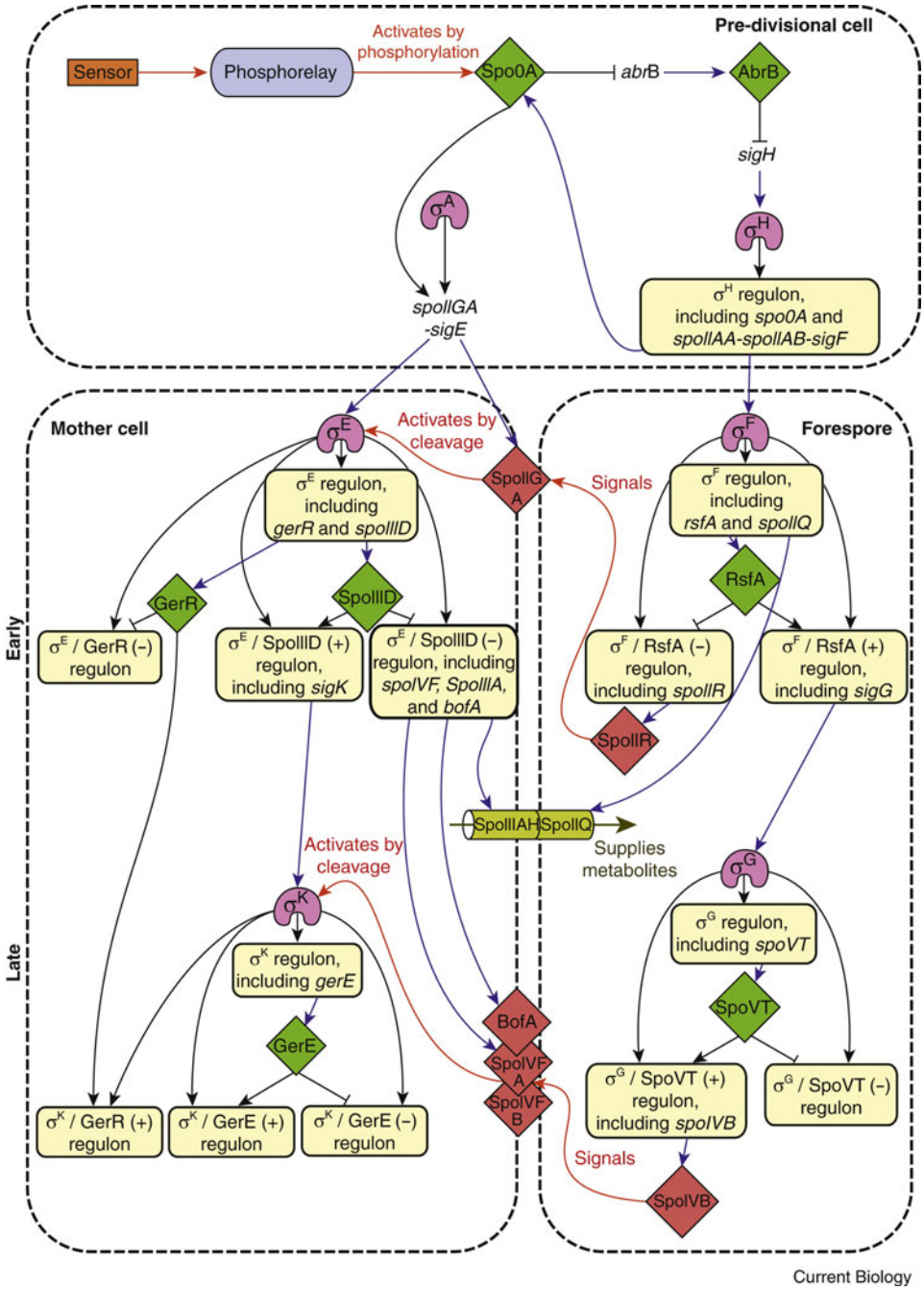


Fig. 16.16

Modular architecture of the sporulation regulatory network in *B. subtilis*. The temporal progression of sporulation is from top to bottom. Each cellular compartment (predivisional cell, forespore, and mother cell) is surrounded by *dotted lines*. Sigma factors are shown in *pink*, transcription factors in *green*, and regulons in *yellow*. Signaling proteins are shown in *red* and the feeding tube in *olive*. Transcriptional regulation is indicated by *black arrows*, gene expression (protein synthesis) by *blue arrows*, and signaling pathways by *red arrows*. Coherent feed-forward loops are indicated by a *plus sign* and incoherent feed-forward loops by a *minus sign* (From de Hoon et al. 2010)

the cell mat and other times fusing with neighboring aggregates. With time, most of the small aggregates disappear leaving only large aggregates. The formation and stability of the large aggregates require the C- and E-extracellular signals along with the Dif chemosensory system.

The large aggregates grow taller by adding tiers consisting of a single layer of cells to the uppermost layer. The cells emerge through a hole on the top tier, and the layer expands to fill the entire surface. This structure eventually becomes encapsulated with polysaccharide. Within the

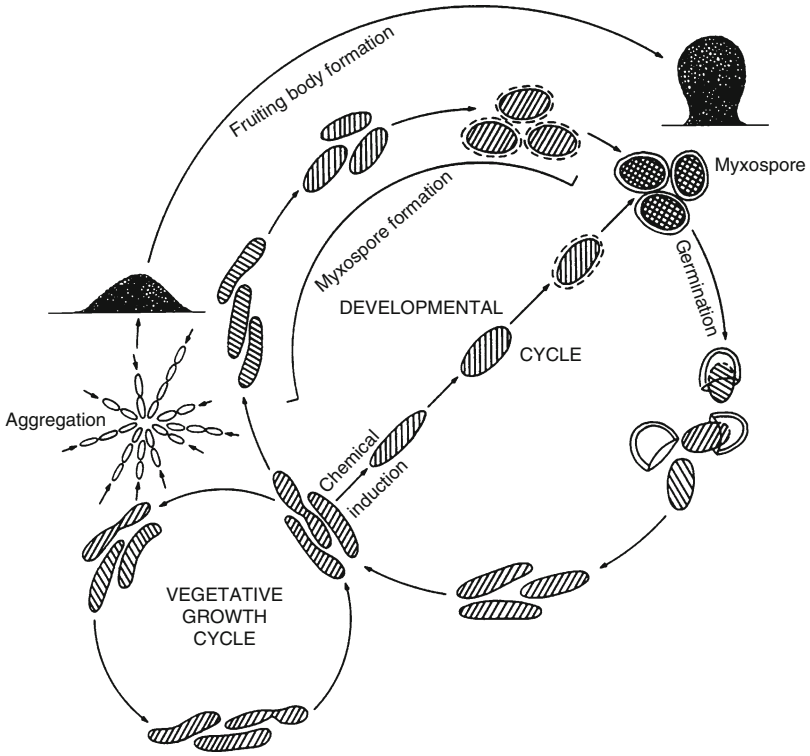


Fig. 16.17
Diagram of the life cycle of *Myxococcus xanthus*. The fruiting body is not drawn to scale, but it is a few hundredths of a mm in diameter, in contrast to the vegetative cells, which are about 5–7 μM (From Dworkin 1985)

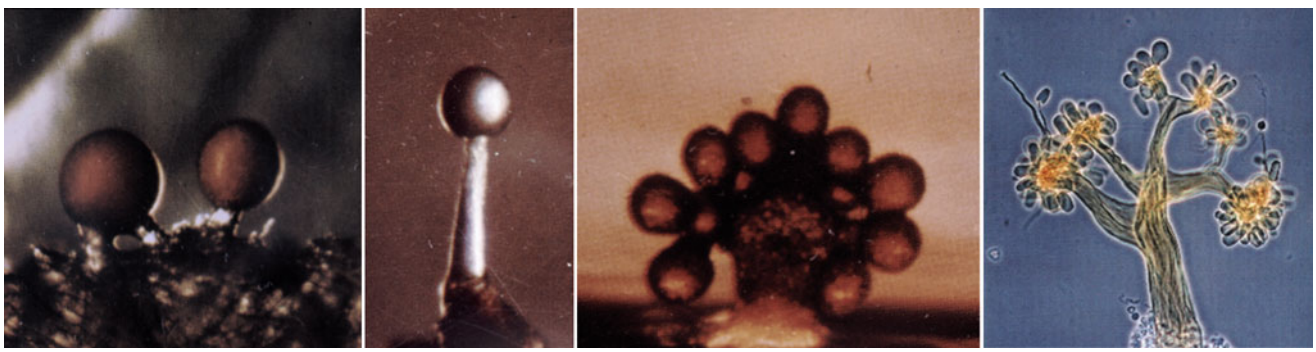


Fig. 16.18
Fruiting bodies. *Myxococcus fulvus*, *M. stipitatis*, *Stigmatella aurantiaca*, *Chondromyces crocatus* (Individual pictures courtesy of Hans Reichenbach)

aggregates, the majority of the vegetative cells undergo programmed cell death. The remaining cells differentiate into myxospores.

The C-signal, E-signal, and Dif chemosensory system govern several stages of myxobacterial development. The E-signal is a lipid containing a branched chain fatty acid that plays a role in fruiting body morphogenesis, programmed cell death, and sporulation. The C-signal is required for the formation of fruiting bodies as well as spores. C-signal transmission leads to transcription of several developmental genes through the phosphorylation of the response regulator FruA. Although FruA is synthesized soon after starvation is sensed, FruA is activated in response to the C-signal. In addition to its role as a transcriptional activator, FruA activates FrzCD, a methyl-accepting chemotaxis protein (MCP). FrzCD relays the signal to FrzE, a response regulator, which controls cell reversals. FrzE∼P also inhibits FrzCD methylation by a negative feedback loop. The Frz-phosphorylated intermediates oscillate forming the "frizillator." FruA∼P levels, initially low because of little C-signaling, set a rhythm to the reversal of gliding direction every eight minutes. When cells collide, the frizillator

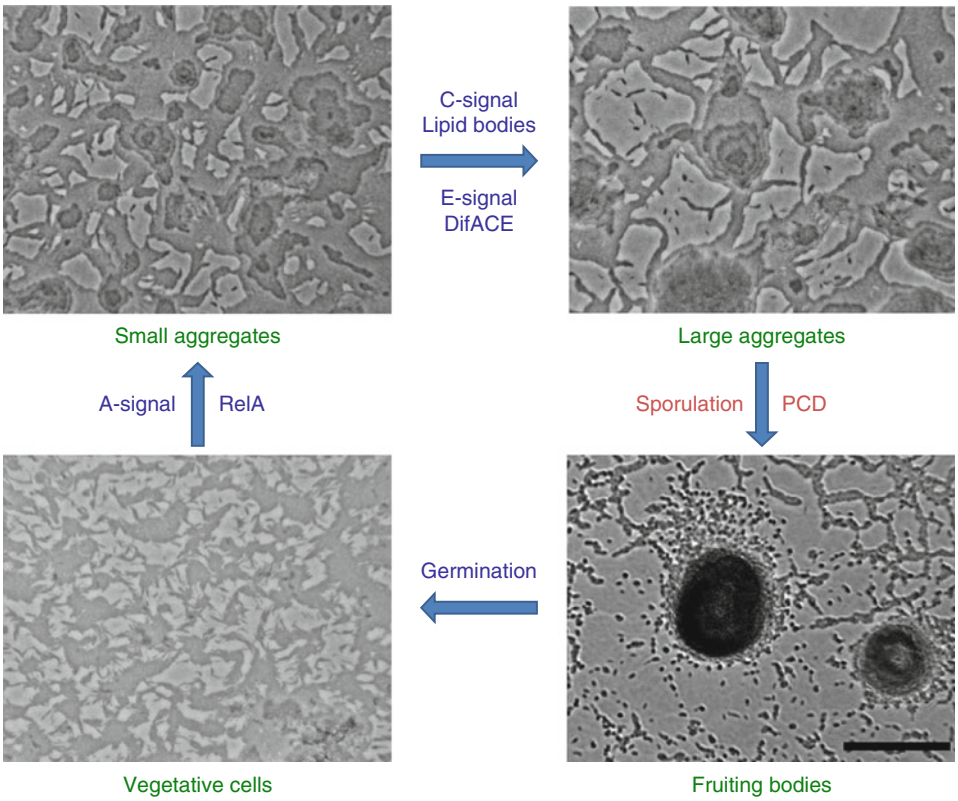


Fig. 16.19

Stages of fruiting body morphogenesis in *Myxococcus xanthus*. Vegetative cells (*lower left*) form a thick biofilm. Cells at the top of the biofilm are arranged in layers visualized as different shades of gray. Fruiting body morphogenesis begins with nutrient limitation detected via a RelA-dependent stringent response. A-signaling is used to determine that a sufficient cell density is available for development to proceed. Morphogenesis begins with vigorous cell movement leading to the formation of many small aggregates raised several layers above the surface of the cell mat (*upper left*). These small aggregates move rapidly, sometimes fusing with other aggregates and sometimes receding into the cell mat. With time, large, spatially stable aggregates form and are extended vertically by adding successive layers to the top of the fruiting body (*upper right*). Inside the aggregates, cells undergo programmed cell death (PCD) and sporulation leading to the production of mature fruiting bodies (*lower right*). Fruiting bodies maintain dormancy until nutrients initiate germination. Bar is 0.1 mm (Modified from data found in Curtis et al. 2007)

becomes synchronized due to increased C-signaling and cells rarely reverse direction leading to streams of cells that move toward aggregation foci. Within the aggregation centers, C-signaling is maximized by cell contact leading to activation of *devRST* operon. *devRST* expression is regulated spatially ensuring sporulation only within fruiting bodies.

The Dif chemosensory system also controls fruiting body morphogenesis and sporulation. DifA, a methyl-accepting chemotaxis protein (MCP); DifC, a CheW-like coupler; and DifE, a CheA-like histidine kinase form a ternary signaling complex. *difACE* mutants form small aggregates that are several layers thick but fail to mature beyond that stage. DifACE are essential for extracellular matrix (ECM) production, S-motility, and chemotaxis to several lipids including one containing a rare fatty acid, 16:1ω5c (a fatty acid of 16 carbons with one point of unsaturation at position 11 from the carboxyl group). S-motility is not the critical output because cells can make fruiting bodies using only A-motility. The DifACE pathway has two sensory inputs mediated by PilA and FibA leading to different outputs for ECM production and lipid chemotaxis, respectively. Either input mediates development, but loss of both inputs eliminates fruiting body morphogenesis.

Whereas the basic program for endospore formation is conserved in all endospore-forming bacteria, there is little conservation of developmental genes among the myxobacteria (Huntley et al. 2011). A set of 95 *M. xanthus* developmental genes was examined for representation in the genomes of other fruiting body forming bacteria. Genes for entire signal transduction pathways important for fruiting body formation in *M. xanthus* are conserved in the closely related species *S. aurantiaca*, whereas only a minority of these genes are conserved in the more distantly related species *S. cellulosum* and *H. ochraceum*. These comparative analyses suggest that the genetic programs for fruiting body formation in *M. xanthus* and *S. aurantiaca* are highly similar in spite of major differences in fruiting body structure and significantly different from the genetic program directing fruiting body formation in *S. cellulosum* and *H. ochraceum*.

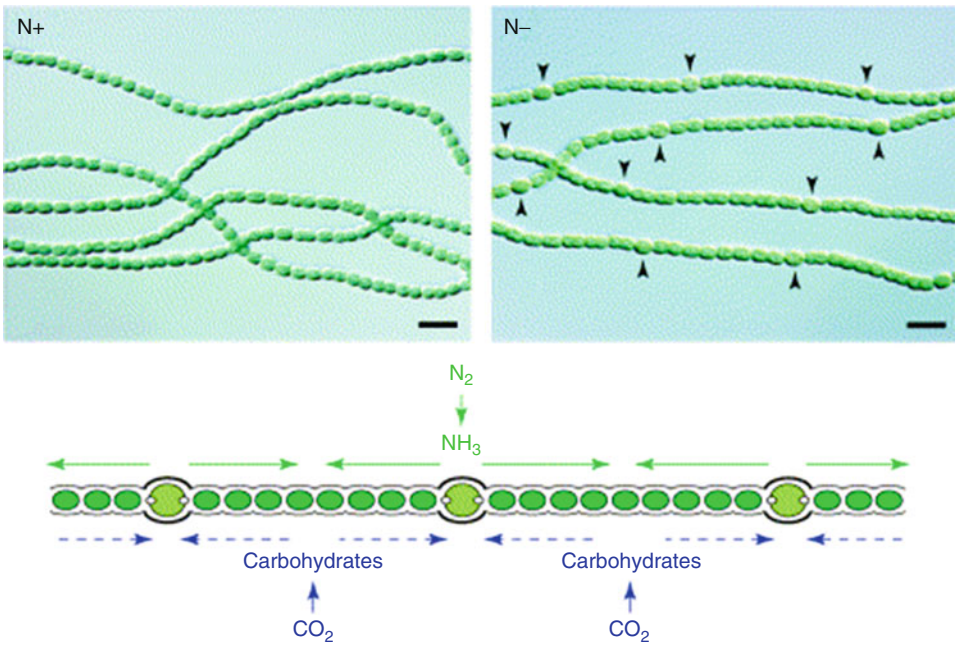


Fig. 16.20
The filamentous cyanobacterium *Anabaena* PCC 7120 grown with (N+) or without (N−) combined nitrogen. Heterocysts are indicated by *arrowheads*. Heterocysts supply fixed nitrogen as glutamine and other amino acids to the neighboring vegetative cells, which supply heterocysts with fixed carbon produced by photosynthesis. Filaments are composed of individual cells, each with its own plasma membrane and cell wall but enclosed by a common outer membrane. The diagram is not drawn to scale (From Golden 2003 #3877)

Nutrient Acquisition

The most prominent examples of specialized cells for nutrient acquisition involve nitrogen fixation in which atmospheric nitrogen (N_2) is converted to ammonia (NH_3). Oxygenic photosynthesis and nitrogen fixation are incompatible processes as nitrogenase is exquisitely sensitive to oxygen. *Anabaena* solves this problem by using a heterocyst to fix nitrogen. The reader is directed to a recent review on heterocysts (Kumar et al. 2010). Heterocysts are larger than vegetative cells due to a thick cell envelope containing glycolipid and polysaccharide that protects nitrogenase from oxygen. Oxygenic photosystem II is dismantled during heterocyst differentiation. Cyanophycin granules are found at poles adjacent to vegetative cells. Vegetative cells supply heterocysts with a source of carbon, possibly sucrose, and glutamate. Heterocysts convert the glutamate to glutamine and other amino acids that are shuttled to the vegetative cells.

Heterocyst formation in *Anabaena* is suppressed in nitrogen-rich media (*Fig. 16.20*). The photosynthetic vegetative cells grow in long filaments and each cell undergoes binary fission to increase the length of the filament. In the absence of nitrate, heterocysts appear at regular intervals. The heterocysts are green in *Fig. 16.21* due to the presence of the *patS-gfp* reporter gene, which produces the green fluorescent protein. The heterocysts supply the vegetative cells with organic nitrogen that diffuses from cell to cell along the chain. Heterocysts are incapable of cell division, and with intervening vegetative cell division, the

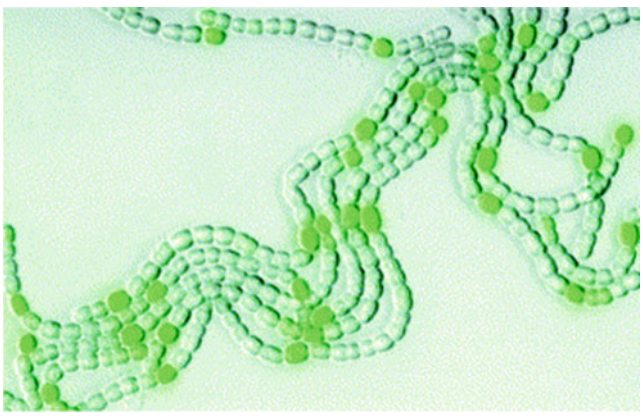


Fig. 16.21
Filaments of *Anabaena* PCC 7120 carrying a *patS-gfp* reporter 27 h after nitrogen step-down. The image is an overlay of a grayscale bright-field micrograph and the corresponding GFP fluorescence micrograph in *green*. The *bright green* and somewhat larger cells are heterocysts (From Golden 2003 #3877)

spacing between the heterocysts increases until vegetative cells some distance from the heterocyst are faced with nitrogen limitation. These nitrogen-stressed vegetative cells differentiate into heterocysts to maintain the proper spacing and the flow of nitrogen. In a *patS* mutant, heterocyst production is excessive relative to organic nitrogen availability. A few heterocysts are found in nitrogen-rich medium, and in nitrogen-limiting medium, the

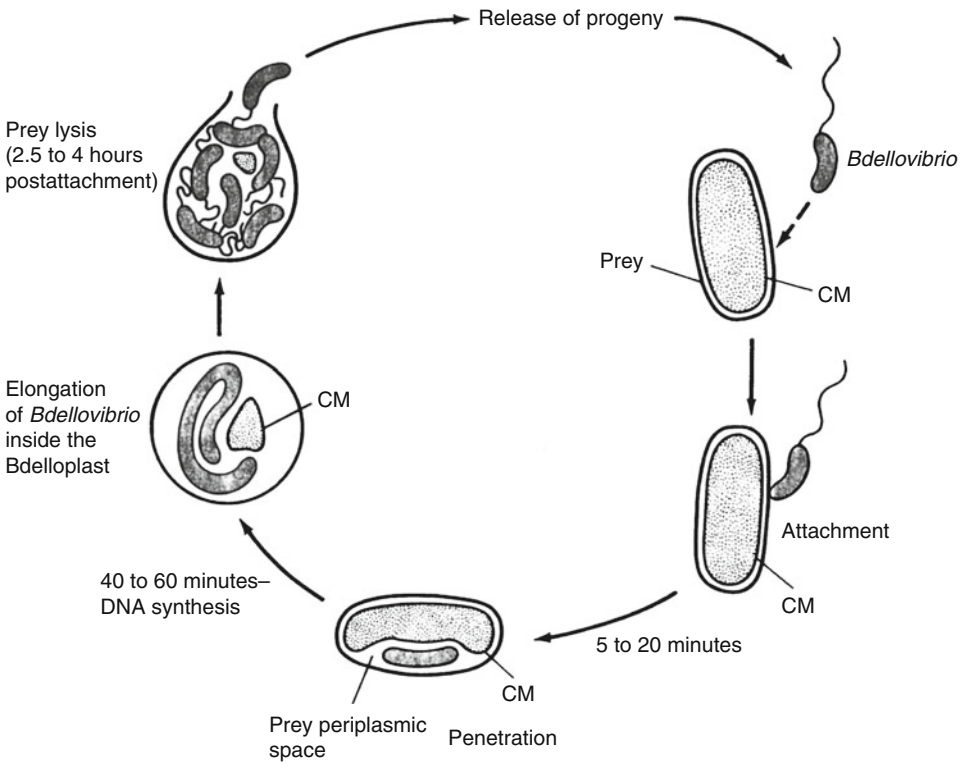
spacing between heterocysts is dramatically decreased and irregular. PatS is an intercellular peptide signal that suppresses heterocyst formation. The prevailing model is that PatS, produced in the heterocyst, diffuses from cell to cell along with organic nitrogen (glutamine and other amino acids) as concentration gradients that lessen with distance from the heterocyst. At a point where both PatS and organic nitrogen diminish below a certain threshold, a new heterocyst is induced.

Heterocyst differentiation begins when a vegetative cell senses nitrogen limitation. The intracellular signal for nitrogen imitation is 2-oxoglutarate, an intermediate in the Krebs cycle. In cyanobacteria, the Krebs cycle is incomplete due to an absence of 2-oxoglutarate dehydrogenase, so the primary function of 2-oxoglutarate is anabolic including serving as the carbon skeleton for ammonium assimilation. NtcA, a transcriptional regulator, responds to excess 2-oxoglutarate by activating many genes involved in carbon and nitrogen metabolism including, indirectly, *hetR*, whose protein product is the master regulator of heterocyst differentiation. HetR is an autoactivator that indirectly induces production of nitrogenase and many of the changes associated with heterocyst differentiation. HetR autoactivation is subject to several levels of control, among them regulation by PatS. The heterocyst inhibitory peptide PatS interferes with HetR DNA-binding activity to diminish the level and activity of HetR.

Dispersal Strategies

While nonmotile cells can be passively dispersed by wind and rain, some organisms produce specialized motile cells to force the issue. Production of dispersal cells is achieved by upregulating genes encoding a motility motor. As observed in *C. crescentus, Vibrio parahaemolyticus*, and *Bdellovibrio bacteriovorus*, this involves generation of flagella. Dispersal cells can also use other types of motility. Baeocytes produced by *Pleurocapsa* cyanobacteria and hormogonia, short motile filaments that are released from filamentous cyanobacteria, move by gliding on solid surfaces without flagella (◉ *Table 16.1*).

Attack Phase Cells. Bdellovibrio bacteriovorus, a member of the δ-proteobacteria, is an obligate intracellular parasite of other gram-negative bacteria (Rendulic et al. 2004; Sockett 2009). *B. bacteriovorus* cells alternate between a motile infectious attack phase and a nonmotile reproductive phase (◉ *Fig. 16.22*). They



◘ Fig. 16.22

Diagrammatic representation of the life cycle of *B. bacteriovorus*. In the predatory phase, *Bdellovibrio* attack phase cells are free-swimming while seeking prey. After collision with a prey cell, *B. bacteriovorus* penetrates the outer membrane and peptidoglycan layer of a gram-negative bacterium. *Bdellovibrio* loses its motility and resides in the periplasm of the prey bacterium. In the growth phase, the host converts into a spherical bdelloplast, while *Bdellovibrio* elongates into a filamentous cell that consumes the prey's nutrients. When the nutrients are exhausted from the prey cell, the filament partitions and the progeny develop into small, highly motile flagellated predator cells. The remainder of the prey cell lyses to release the progeny (From Brock and Madigan 1988 with permission of Prentice-Hall, Inc., Englewood Cliffs, NJ)

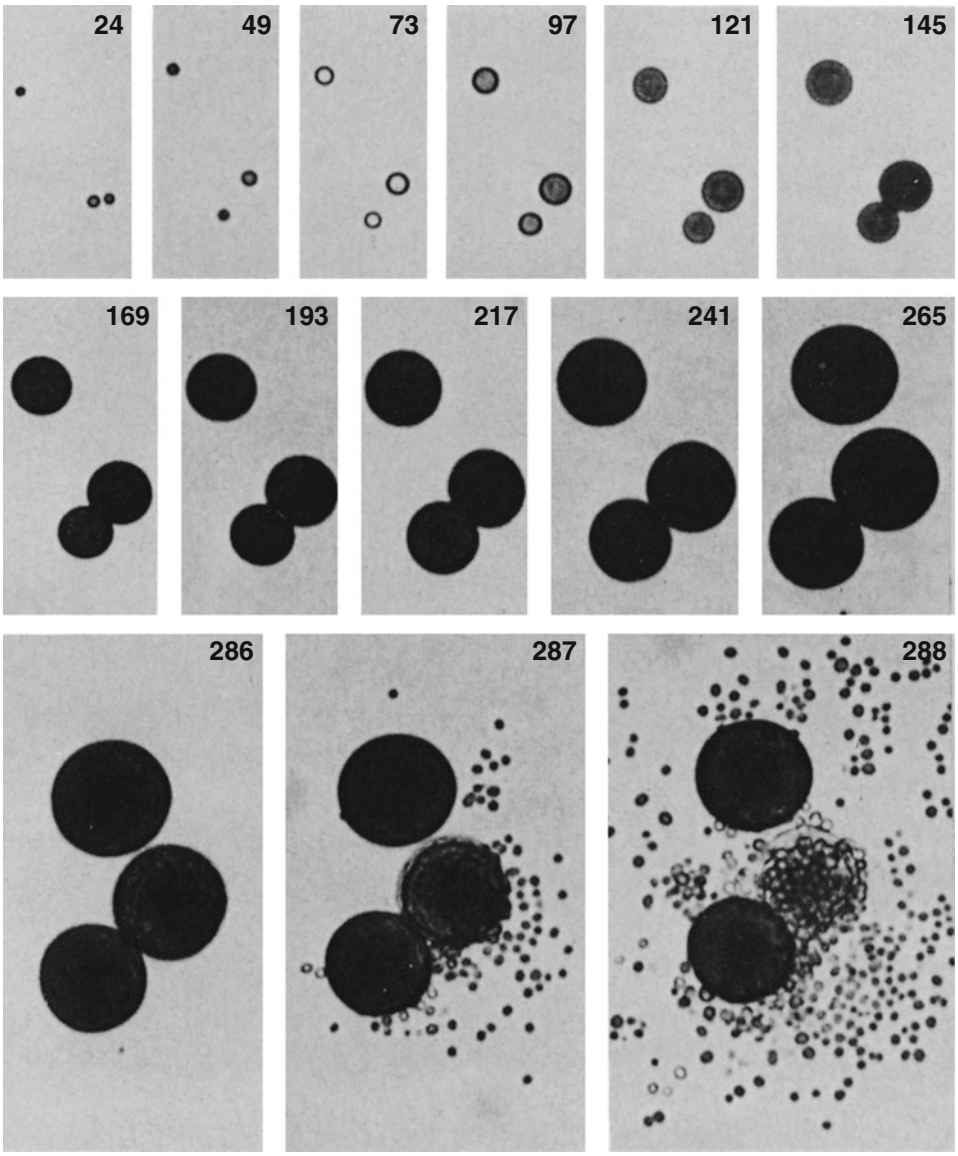


Fig. 16.23
Phase contrast photomicrographs illustrating the development of *Dermocarpa*. The *number* on each photo indicates the elapsed time in hours since the initial observation (From Waterbury and Stanier 1978)

invade the host cell by forming a hole in the outer membrane where they reside in the periplasm (the region between the inner and outer membrane) feeding on biopolymers with hydrolytic enzymes. Greater than 200 genes encoding lytic enzymes have been found in the genome. Within three hours of invasion, the host cell is full of attack phase cells that are released by lysing the host.

Attack phase cells are motile using a single polar flagellum. Whether they actively seek prey cells or happen upon them by chance remains unknown. *B. bacteriovorus* probably attaches to prey cells using type IV pili since inactivation of the pilin gene eliminates predation (Evans et al. 2007). A small opening in the prey cell outer membrane and peptidoglycan layer is made then resealed once *Bdellovibrio* is inside the host cell. Growth and DNA replication occur without cell division to form a multinucleate filament. *B. bacteriovorus* transforms the shape of the prey cell to a spherical, osmotically stable bdelloplast where the *B. bacteriovorus* transports nutrients from the host cytosol. *B. bacteriovorus* can synthesize only 11 amino acids and is dependent on host cell amino acids for protein synthesis. The filamentous *B. bacteriovorus* cell undergoes multiple septation events to generate many progeny. The progeny develop flagella then dissolve the outer membrane and peptidoglycan layer of the bdelloplast to emerge as mature attack phase cells.

Baeocytes. The pleurocapsalean cyanobacteria reproduce by multiple fission to produce many motile baeocytes (*Fig. 16.23*). The baeocyte becomes covered by a thick, fibrous sheath and increases in size as much as 1,000-fold in some

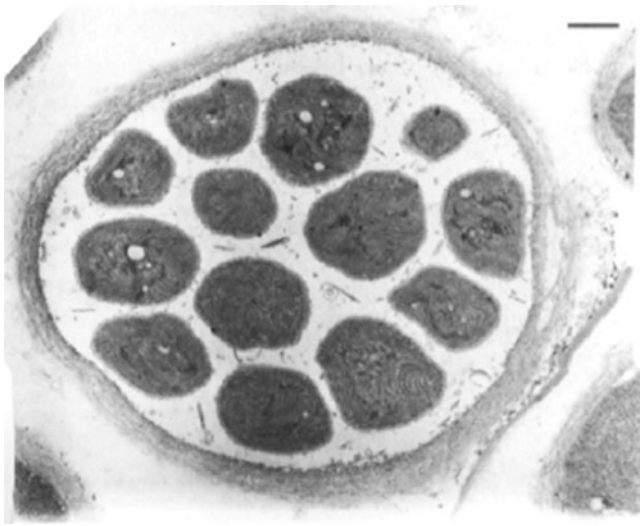


Fig. 16.24
Thin section of a *Dermocarpa* cell. The cell has undergone multiple fission events and is filled with baeocytes, each of which is surrounded by layers of peptidoglycan and outer membrane. Bar, 1 μm (From Waterbury and Stanier 1978)

species such as *Dermocarpa*. When the maximum size has been reached, the cell undergoes multiple fissions within the fibrous sheath (Fig. 16.24). The parental cell then ruptures, releasing numerous small baeocytes. The baeocytes are phototactic and motile by gliding until the cells become covered by the fibrous sheath. At this point, they tend to attach to a solid surface. Unfortunately, little work is done with this intriguing system.

Hormogonia. The most carefully examined dispersal cell using gliding motility is the hormogonium, produced by several filamentous cyanobacteria (Meeks and Elhai 2002). Hormogonia are short filaments composed of nongrowing cells that are motile on surfaces by a mechanism that remains unknown. Cyanobacteria form symbiotic associations with a wide range of eukaryotic hosts including plants, fungi, sponges, and protists. The most carefully studied cyanobacterial symbioses are those with plants in which the cyanobacteria infect the roots, stems, leaves, and, in the case of the liverworts and hornworts, the thallus. The symbionts are usually *Nostoc* spp, that gain entry to the host by means of hormogonia where they resume filamentous growth and develop enhanced N_2 fixation, with much of the fixed nitrogen being destined for the plant.

The differentiation of hormogonia results from a round of synchronous cell division that decreases cell size followed by fragmentation of the filament at the heterocyst–vegetative cell junctions to release short filaments lacking heterocysts. Gene expression during hormogonia differentiation in *Nostoc punctiforme* was examined with DNA microarrays (Campbell et al. 2007). The number of genes expressed in hormogonia producing cells is nearly fivefold higher than those expressed in akinete-forming or nitrogen-fixing cultures. This result is startling because hormogonia are nongrowing and unable to fix nitrogen. The upregulation of 944 genes (out of 2,935 total) suggests that hormogonia are metabolically active. Of the upregulated genes, 85 are involved in signal transduction, 18 are involved in chemotaxis, and 20 are involved in transcriptional regulation arguing that hormogonia are highly tuned to the environment. This result may reflect the fact that the plant exerts strong influence over the differentiation and colonization processes (Meeks and Elhai 2002).

Swarmer Cells. The term "swarmer cell" is ambiguous in that it can refer to several different types of cells. Three examples will be offered. The first uses *V. parahaemolyticus* as the model organism but is represented by a wide variety of Proteobacteria that use swimming cells to move in liquid and swarmer cells to move on solid surfaces. The other two examples, *Rhodomicrobium* and *Caulobacter*, are more similar to each other than to *Vibrio*.

V. parahaemolyticus exists as a swimmer (vegetative) cell in liquid environments or as a swarmer cell on solid surfaces, allowing it to colonize a variety of niches (Fig. 16.25). Swimmer cells are small, uninucleate, and have a single polar flagellum. Swarmer cells are 20–30 times larger than swimming cells and possess a large number of lateral flagella that differ from the polar flagellum. The polar flagellum contains four flagellin subunits, FlaA, B, C, and D, while the lateral flagella contain a single flagellin protein LafA. The polar flagellum is protected by a membranous sheath, which is absent in the lateral flagella. Although the energy used for both types of motility is the electrochemical gradient, the coupling ions are different. The polar flagellum utilizes Na^+ ions, while the lateral flagella use H^+.

Swarmer cell differentiation is induced by growth on solid surfaces or viscous environments because resistance to polar flagellum rotation signals differentiation. Swarmer cells are also differentiated in response to iron limitation. Cell division is inhibited but not cell growth, leading to polynucleated, elongated cells. On solid surfaces, swarming occurs for several hours and then ceases as rapid cell division produces swimmer cells by a process known as consolidation. Concentric rings or terraces are formed on agar plates due to repeated cycles of swarming and consolidation.

Rhodomicrobium vannielii is a phototrophic, budding bacterium that produces both exospores and motile swarmer cells in addition to nonmotile vegetative cells. The polarly flagellated swarmer cell is, like its counterpart in *Caulobacter*, a nongrowing cell whose function is to maximize dispersal. Swarmer cell formation in *R. vannielli* is induced by conditions of low light intensity and high levels of CO_2. At higher light intensities, the swarmer cells shed their flagella and undergo morphogenetic conversion to the reproductive budding phase.

The strategy of alternating swarmer and stalked cells in *Caulobacter* (described in section "*Caulobacter*") is similar but different in a fundamental way. A *Caulobacter* stalked cell cannot give rise to another stalked cell but only to a swarmer cell whose production is an obligate output of the cell division cycle. The sessile, budding cell of *R. vannielii*, on the other hand, has the option of either producing a swarmer cell, or continuing to produce cells connected by hyphae. *R. vannielli* can also generate a resistant, resting exospore as described in section

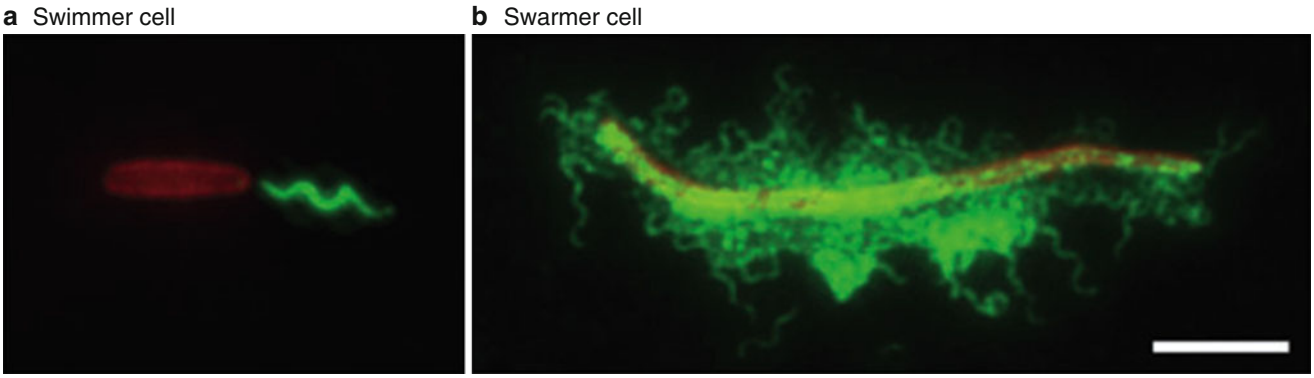


Fig. 16.25
Swimmer and swarmer cells of *V. parahaemolyticus*. LM5674 (wild-type) swimmer cell grown in liquid (a) and swarmer cell grown on a surface (b) are profoundly different. Cells were fixed and examined by immunofluorescence microscopy. Both panels are of the same magnification and the bar indicates 5 μm. Cells were stained with membrane dye FM 4–64 (*colored red*) and anti-polar flagellin antiserum (a, *colored green*) or anti-lateral flagellin antiserum (b, *colored green*) (From Gode-Potratz et al. 2011)

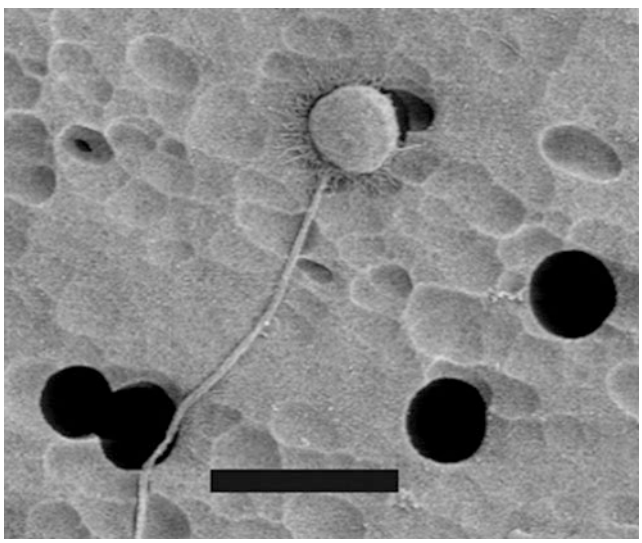


Fig. 16.26
Scanning electron micrograph of a *Kineococcus radiotolerans* SRS30216^T zoospore exhibiting a single flagellum. Bar, 2 μm (From Phillips et al. 2002)

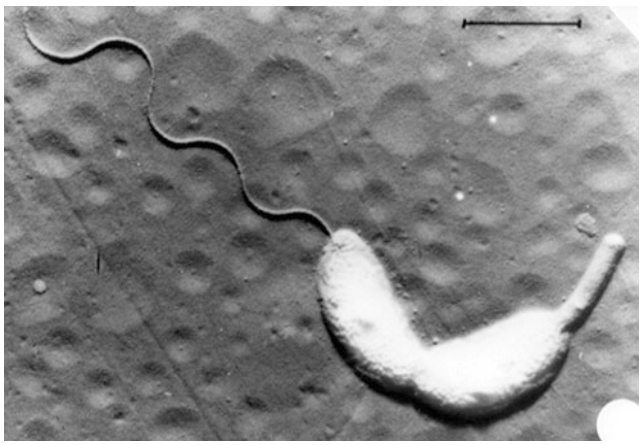


Fig. 16.27
Electron micrograph of a shadowed cell of *Caulobacter crescentus*. The stalked mother cell is in the process of dividing, leading to the formation of a flagellated swarmer cell. Bar, 1 μm (From Poindexter 1964)

"Differentiation Leading to Dormancy". In addition to this remarkable panoply of developmental options, *R. vannielli* can grow either anaerobically as a phototroph or aerobically as a chemotroph, so it is an extremely versatile organism.

Zoospores. The production of motile zoospores is widespread but patchy among members of the Actinobacteria (*Fig. 16.26*). *Kineosporia* zoospores are produced at the tips of substrate hyphae and in clusters on sporangioles, though there is a great deal of variation in the manner in which zoospores are produced in other genera. A question of interest is how the zoospores maintain dormancy and at the same time exhibit rapid movement and tactic behavior. *Kineosporia* SR11 zoospores move at remarkable speeds of up to 160 μm/s and can move up chemical gradients of a variety of inorganic compounds (Radajewski and Duxbury 2001). The *Kineococcus* genome contains complete pathways for flagellar biogenesis and chemotaxis arguing that zoospore dispersal is designed to locate new niches for growth (Bagwell et al. 2008).

Caulobacter

During the *C. crescentus* cell cycle, a stalked parent always produces flagellated progeny (*Fig. 16.27*). A comprehensive review of this dimorphic life cycle is found in Curtis and Brun (2010) and can be divided into cyclic and noncyclic phases

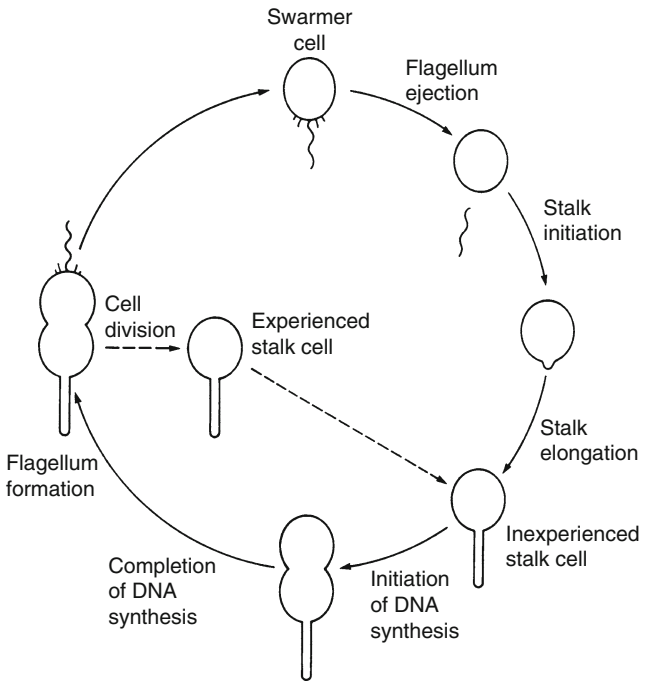


Fig. 16.28

Diagram of the life cycle of *Caulobacter crescentus*. The cyclic developmental program begins with a stalked cell with an adhesive holdfast at the tip of the stalk. The stalked cell enters S phase, a cell state where it is competent for DNA replication. As the cell grows and replicates its DNA, it becomes a predivisional cell. During this time, the cell becomes incompetent for DNA replication, entering the G2 phase. In the late predivisional stage, a flagellum is formed at the swarmer cell pole. After compartmentalization, flagellar rotation is activated and pili are produced. Cell separation leads to two different cell types. One cell is a stalked cell, which reenters the cyclic developmental program and S phase, completing the circle. The other cell is a swarmer cell. The swarmer cell cannot replicate its chromosome yet is distinct from the predivisional cell and therefore is in a separate phase, referred to as G1. The holdfast is formed predominantly during the swarmer cell stage. Later, the swarmer cell differentiates into a stalked cell. This differentiation comprises the noncyclic developmental program. (From Dworkin 1985)

(*Fig. 16.28*). The cyclic phase begins with a stalked cell using the terminal holdfast to attach to a substrate. The stalked cell initiates chromosome replication, and by analogy with the eukaryotic cell cycle, this is referred to as the S phase. At the conclusion of DNA replication and growth, the cell emerges from S phase into the predivisional (G2) phase. In late G2, a flagellum forms at the opposite cell pole from the stalk and begins rotating (*Fig. 16.27*). Pili are produced on the same pole. Following cell division, the flagellated swarmer cell swims away. The stalked cell repeats the cycle, each time producing a swarmer cell. The cyclic portion of the life cycle is controlled by three master regulators, CtrA, GcrA, and DnaA, to move the cell through the cell cycle in a variety of fascinating ways. The complete regulatory circuit involves approximately 550 cell cycle–dependent genes.

After swimming for some time, the swarmer cell grows a holdfast at the same pole as the flagellum, sheds the flagellum, and eventually grows a stalk to become a new stalked cell. At this point, it enters the cyclic developmental phase. CtrA is a response regulator that regulates chromosome replication initiation. Phosphorylated CtrA (CtrA∼P) interacts with DNA in the origin of replication (*oriC*) to block initiation of replication locking the swarmer cell into the G1 phase. The swarmer to stalked cell transition utilizes the unusual response regulator PleD to produce a second messenger, cyclic diguanylic acid (c-di-GMP). PleD activity is needed to degrade the flagellar anchor protein FliF, which coincides with flagellum ejection. Less is known about the mechanisms behind pilus loss, holdfast induction, and stalk formation. Stalk formation has been difficult because there seem to be two pathways for making a stalk as there are no stalkless mutants. The final step in the transition from swarmer cell to stalked cell involves becoming replication competent. This event also begins the cyclic portion of the life cycle for all stalked cells.

The mechanism by which the stalked cell produces the swarm cell is known with stunning clarity and includes temporal induction of cell cycle events and spatial control of protein localization into the stalked cell compartment and the swarmer cell compartment. CtrA is degraded by ClpXP, an ATP-dependent protease releasing the major block to chromosome replication. Degradation of CtrA is accompanied by DnaA synthesis. DnaA binds to *oriC* to initiate DNA replication. Free DnaA, which is present only during the start of each cell cycle, also acts as a transcriptional activator of GcrA expression to activate genes required for DNA replication. CtrA also represses transcription GcrA, and proteolysis of CtrA enables transcription of GcrA. DnaA also acts as a transcriptional activator of cell division initiator FtsZ, which localizes at the site of cell division.

Cell polarity governs correct localization of the flagellum and stalk in the predivisional cell. Cell polarity is achieved with the regulators of polarity, the DivJ and PleC histidine kinases, and the DivK response regulator. The cyclic developmental cycle begins with DivJ and DivK∼P localized to the stalked pole. DivL and PleC are delocalized in the inner membrane. DivK phosphorylation leads to inactivation of CtrA, which, along with DnaA, leads to *gcrA* transcription. DnaA and GcrA produce PodJ, which localizes to the nascent swarmer pole and serves as a localization factor for PleC. Now, DivJ and PleC are located at opposite poles and mark the stalked pole and the pole that will develop the flagellum, respectively. DivK is phosphorylated by DivJ at the stalked pole and localizes there. Some DivK∼P also diffuses to the swarmer pole but is dephosphorylated by PleC causing it to diffuse back across the cell where it becomes rephosphorylated by DivJ at the stalked pole. DivK phosphorylation/dephosphorylation cycling continues as the cell cycle progresses. After cytokinesis, DivJ and PleC enzymatic activities are separated from each other. As a consequence, DivJ activity leads to DivK phosphorylation in the stalked cell, which then localizes at the stalked pole. In the swarmer cell compartment, PleC

activity leads to DivK∼P dephosphorylation and delocalization. The phosphorylation state of DivK affects activation/inactivation of CtrA in each cell compartment. DivK∼P favors CtrA inactivation encouraging further rounds of cell division. In the swarmer cell, DivK favors CtrA activation blocking chromosome replication initiation and reproduction.

Conclusion

The startling diversity of prokaryotic developmental cycles attests at once to the ability of specialized cells to enhance survival of the species in ever-changing environments. Differentiation of multiple cell types achieves a division of labor that maximizes the resources of these simple but cunning creatures. One may justify an interest in prokaryotic life cycles solely on the ground that locked within them is a vast array of undiscovered secrets stemming from the delicate interactions between an organism and its environment. The spatial and temporal cues and their exquisitely balanced sensory pathways manifested in the systems described in this chapter are but the beginning of a journey of discovery that will enlighten and entertain for decades to come.

Acknowledgment

This work was supported by National Science Foundation Grant MCB0742976.

References

Abdelrahman YM, Belland RJ (2005) The chlamydial developmental cycle. FEMS Microbiol Rev 29(5):949–959

Angert ER, Losick RM (1998) Propagation by sporulation in the guinea pig symbiont *Metabacterium polyspora*. Proc Natl Acad Sci USA 95(17):10218–10223

Angert ER, Brooks AE et al (1996) Phylogenetic analysis of *Metabacterium polyspora*: clues to the evolutionary origin of daughter cell production in *Epulopiscium* species, the largest bacteria. J Bacteriol 178(5):1451–1456

Bagwell CE, Bhat S et al (2008) Survival in nuclear waste, extreme resistance, and potential applications gleaned from the genome sequence of *Kineococcus radiotolerans* SRS30216. PLoS One 3(12):e3878

Berleman JE, Bauer CE (2005) Involvement of a Che-like signal transduction cascade in regulating cyst cell development in *Rhodospirillum centenum*. Mol Microbiol 56(6):1457–1466

Brock TD, Madigan MT (1988) Biology of microorganisms, 5th edn. Prentice-Hall, Englewood Cliffs, 722

Campbell EL, Summers ML et al (2007) Global gene expression patterns of *Nostoc punctiforme* in steady-state dinitrogen-grown heterocyst-containing cultures and at single time points during the differentiation of akinetes and hormogonia. J Bacteriol 189(14):5247–5256

Cano RJ, Borucki MK (1995) Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268(5213):1060–1064

Curtis PD, Brun YV (2010) Getting in the loop: regulation of development in *Caulobacter crescentus*. Microbiol Mol Biol Rev 74(1):13–41

Curtis PD, Taylor RG et al (2007) Spatial organization of *Myxococcus xanthus* during fruiting body formation. J Bacteriol 189(24):9126–9130

Cutlip RC (1970) Electron microscopy of cell cultures infected with a chlamydial agent causing polyarthritis of lambs. Infect Immun 1(5):499–502

de Hoon MJ, Eichenberger P et al (2010) Hierarchical evolution of the bacterial sporulation network. Curr Biol 20(17):R735–R745

Dworkin M (1985) Developmental biology of the bacteria. Benjamin/Cummings, Menlo Park

Evans KJ, Lambert C et al (2007) Predation by *Bdellovibrio bacteriovorus* HD100 requires type IV pili. J Bacteriol 189(13):4850–4859

Gimmestad M, Ertesvag H et al (2009) Characterization of three new *Azotobacter vinelandii* alginate lyases, one of which is involved in cyst germination. J Bacteriol 191(15):4845–4853

Gode-Potratz CJ, Kustusch RJ et al (2011) Surface sensing in *Vibrio parahaemolyticus* triggers a programme of gene expression that promotes colonization and virulence. Mol Microbiol 79(1):240–263

Golden JW, Yoon HS (2003) Heterocyst development in *Anabaena*. Curr Opin Microbiol 6(6):557–563

Hitchins VM, Sadoff HL (1970) Morphogenesis of cysts in *Azotobacter vinelandii*. J Bacteriol 104(1):492–498

Huntley S, Hamann N et al (2011) Comparative genomic analysis of fruiting body formation in myxococcales. Mol Biol Evol 28(2):1083–1097

Kumar K, Mella-Herrera RA et al (2010) Cyanobacterial heterocysts. Cold Spring Harb Perspect Biol 2(4):a000315

McCormick JR (2009) Cell division is dispensable but not irrelevant in *Streptomyces*. Curr Opin Microbiol 12(6):689–698

Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66(1):94–121, Table of contents

Moir A (2006) How do spores germinate? J Appl Microbiol 101(3):526–530

Nichols JM, Adams DG (1982) Akinetes. In: Carr NG, Whitton BA (eds) The biology of the cyanobacteria. University of California Press, Berkeley, pp 387–412

Olsson-Francis K, de la Torre R et al (2009) Survival of Akinetes (resting-state cells of cyanobacteria) in low earth orbit and simulated extraterrestrial conditions. Orig Life Evol Biosph 39:565–579

Phillips RW, Wiegel J et al (2002) *Kineococcus radiotolerans* sp. nov., a radiation-resistant, gram-positive bacterium. Int J Syst Evol Microbiol 52(Pt 3): 933–938

Poindexter JS (1964) Biological properties and classification of the *Caulobacter* group. Bacteriol Rev 28:231–295

Popham DL (2002) Specialized peptidoglycan of the bacterial endospore: the inner wall of the lockbox. Cell Mol Life Sci 59(3):426–433

Radajewski S, Duxbury T (2001) Motility responses and desiccation survival of zoospores from the Actinomycete *Kineosporia* sp. Strain SR11. Microb Ecol 41(3):233–244

Reed WM, Titus JA et al (1980) Structure of *Methylosinus trichosporium* exospores. J Bacteriol 141(2):908–913

Rendulic S, Jagtap P et al (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303(5658):689–692

Setlow P (2006) Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. J Appl Microbiol 101(3):514–525

Setubal JC, dos Santos P et al (2009) Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes. J Bacteriol 191(14):4534–4545

Sockett RE (2009) Predatory lifestyle of *Bdellovibrio bacteriovorus*. Annu Rev Microbiol 63:523–539

Vreeland RH, Rosenzweig WD et al (2000) Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature 407(6806):897–900

Waterbury JB, Stanier RY (1978) Patterns of growth and development in Pleurocapsalean cyanobacteria. Microbiol Rev 42(1):2–44

Whittenbury R, Dow CS (1977) Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. Bacteriol Rev 41(3):754–808

Whittenbury R, Davies SL et al (1970) Exospores and cysts formed by methane-utilizing bacteria. J Gen Microbiol 61(2):219–226

Whitworth DE (2008) Myxobacteria: multicellularity and differentiation. American Society for Microbiology Press, Washington, DC

Willey JM, Willems A et al (2006) Morphogenetic surfactants and their role in the formation of aerial hyphae in *Streptomyces coelicolor*. Mol Microbiol 59(3):731–742

17 Life at High Temperatures

Rainer Jaenicke · Reinhard Sterner
Institut für Biophysik und physikalische Biochemie, Universität Regensburg, Regensburg, Germany

Introduction

In contrast to the simplistic definition of life as the quality that distinguishes a vital and functional being from a dead body, present-day biological sciences are mechanistically oriented, that is, cells and their inventory are functionally determined by the nonvitalist principle that living matter is composed of chemical substances obeying the fundamental laws of physics. Any biological function, including ecological adaptation, differentiation, and behavior, can be described in terms of the structures of those substances and the reactions that they undergo. However, one apparent difference between the life sciences, on the one hand, and physics or chemistry, on the other, deserves mentioning: Physics and chemistry study the unchanging properties of matter and energy, while the subject matter of biology (presently known organisms) is evolving, that is, includes only a subset that has managed to produce descendents under the changing physical conditions of the biosphere.

Within the framework of biology and physical biochemistry, life refers to cellular organisms whose characteristics are (1) the capacity for metabolism (energy transformation), (2) growth, (3) response to stimuli, and (4) reproduction. Their constituent building blocks comprise a relatively small number of complex biomolecules (proteins, nucleic acids, carbohydrates, and lipids), with the first two serving as substrates for the process of evolution. Evolution occurs because natural selection favors, among all the combinations available, those individuals whose characteristics increase their reproduction in a particular environment. At this point, the physical or chemical conditions of the environment come into the play. As a consequence of the stochastic mechanism of "successful adaptation" to changing environmental conditions, all organisms are phylogenetically related to one another; in addition, they share most of the basic biochemical processes involved in replication, transcription, and translation and in the basic reactions governing metabolic and energy-transfer pathways. Thus, fundamental biochemical and biophysical problems may be studied in whatever organism is practical or convenient. In the context of this chapter, the specific properties of biomolecules from thermophilic microorganisms may provide us with a deeper understanding of general mechanisms underlying differences in the stability of proteins, nucleic acids, and lipids, as well as in their metabolic turnover.

Dedicated to the memory of Professor John T. Edsall (3 November 1902–12 June 2002), one of the founders of physical biochemistry: scientist, teacher and mentor.

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_55,

Adaptation to Extremes of Physical Conditions

Among the three alternative responses (avoidance, compensation or detoxification, and mutative adaptation) of microorganisms to extreme physical conditions, only mutative adaptation can cope with high temperature and high hydrostatic pressure, simply because cells in their natural aqueous environment are isothermic and isobaric (Jaenicke 1981, 1990). At this point, we include high pressure as a second variable because in many cases the term "environmental extremes" refers to a whole set of factors, for example, high pressure and low or high temperature in deep-sea hydrothermal vents (Somero 1992; Kelley et al. 2001) or low pH and high temperature in acid solfataras (Brock 1986; Stetter 1996, 1998). In addition, physicochemical parameters may have an indirect effect on an organism, for example, temperature effects on the solubility of gases or on the viscosity and ionization of the aqueous medium. In such cases, in vitro experiments can easily compensate for such perturbations, this way eliminating indirect effects.

Considering mutative adaptation to extreme conditions, it is obvious that to grow and reproduce, the whole inventory of an extremophile needs to be adapted; fitness to survive in the competitive situation of a given environment is defined by the least stable constituent of a species. In the context of thermophilic adaptation, the example of temperature-sensitive point mutants stresses this argument.

High temperature can be defined as the upper temperature range in which mesophilic organisms do not survive while specifically adapted hyperthermophilic organisms grow and multiply, not simply tolerating the high temperature but requiring it as their standard physiological condition. Commonly, species diversity in extreme environments is distinct from that in mesophilic environments and therefore may be used as a criterion for extremophilism. In extreme environments (with low species diversity), often whole taxonomic groups are missing. For example, in saline and thermal lakes, as well as in hydrothermal vents, there are no vascular plants or vertebrates; in the most extreme high-temperature environments, only prokaryotic microorganisms have been discovered so far. Here, the low species diversity may sometimes be limited by extreme conditions to a few or even one species (Brock 1978). In analyzing this phenomenon, we may ask how and at which level the extreme environment interferes with the normal growth of mesophilic organisms; in turn, to discover the essential characteristics of thermophiles, studying the adaptive mechanisms decelerating or even inhibiting the growth of thermophiles at suboptimal temperature seems most promising. In the following, no strict distinction between thermophiles and hyperthermophiles will be made, because the limits are not well defined. Commonly, the temperature boundary of thermophiles is ca. 60 °C, while hyperthermophiles show optimal growth temperatures of 80 °C or above. Both estimates refer to growth, not survival. Needless to say, many bacteria, especially those capable of forming endospores, can tolerate temperatures much higher than those needed for optimal growth. However, it is the temperature range over which a microorganism is able to maintain growth and proliferation that is essential for evolution. The temperature range in the biosphere reaches from −80 °C (in the Arctic [to approximately 65°N latitude] and in the Antarctic) to +350 °C near white or black smokers in deep-sea vents. The upper temperature limit that still allows growth and proliferation of microorganisms is difficult to determine because of the extreme in situ turbulence in volcanic areas of the ocean. Evidently, "black smoker" bacteria alleged to grow at 250 °C and 265 bar are in fact merely the subject of Jules Verne fantasy (Bernhardt et al. 1984).

Pyrolobus fumarii may serve as a well-established example for bacterial life at the upper temperature limit of viability. Its physiological characteristics are growth between 90 °C and 113 °C, with an optimum at 106 °C (1-h doubling time at pH_{opt} = 5.5 and $[NaCl]_{opt}$ = 1.7 %), and no growth at 85 °C and 115 °C (Blöchl et al. 1997). The recent new world record, a Pyrodictium- and Pyrobaculum-related "Strain 121" with a temperature of maximal growth at 121 °C and a survival half-life of ∼6 min at 130 °C, draws a new line (Kashefi and Lovley 2003). However, considering the rise of T_{max} during the last 40 years (Covan 2004), it is obvious that there is a sigmoidal time course that extrapolates to the expected upper temperature limit of viability around 140 °C. The physical reasons are twofold: (1) Crucial biomolecules undergo hydrothermal decomposition, and (2) the energetic costs of repair and resynthesis become unsustainable (Bernhardt et al. 1984; White 1984; Stetter 1998; Jaenicke and Böhm 1998; Jaenicke 2000a).

Since water in its liquid state is a necessary requirement for biological function in terms of the above criteria, the freezing temperature of homogeneous nucleation (−40 °C) defines the lower limit at which life can exist. Chill conditions in the aqueous cytosol can persist down to this temperature and even below (Franks et al. 1990). Commonly, freezing damages cells irreversibly. In contrast, dehydration, for example, in seeds and other dormant states of cells and tissues, allows various forms of cryptobiosis. Generally speaking, these limits are based on (1) the effect of low temperatures on the weak interactions between biomolecules (especially on the self-organization of proteins and lipids) and (2) metabolic and/or protective regulation mechanisms involving compatible solutes and other forms of stress response. For details regarding cold tolerance, resistance, acclimation, adaptation, and cryptobiosis, see Crow and Clegg (1978), Finegold (1986), Laws and Franks (1990), Carpenter et al. (1993), Graumann and Marahiel (1996), Marshall (1997), Thieringer et al. (1998), Phadtare et al. (1999), Cavicchioli et al. (2000), Zachariassen and Kristiansen (2000), and Clegg (2001).

Over the whole biologically relevant temperature range, from psychrophiles up to hyperthermophiles, it is essentially impossible to predict how temperature changes may affect viability. Considering the complexity of metabolic pathways, the kinetics of each single step in any linear or cyclic reaction sequence may become rate limiting, either by its own high activation energy or by product inhibition. As evolution has produced efficient coupling mechanisms whereby products

Table 17.1
Anomalies of water in comparison with other solvents

Substance	F_p (°C)	K_p (°C)	ΔH_{vap} (cal/g)	c_p (cal/g·degrees)	σ (erg·cm²)	ε	η (poise)
H_2O	0	100	585	1.00	78	80	1.00
NH_3	−78	−34	296	1.125	18	15	0.27
CH_3CH_2OH	−114	78	204	0.58	22	24	1.20
CH_3COOH	17	118	96	0.47	28	7	1.22
CH_3COCH_3	−95	56	112	0.53	14	21	0.32
C_6H_6	5	80	104	0.41	29	2.3	0.65

F_p melting point, K_p boiling point, ΔH_{vap} specific enthalpy of vaporization, c_p specific heat capacity, σ surface tension at 25 °C, ε dielectric constant at 25 °C, and η viscosity. Data for σ, ε, and η refer to 25 °C, except for liquid NH_3, which was investigated at −34 °C

of reactions become the substrates for subsequent reactions, temperature perturbation will necessarily lead to a decrease in coupling efficiency. This holds because the relative reaction rates are determined not only by the specific differences in the temperature coefficients of ligand binding and enzyme turnover but also by the stability of the cellular microcompartmentation, for example, in multienzyme complexes and by a wide variety of transport processes (Franks 1985–1990; Jaenicke 1990).

Water

General Properties

Because it is ubiquitous and the main component in the cell, water might be regarded as a mere space filler in living organisms. Actually, Thales of Miletus with more insight praised water as the basic element. As the various aspects of the physics, chemistry, and biology of water have reached encyclopedic dimensions (Franks 1975–1982; Franks 1985–1990), this subject cannot be detailed to any great extent in this chapter. Instead, we will focus on liquid water and its significance in the context of the structure-function relation of biomolecules and their intrinsic and extrinsic stability.

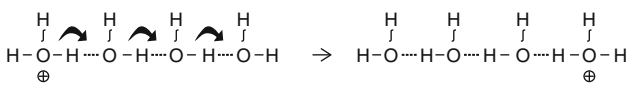
Evidently, from a physicochemical point of view, water is involved in biological processes as (1) medium in natural biotopes, (2) solvent within the cell, and (3) reactant or product in all biochemical and biophysical reactions. In the life cycle of the cell, this holds from the biosynthesis of proteins, nucleic acids, lipids, and carbohydrates to their degradation. Beyond this "housekeeping" part of life, water is of critical importance in the formation and maintenance of macromolecular and supermolecular structures; it determines not only the structure of the aforementioned biomolecules and their cellular compartmentation (e.g., in membranes) but also their function. For this reason, most cells have evolved mechanisms to control their water balance to avoid osmotic stress under extreme physical conditions (salt stress, desiccation, freezing, etc.; Hochachka and Somero 1973).

When compared with other common solvents, liquid water exhibits unique properties (*Table 17.1*).

Its anomalously high melting and boiling points, heat capacity, enthalpy of melting and vaporization, and its high surface tension all indicate that the forces of attraction between molecules in the liquid state must be significant. As a consequence of this internal cohesion, the molecular mobility and fluidity of water remain unchanged, even if aqueous solutions are confined to subnanometer films or pores (Raviv et al. 2001). This property is attributable to the structure of the H_2O molecule itself (*Fig. 17.1a*): The O atom shares an electron pair with each of the two H atoms. Owing to the repulsion of the paired electrons by the unpaired ones, the H-O-H bond angle of 104.5° deviates from the tetrahedral arrangement (109.5°), which is a characteristic of ordinary hexagonal ice (Pauling and Hayward 1964). As a consequence, the H_2O molecule possesses a high dipole moment (1.84 Debye) and a high dielectric constant, thus favoring dipole-dipole interactions involving hydrogen bonds (see below).

As shown in *Fig. 17.1b and c*, the water molecule has the ability to form four H-bonds, with two proton donor and two proton acceptor sites. The spatial disposition of these sites gives a clear visual conception of the geometry within each cluster.

To what extent does such ordered molecular arrangement persist in the liquid, bearing in mind that (compared to the covalent H-O-H bonds) the hydrogen bond is only a weak interaction? In this context, two observations are important: (1) As shown by the anomalously high conductivity of protons (= hydronium ions H_3O^+) in aqueous solution, the distinction between covalent and noncovalent bonds in a water cluster is blurred, since proton conductivity is "charge transfer without mass transport" according to



The reason is that in bulk water the transition of a bound proton to a neighboring free electron pair in a hydrogen bond takes less than 10^{-12} s (<1 ps [ps]); thus, the lifetime of a given $(H_2O)_n$ arrangement is adequately described as a "flickering cluster." (2) The enthalpies of melting and vaporization of ice and water allow an estimate of H-bonding in liquid water to be given: Close to the melting point,

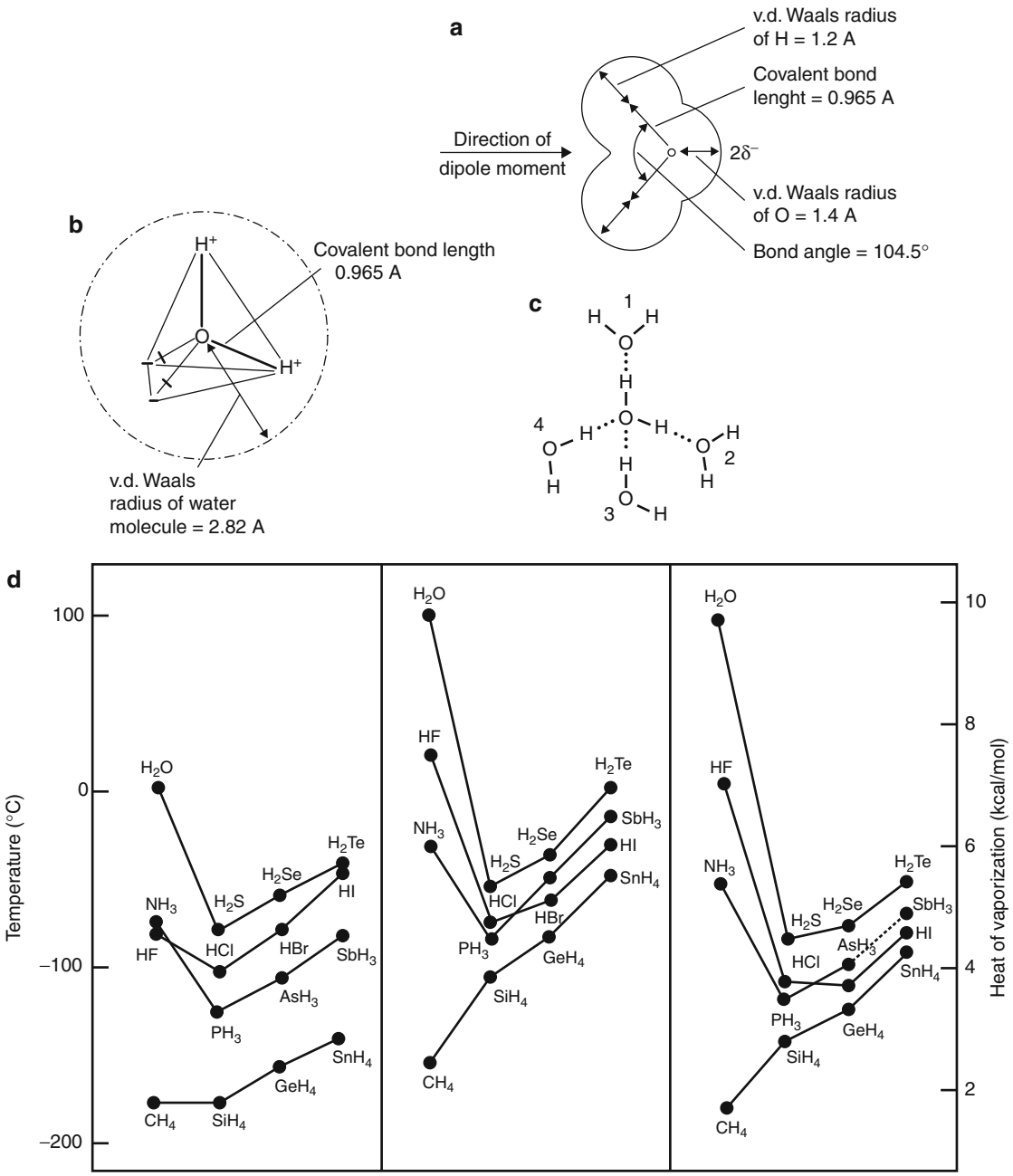


Fig. 17.1

The water molecule and its anomalies. (a) Ball-and-stick crystallographic model giving the bond angles and lengths. (b) Four-point-charge model with the oxygen atom placed in the center of a regular tetrahedron with vertices occupied by two positively charged hydrogen atoms and two negatively charged electron pairs. The distance of closest approach of two molecules (van der Waals radius) is 0.282 nm. (c) Schematic view of the tetrahedral hydrogen bonding around a water molecule in ice; molecule (1) and (2) and the central H_2O molecule are in the plane of the paper, while (3) and (4) are above and behind it. (d) Melting points (*left*), boiling points (*middle*), and heats of vaporization (*right*) of the isoelectronic sequences of hydrides in various rows of the periodic table. All three plots illustrate the effect of the anomalous interatomic forces between water molecules (Data from Pauling (1940), cf. Edsall and Wyman (1958))

around 85 % of the H-bonds in ice are still intact in the liquid state, whereas at the boiling point, single H_2O molecules prevail (Edsall and Wyman 1958). Obviously, thermal energy opposes the structural forces so that physical parameters as well as solutes of various kinds are expected to easily perturb water structure. In turn, water is capable of modifying the intermolecular interactions between solute molecules as well as intramolecular interactions within each solute macromolecule. Thus, the biological significance of water stems from the intimate details of the compromise between

Table 17.2
Physical properties of liquid water at varying temperature

	−25 °C	+25 °C	100 °C
Density ($g \cdot cm^{-3}$)	0.987	0.996	0.958
Heat capacity C_p ($J \cdot [mol \cdot K]^{-1}$)	80	75	76
Isothermal compressibility (10^6 MPa^{-1})	720	440	490
Hypersonic sound velocity ($m \cdot s^{-1}$)	1,220	1,480	1,540
Dielectric constant	102	79	65
Self-diffusion coefficient ($10^5 \cdot cm^2 \cdot s^{-1}$)	0.32	2.2	8.4
Viscosity (mPa·s)	6.5	0.89	0.28
pKw[a]	17.3	14.0	12.3

[a]Refers to the changed dissociation of water into H^+ and OH^- at varying temperature (Data taken from the first volume of Franks (1975))

water-water and water-solute interactions; the quantitative treatment of these interactions in terms of potential functions and activation profiles in the given multicomponent system is presently not feasible.

Temperature Dependence

As taken from its pressure/temperature (p/T) phase diagram over the whole biologically relevant range of hydrostatic pressure (<110 MPa, 1.1 kbar ~ 1,100 atm), water either is in its liquid state or is hexagonal phase I ice. Owing to its exceptionally low density, the latter shows a decrease of its freezing point with increasing pressure. Over the whole p/T range, there is no significant effect on the clustering of water molecules: Evidently, pressure alone does not break H-bonds (Groß and Jaenicke 1994). Focusing on isobaric conditions at atmospheric pressure, *Table 17.2* summarizes the change of some important physical properties of water over the temperature range between −25 °C and 100 °C, with +25 °C representing the common seasonal temperature in the natural environment of mesophilic species. It is obvious that the temperature dependences are not linear but become more pronounced at low temperatures. In fact, many physical properties of water appear to diverge at −45 °C (Franks 1985; Franks et al. 1990).

Apart from the temperature effects on water structure in terms of cluster size and water-solute interactions, from the biochemical point of view, the most relevant change that takes place at varying temperature refers to the changed dissociation of water into H^+ and OH^-, described by the equilibrium constant K_w or its negative logarithm pK_w. In an aqueous environment, the solvent acts as conjugate acid or base, and any change in K_w or pK_w will produce changes in the respective dissociation constants K_d or pK values of acids and bases that define ionization equilibria in solution. As both H^+ and OH^- are involved in most cellular processes (condensation, hydrolysis, reduction/oxidation, and membrane transport), it is likely that the large decrease in K_w with increasing temperature will affect equilibrium and kinetic processes. With increasing temperature, the dissociation of water increases, that is, pK_w decreases. Most biopolymers are polyelectrolytes, but the pK values of their ionizable groups do not necessarily show the same temperature dependence observed for pK_w. Therefore, both their net charge and the state of ionization of crucial functional groups will change with temperature.

Thus, their conformational stabilities and biological activities may be affected in a complex way because any such influences would presumably be compounded by changes in the dielectric properties of the solvent, especially in structures with high charge density such as hyperthermophilic proteins, nucleotides, and sulfated polysaccharides.

Hydration

The common knowledge that living cells and tissues contain around 70 % water means that all cellular components interact with water; their native conformation results from the balance between intra- and intermolecular forces, on the one hand, and forces resulting from interactions with the aqueous solvent, on the other. Since all major biomolecules and water have the strongly polar hydroxyl group in common, it is obvious that what was called "clustering" is not restricted to H_2O but also holds for proteins, nucleic acids, carbohydrates, and fatty acids and their constituents. Here, from the energetic point of view, the stabilizing effect of H-bond formation within a biomolecule is expected to be marginal, because most of the energy gained by forming the new "stabilizing bond" has to be paid by the breaking of a preexisting solute-water bond. Evidently, multiple H-bonds in cooperative units such as α-helices or strands of nucleic acids, as well as additional contributions (e.g., from hydrophobic constituents), may accumulate to reach a high energy of stabilization, frequently referred to as "conformational energy" (Kauzmann 1959; Franks 1975–1982; Franks 1985–1990; Dill 1990; Jaenicke 1991b; Pace et al. 1996).

In the case of carbohydrates and fatty acids, the dominance of the polar hydroxyl and carboxyl groups is evident. They are responsible for the high solubility of sugars and other oligo- and polyhydroxy compounds as well as uronic acids, *N*-acetyl glucosamine, etc. (Suggett 1975; Franks and Grigera 1990).

Conjugated with proteins, they allow the solubility and stability properties of their partner molecules to be modified (Kern et al. 1992, 1993). The net result of the aqueous environment for proteins is the protection of the nonpolar polypeptide core from the polar solvent. In nucleic acids, the situation is more complex: Here, secondary- and tertiary-structure formation is the result of an equilibrium between (1) electrostatic repulsion of the negatively charged phosphate groups along the linear polyelectrolyte, (2) stacking interactions and hydrogen bonding between the nucleotide bases, and (3) the conformational energy of the sugar-phosphate backbone. In its preferred conformation, the two polynucleotide strands in a duplex expose their deprotonated phosphates to the dielectric screening by the solvent, this way promoting the stacked arrangement of adjacent bases. As a result, a hydrophobic core is created in which H-bonds between the bases as well as additional sugar-base and sugar-sugar interactions are favored. The aqueous solvent contributes to the stability by (1) screening the charges of the phosphates, (2) hydrogen bonding to the polar exocyclic atoms of the bases, and (3) influencing the conformations of nucleotide constituents with methyl groups via nonpolar interactions (see below). Besides, because of the periodicity of the helical conformations, local binding sites of firmly bound structural water and linear arrangements of "bridges" of water molecules (involving nucleic acid polar atoms) can lead to favored structural arrangements with high conformational stability (Saenger 1984; Westhof and Beveridge 1990).

In spite of the well-established fact that the aqueous solvent is essential in accomplishing and maintaining the native state of biopolymers, so far theoretical treatments involving either distribution functions of the various intra- and intermolecular increments of stabilization or energy functions aimed at structural parameters of hydration have been of limited success. On the other hand, a wealth of experimental data has been accumulated applying a wide variety of techniques: X-ray and neutron diffraction, hydrogen-deuterium exchange, Raman, infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy, sorption/desorption of water vapor, calorimetry (including heat capacity calorimetry), dilatometry, sedimentation analysis, viscometry, and other hydrodynamic methods. The reason why all of them "gave a better view of the experimenters' interests than of water structure" (G.A. Jeffrey) is that there is no clear definition of "hydration" (or "solvation" in general); in addition, the problem of how to separate the contributions of the solute from the background of the solvent and from potential conformational effects in a highly dynamic system is still unresolved.

Considering the various experimental approaches, the most detailed information is expected from high-resolution X-ray data and solution NMR (Westhof and Beveridge 1990; Otting et al. 1991; Frey 1993; Goodfellow et al. 1994). Comparing the results, it is important to note that in the crystalline state, lattice forces may affect the number and position of spatially well-defined water molecules, so that in different crystal forms, not all binding sites are conserved (Zhang and Matthews 1994). NMR measurements allow two qualitatively different types of hydration sites to be distinguished: (1) a small number of interior water molecules (with residence times of 10^{-2}–10^{-8} s and X-ray coordinates in the crystal) and (2) surface hydration (with residence times $<10^{-9}$ s, not necessarily fixed in the crystal structure; Otting et al. 1991). How the latter type of hydration compares to the hydrodynamically relevant bound water is still unresolved. Regarding hydration/dehydration at elevated temperature, phase separation and neutron scattering measurements have been applied mainly to quantify macromolecular interactions, especially in protein mixtures. Available data are rudimentary and far from being understood in quantitative terms (Benedek 1997; Tardieu et al. 1999; Jaenicke and Slingsby 2001). In qualitative terms, at this point, theoretical approaches are promising in interpreting available experimental data: Using the RNA duplex $r(CpG)_{12}$ as a structurally well-defined model (Conte et al. 1996; Gyi et al. 1998), molecular dynamics simulations at 5 → 40 °C gave evidence for a significant decrease in the residence time of water molecules and potassium ions bound in the first coordination sphere of the duplexes, indicating decreased order in the solvent around the solute with increasing temperature (Auffinger and Westhof 2002; E. Westhof, personal communication). In general, unfolding and/or aggregation of the solute upon melting lead to a drastic further release of water (Jaenicke 1971; Lauffer 1975; Jaenicke and Seckler 1997).

The present knowledge of the role of water in connection with the stability and activity of biomolecules may be summarized as follows:

1. *Dielectric constant (ε).* The formation and maintenance of cellular components occur in the presence of excess water; thus, the weak interactions responsible for their various functions are governed by the energetics of solvated partners in a strong dielectric, dielectric (∼80), not by interactions in vacuo (= 1) (Dill 1990).
2. *Temperature effects on biomolecules and water.* High temperatures alter the energetic properties (e.g., vibrational modes) of biomolecules in their aqueous solvent, which itself shows a strong temperature dependence in its interaction parameters (cf. ❯ *Table 17.2*); both levels are intertwined in a complex way.
3. *Hofmeister effects.* In the case of high charge densities on the surface of polyelectrolytes, as well as in the presence of high salt concentrations (e.g., in halophiles), electrostatics are complicated by the clustering of water around the charged groups and by the competition of (counter-)ions for their own water of hydration (Jaenicke 1991b). At this point, the fundamental laws of electrochemistry have to consider the specific effects of the size, charge, and hydration of each individual electrolyte, which taken together determine the solubility of polyelectrolytes (salting-in and salting-out effects) and many other physicochemical and biological phenomena. The immediate cause of the "Hofmeister series of cations and anions" is the differences in hydration attributable to the intensities of the electrostatic field

around each specific ion (von Hippel and Schleich 1969). The "Hofmeister series" was first reported by Hofmeister (1888) for the coagulation of lyophilic colloids and later for many other physical, chemical, and biological phenomena (Edsall and Wyman 1958). Ordering cations and anions according to their capacity to promote the solubility of neutral and alkaline proteins, the following two series are observed: $Li^+ < Na^+ < K^+ < NH_4^+ < Mg^{2+}$ and $SO_4^{2-} < PO_4^{3-} < CH_3COO^- < citrate^{3-} < Cl^- < ClO_4^- < Br^- < I^- < SCN^-$. The solubilizing or precipitating effects can be explained in terms of the competition for water between a polyelectrolyte (here the protein) and excess electrolyte in the solvent (Collins and Washabaugh 1985; Baldwin 1996; Jaenicke and Seckler 1997). One important Hofmeister effect is that guanidine denaturation depends on the anion. While guanidinium sulfate ($[Gdm]_2SO_4$) has no denaturing effects, GdmCl is a strong denaturant and GdmSCN is the strongest chaotropic agent.

4. *Volume effects of solutes.* Considering the "structure-making effects" of biomolecules in water, peripheral charges and nonpolar groups exposed to the aqueous solvent show an anomalous increase in solvent density (decrease in volume), "electrostriction" in the case of ions and "iceberg formation" in the case of exposed nonpolar groups (Kauzmann 1959; Jaenicke 1981; Groß and Jaenicke 1994).
5. "*Hydration numbers.*" Attempts to quantify both the hydrophobicity and hydration of amino acid residues can be summarized by the following series (with hydration estimated by the number of moles of H_2O per mole of amino acid residue in parenthesis): Phe (0); Cys, Gly, Ile, Leu, Met, and Val (1); Ala (1.5); Arg^+, Pro, and Tyr (3); His^+ and hydroxyproline (4); Lys^+ (4.5); Asp^- (6); and Glu^- and Tyr^- (7.5) (Kuntz 1971; Kuntz and Kauzmann 1974; Kyte and Doolittle 1982).
6. *Types of "bound water."* In comparing tabulated hydration data from X-ray crystallography, NMR (see above), and thermodynamic and hydrodynamic measurements, it becomes clear that different experimental approaches "see different types of bound water molecules." Most of the respective terms are self-explanatory; since space does not permit a detailed discussion, key references may suffice: structural hydration (the number and positions are determined from X-ray and NMR coordinates), hydrodynamic hydration (the amount of "hydration shell" migrating with the solute is determined in sedimentation/diffusion experiments and viscometry), low-temperature hydration (estimated, e.g., as "non-freezable water"), and preferential hydration (measured by thermodynamic methods in the presence of low molecular weight additives competing with the macromolecular component for its hydration) (Tanford 1961; Kuntz and Kauzmann 1974; Franks, 1975–1982; Franks 1985–1990; Franks and Eagland 1975; Eisenberg 1976; Timasheff 1995; Timasheff and Arakawa 1997).

   In the case of proteins, quantitative data vary between 0.25 g and 0.40 g of H_2O per g of protein. A rough estimate of the bound water surrounding the protein whose properties differ from those of the bulk water can be obtained as the sum of the hydration numbers of the constituent amino acids (see 5 above); this holds despite the fact that part of the amino acid residues are buried in the protein interior and not accessible to the solvent.
7. *Dehydration causes denaturation.* If the structure-function relation of lysozyme is taken as a model, it becomes clear that dehydration below the limiting value of 0.25 g of H_2O per g of protein causes reversible deactivation paralleled by drastic changes of all available physical characteristics (Careri et al. 1980).
8. *Residual hydration of proteins.* Complete dehydration (e.g., by freeze-drying or high-temperature dry-weight determination) cannot be accomplished. Even dry biopolymers still contain residual water of the order of 5–10 %; before the dry state is reached, chemical modification (such as deamidation) occurs.
9. *X-ray structures yield biologically relevant information.* Biopolymer crystals investigated by X-ray crystallography contain ca. 50 % aqueous mother liquor filling the space between the single molecules; thus in the crystal, there is sufficient water available to guarantee complete hydration, that is, native-like conditions. From this we may conclude that three-dimensional (3D) structures based on crystallographic data are biologically relevant.
10. *Structure determination at high temperature.* Regarding the structural analysis of macromolecular components from thermophiles and hyperthermophiles at elevated temperature, only sparse high-resolution data have been reported. It would be desirable to develop the necessary methods to bridge this gap in order to gain insight into the correlation of stability and molecular flexibility up to the temperature limit of viability.

Stability of Biomolecules

Intrinsic Versus Extrinsic Stability

The physical limits of life at high temperature are defined by the temperature dependence of the interatomic forces involved in the covalent and noncovalent stabilization of the molecular inventory of the cell. Except for membranes, the lipids of which often are anomalous phytanyl ethers instead of fatty-acid esters (see below), the inventory of thermophilic and hyperthermophilic cells consists of the same building blocks as those found in mesophilic cells. The occurrence of covalent modifications such as methylation and glycosylation of biopolymers from hyperthermophiles has been frequently reported; whether they are biologically relevant molecular strategies of stabilization is still unclear (Vieille and Zeikus 2001; see sections on "Adaptive Stabilization Mechanisms of Nucleic Acids" and "Adaptive Stabilization Mechanisms of Lipids and Membranes" in this chapter).

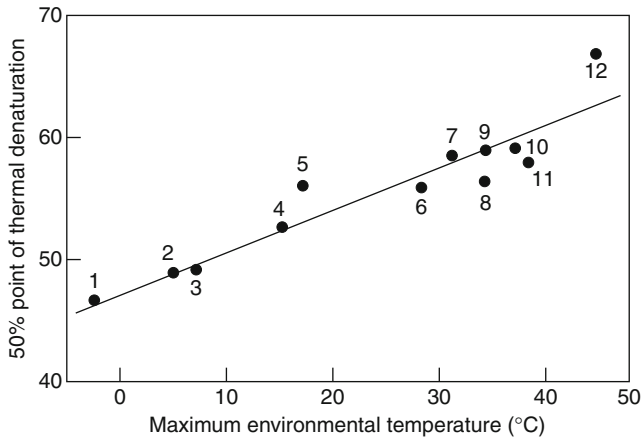


Fig. 17.2

The thermal stabilities of eye lens crystallins increase with the respective physiological temperatures of vertebrates. Coordinates refer to the 50 % points of thermal denaturation and the adaptation temperatures of the following species: *1 Pagothenia borchgrevinki* (Arctic fish), *2 Coryphaenoides armatus* (deep-sea fish), *3 Coryphaenoides rupestris* (deep-sea fish), *4 Oncorhynchus mykiss* (rainbow trout), *5 Cebidichthys violaceus* (tidepool fish), *6 Rana muscosa* (frog), *7 Alticus kirkii* (Red Sea fish), *8 Rana erythraea* (frog), *9 Gekko gekko* (lizard), *10 Rattus norwegicus* (rat), *11 Tropidurus hispidus* (reptile), and *12 Dipsosaurus dorsalis* (desert iguana) (Data from McFall-Ngai and Horwith (1990))

Mutational Adaptation

Given the conventional set of canonical nucleic acid bases and amino acids, the general response to evolutionary stress is the selection for beneficial mutations on the genome level. In the case of thermophilic adaptation, these mutations lead to an enhancement of the intrinsic stability of the protein inventory.

At high temperature, the integrity of nucleic acids is threatened by strand separation and chemical damage to the nucleotide chains. Mechanisms providing intrinsic stabilization comprise an increase in G + C content of tRNAs and rRNAs and posttranscriptional modification. Extrinsic stabilization may be provided by specific salts or histone-like proteins and by efficient repair systems (Grogan 1998; di Ruggiero et al. 1999; for details, see section on "Adaptive Stabilization Mechanisms of Nucleic Acids" in this chapter).

Considering proteins, the thermal stabilities of the orthologous homologs are found to be positively correlated with the maximal environmental temperature (see also Alexandrov 1969; Hochachka and Somero 1984, and Dahlhoff and Somero 1993; *Fig. 17.2*).

Thus, the balance between stabilizing and destabilizing forces is adjusted during evolution such that homologous proteins from different species retain similar conformational stabilities at their respective physiological temperatures (Jaenicke 1991b; Somero 1995, 2000). Generally, both thermophilic and hyperthermophilic proteins exhibit high intrinsic stabilities and long denaturation half-lives of the order of hours close to the boiling point of water (Jaenicke et al. 1996; Daniel and Cowan 2000; Jaenicke and Böhm 2001). Basically, the natural amino acids would allow the formation of proteins with stabilities exceeding the magic upper temperature limit of ~115 °C (de Grado 1988; van den Burg et al. 1998). What constrains evolution for maximum thermal stability becomes obvious, keeping in mind that proteins are fundamentally multifunctional, combining the capacity to fold, serve a wide range of functions, and be degradable at the same time. As a consequence, protein evolution is a compromise between rigidity (stability) and flexibility (function, regulation, and turnover; Wetlaufer 1980; Somero 1995; Jaenicke 2000a, b). At this point, it needs to be mentioned that the correlation between molecular flexibility and function (i.e., catalytic activity) is ambiguous and cannot be generalized because conformational stability is a global property, whereas the influence of flexibility on stability may be either global or local, as shown by kinetic unfolding experiments (Jaenicke 1999; Bieri and Kiefhaber 2000; Jaenicke and Lilie 2000; Wright and Baldwin 2000). The question of how local motions involved in the catalytic reaction are correlated with the fast anharmonic global dynamics, monitored spectroscopically or by X-ray analysis, is still open (Daniel et al. 1998, 1999). Comparing the Arrhenius activation energy and thermal stability of various enzymes, a new intrinsic thermal parameter, *Teq*, was defined that arises from the *T*-dependent equilibrium between the active and inactive enzyme at its true temperature optimum (Peterson et al. 2004). Beyond *Topt*, the decrease in enzyme activity, induced by the *T*-dependent shift in the equilibrium, is up to two orders of magnitude greater than what occurs through irreversible thermal denaturation. *Teq* is central to the physiological adaptation of an enzyme to its environmental temperature, linking the molecular, physiological, and environmental aspects of adaptation.

Disulfide Bonds

Disulfide bonds are known to be of utmost importance in stabilizing proteins such as hormones, plasma proteins, or hydrolases and their inhibitors (Cecil and McPhie 1959; Cecil 1963; Friedman 1973; Schulz and Schirmer 1979; Fersht 1998a; Branden and Tooze 1999). Because the environment inside typical cells is reducing, cystine cross-links are rarely found in intracellular proteins; in those exceptional cases where they are found, they usually exist transiently, playing roles in redox signaling or disulfide exchange, rather than serving to stabilize proteins, as they do outside the cell.

The suggestion that cystine might be essential in stabilizing cytosolic proteins in thermophiles was raised by a series of crystal structures of archaeal and bacterial proteins, for example, elongation factor Ts from *Thermus thermophilus* (Jiang et al. 1996), TATA-box binding protein from Pyrococcus woesei (De Decker et al. 1996), triosephosphate isomerase from *Thermotoga maritima* (Maes et al. 1999), adenylosuccinate

lyase from *Pyrobaculum aerophilum* (Toth et al. 2000), ferric reductase from Archaeoglobus fulgidus (Chiu et al. 2001), and ferredoxin Fd1 from *Aquifex aeolicus* (Meyer et al. 2002). Based on this structural evidence, computational genomics and proteomics were applied to do a careful sequence-structure mapping study over the completely sequenced microbial genomes (Mallick et al. 2002). As a result, it turned out that in the case of the intracellular proteins of certain hyperthermophilic archaea, especially the two crenarchaea *Pyrobaculum aerophilum* and *Aeropyrum pernix*, there is a clear preference for even numbers of cysteine residues that are mapped within disulfide bonding distance: More than 40 % of the cysteine residues in *Pyrobaculum aerophilum* are predicted to be involved in disulfide bonds. Experimental findings support the computational results (T. O. Yeates, 2002, personal communication).

Stabilizing Additives and Molecular Chaperones

Apart from the intrinsic stabilization coming from contributions of intra- and intermolecular interactions within and between biomolecules, additional extrinsic stability increments may come from ligand binding, preferential solvation in the presence of high concentrations of compatible solutes, crowding, and the action of molecular *chaperones*. Circumstantial evidence indicates that crowding effects within the living cell may extend the temperature range of stability significantly (Hochachka and Somero 1973; Carpenter et al. 1993; Somero 1995; Timasheff 1995; Blöchl et al. 1997; Jaenicke 2000a; Minton 2000).

The discussion of intrinsic and extrinsic stability would be incomplete without mentioning molecular chaperones as accessory components involved in the stabilization of proteins at the borderline between self-organization and destruction. Functionally they are known to promote the long-term stability of proteins by regulating the kinetic partitioning of polypeptides between proper folding and association, on the one hand, and misfolding and subsequent aggregation or degradation, on the other (Jaenicke 1987, 2004; Zettlmeissl et al. 1979; Goldberg et al. 1991; Kiefhaber et al. 1991; Jaenicke and Seckler 1997). Using the primary meaning of the word, molecular chaperones avoid the "illegitimate interactions" between nascent or folding chains by keeping the level of aggregation-competent polypeptide chains below a critical concentration, either by complex formation or by "iterative annealing," without becoming integral parts of the final native structure. The binding energy that drives the formation of the complex between the protein substrate and its chaperone may be used to rescue nascent or folding chains already on an off pathway of proper folding (Beissinger and Buchner 1998; Burston and Saibil 1999; Jaenicke and Lilie 2000; Leroux and Hartl 2000; Walter and Buchner 2002).

From their designation as "heat-shock proteins" (HSPs) (or "thermosomes" in the case of thermophilic archaea), one might expect that close to the limit of viability they would represent prototypes of proteins showing exorbitantly high intrinsic stability. However, as in the case of mesophiles, the term is a misnomer: Heat-shock proteins are ubiquitous both in mesophiles and extremophiles, also under physiological conditions. Ubiquitous refers not only to the three phylogenetic domains, eukarya, bacteria, and archaea, but also to all the branches of the phylogenetic tree. In the case of (hyper-)thermophilic archaea, this means that all phyla, including the newly discovered Nanoarchaeota (Huber et al. 2002), contain HSPs or thermosomes and other types of chaperones (Baross and Holden 1996; Waters et al. 2003; Laksanalamai and Robb 2004).

Considering the concise definition of molecular chaperones as "any protein that transiently interacts with and stabilizes an unstable conformer of another protein, facilitating its folding, assembly and interaction with other cellular components, as well as its intracellular transport or proteolytic degradation" (Leroux and Hartl 2000), it is obvious that accessory proteins that assist protein folding, compartmentation, turnover, etc., must be of utmost importance in thermophiles. As a matter of fact, representatives of most chaperone families, Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, GimC (prefoldin), and Hsp 16.5 (sHsp), have been isolated and studied in detail, in certain cases to the level of high-resolution 3D structures and systematic investigations of their structure-function relationship. From the physicochemical and functional point of view, they do not exhibit anomalous characteristics, except for differences in their quaternary structure and their ATP requirement. Space limits do not permit a detailed presentation of the rapidly growing field. Some aspects will be discussed in connection with the adaptive stabilization mechanisms of proteins. For reviews, see Fink and Goto (1997), Bukau (1999), Pain (2000), and Kiefhaber and Buchner (2004), and for special systems, Trent et al. (1991), Phipps et al. (1991), Trent (1996), Baross and Holden (1996), Trent et al. (1997), Andrä et al. (1998), Kim et al. (1998, 2003), Huber et al. (2002), and Laksanalamai and Robb (2004). At this point, we focus our attention to only a few general observations that are connected with systematic, structural, cell biological, and mechanistic aspects:

1. *Systematics*. The paradigm for chaperone-assisted protein folding has been the *group I* GroE system from *Escherichia coli*, which is generally found in bacteria and eukaryotic organelles of bacterial origin (Sigler et al. 1998; Hartl and Hayer-Hartl 2002). The *group II* homologs in archaea (and the cytosol of eukaryotes) show a number of distinct features, as expected from the low sequence identity (<25 %): Group I forms cages made up of seven-membered rings of Hsp60 subunits (as chaperone) and Hsp10 subunits (as co-chaperone), whereas *group II* consists of eight- or nine-membered hetero-oligomeric rings, with no general co-chaperone. The domain organization (equatorial ATPase domain, apical *recognition* domain, and intermediate *connecting* domain) has been conserved between the two groups. However, in the "protrusion region" of the apical domain, significant differences have been discovered that allow the binding of the substrate protein without the help of the *group I* co-chaperone to be explained (Heller et al. 2004).

2. *Beyond systematics: horizontal gene transfer.* As one would expect after the discovery of horizontal gene transfer, nature does not follow simple systematic rules. In the case of *group I* and *group II* chaperones, an archaeon has been found in which both types of HSPs, group I and group II, coexist. Inspecting the complete genomes of several species of the genus Methanosarcina, the first archaeal genomes were identified to contain both the GroEL/GroES (*group I*) and the thermosome/prefoldin (*group II*) genes. Both chaperones are coexpressed in the cytosol; under heat stress, they are moderately induced. The GroE proteins show the structural features of their bacterial counterparts, whereas the thermosome contains three paralogous subunits (α, β, and γ) which assemble at a molar ratio 2:1:1. As shown in vitro, the ATP- and ADP-dependent assembly reaction is regulated by the β subunit. The role of the two chaperones in one and the same cellular compartment with respect to substrate specificity and protein sorting from the ribosome to the proper chaperone machine is still enigmatic (Klunker et al. 2003; Figueiredo et al. 2004).
3. *Mechanism.* The functional significance of the structural flexibility ("plasticity") of proteins has been a well-established paradigm in the elucidation of enzyme mechanisms, self-assembly processes, molecular mechanics, etc. In the case of assisted protein folding, the molecular machines nature has developed during evolution are absolutely unique: Binding a nascent or (re-)folding polypeptide chain via aggregation-competent hydrophobic core residues in a hydrophobic cage-like protein assembly, altering the hydrophobic surface of the cage into a hydrophilic one by closing the lid, and now allowing the secluded substrate polypeptide to find its energy minimum in the natural microenvironment sound like a magician's trick, but that is what the thermosome manages to achieve (Gutsche et al. 1999; Bosch et al. 2000).
4. *Expression level.* The level of chaperone expression may vary over a wide range. In Pyrodictium occultum, a shift from 102 °C to 108 °C has been reported to enhance the level of two bitoroidal hexadecameric ATPases of 56 kDa and 59 kDa with optimal activity at 100 °C to 80 % of the total cytosolic protein concentration (Phipps et al. 1991; Baross and Holden 1996). Similarly, electron micrographs of T-stressed *Sulfolobus shibatae* cells display a dense filamentous network of bitoroidal octodecamers, which suggests chaperone assemblies play a cytoskeletal role in archaea (Kagawa et al. 1995; Trent et al. 1997). On the other hand, the quantitative assessment of the role of the GroE system in protein folding in *Escherichia coli* suggested that there is sufficient GroEL to facilitate the folding of no more than 5 % of all of cellular proteins within the cell (Lorimer 1996); interestingly, overexpression of GroEL to high cellular levels in *Escherichia coli* does not inhibit cell growth (R. Rudolph, 2004, personal communication).
5. *Function of recombinant thermosomes.* The hyperthermophilic thermosomes from Pyrodictium occultum (*P.o.*) and Methanopyrus kandleri (M.k.) are members of the Hsp60 family. They form high molecular mass complexes, arranged in two rings of eight subunits each, stacked back to back, without Hsp10 as a co-chaperone. In *P.o.* two types of subunits (α and β) participate in the formation of the cage; they seem to alternate within each of the two rings. Overexpressing the two polypeptides separately and jointly in Escherichia coli yields authentic hexadecameric quaternary structures for all three-all-α, all-β, and α + β. All three exhibit ATPase activity and bind denatured protein substrates, inhibiting their heat aggregation. At temperatures up to 55 °C, no release of renatured substrate was detectable. For technical reasons, experiments at physiological temperature were not feasible (Minuth et al. 1998). Switching to *M.k.* avoided the ambiguities caused by the hetero-polymeric quaternary structure of the *P.o.* thermosome. Strangely enough, its synthesis is not increased upon heat shock, and its ATPase activity depends on NH_4^+. The homo-hexadecameric recombinant protein is authentic and shows chaperone-like activity; again, no release of the substrate polypeptide chains is detectable at temperatures up to 60 °C (Minuth et al. 1999).
6. *Structural studies at high temperature.* As taken from the thermosome example, collecting structural and functional data at the temperature limits of hyperthermophiles would be highly desirable. In this context, novel approaches have been developed. For example, in the case of the above mechanism of *group II* archaeal chaperones (see point 1), the functional details were deduced from differences between X-ray and solution-NMR data at varying temperature (Heller et al. 2004). Unfortunately, for both experimental approaches, presently data collection at temperatures close to or beyond the boiling point of water is impeded by crystallization problems and line broadening as well as signal overlap. In the case of NMR, using dipolar couplings allowed the loss of NOE information at high temperature to be compensated; on the other hand, assigning and separating single resonances of aromatic amino acids was facilitated by selective ^{19}F-labeling of Trp residues. Using the cold-shock protein (Csp) from *Thermotoga maritima* as a model, both methods were applied to extend structural studies into the physiological temperature regime. Taking the room-temperature structure as a reference, most significant alterations at high temperature occur in regions of the molecule that have been modeled as binding sites for single-stranded DNA, in agreement with the idea that *Tm*Csp plays a central role in the regulation of gene expression under cold-shock conditions (Jung et al. 2004). ^{19}F tryptophan labeling was used to study the folding of *Tm*Csp over a wide temperature range. In combination with stopped-flow experiments at lower temperatures, global line-shape analysis showed that the folding rate of *Tm*Csp closely resembles data collected for mesophilic Csps. However, the unfolding rate constant of *Tm*Csp is two orders of magnitude lower over the entire temperature range. Thus, stability differences are solely due to differences in the unfolding rates of the mesophilic and thermophilic proteins

(Sterner and Liebl 2001). A thermodynamic analysis points to an important role for entropic factors in the stabilization of *Tm*Csp relative to its mesophilic counterparts (Schuler et al. 2002).

7. *Small heat-shock proteins (sHSPs).* sHSPs from thermophilic and hyperthermophilic organisms form multimeric complexes with (occasionally heterodisperse) molecular masses ranging from 200 kDa to more than 1 MDa. Although they show high diversity, the majority of acid sHSPs share amino acid sequence similarity with the vertebrate eye lens α-crystallins; both groups are molecular chaperones (Jacob et al. 1993; Jaenicke and Creighton 1993). Presently available sparse structural data suggest that their monomeric structures share a common building-block structure (van Montfort et al. 1992). The mechanism of action seems to be defined by the individual quaternary structure; evidently, dissociation/association reactions play a role in the regulation of chaperone activity (Laksanalamai and Robb 2004). In contrast to the acidic chaperones (IP ∼ 4.6), basic sHSPs (IP ∼ 9) are involved in nucleotide binding (Korber et al. 2000).

Measuring Thermodynamic Stability

The conventional introduction to the subject, with its emphasis on heat engines, is almost certain to convince the student that thermodynamics is sheer sophistry and unrelated to the real business of biochemistry. But an understanding of some of the ideas of thermodynamics is important to discover how molecules make organisms work (van Holde 1985).

The thermodynamic stability, for example, of nucleic acids or proteins, can be quantified by measuring the temperature- or denaturant-induced unfolding, excluding irreversible side reactions such as chemical modifications or aggregation (Tanford 1968; Tanford 1970; Privalov 1979; Jaenicke and Seckler 1997). To illustrate the procedure, we assume an N U equilibrium transition of a monomeric globular protein from its native (N) to the denatured state (U). The free energy of conformational stability is the difference between the free energies of the unfolded and the folded states

$$\Delta G_{stab} = G_{unfolded} - G_{native} \qquad (17.1)$$

as well as the Gibbs-Helmholtz equation

$$\Delta G_{stab} = \Delta H_{stab} - T\Delta S_{stab} \qquad (17.2)$$

where T is the absolute temperature and ΔH_{stab} and ΔS_{stab} are the enthalpy and entropy differences between the unfolded and the folded states

$$\Delta H_{stab} = H_{unfolded} - H_{native} \qquad (17.3)$$

can be determined either directly from calorimetric experiments (ΔH_{cal}) or from a "van't Hoff plot," that is, from the temperature dependence of the apparent equilibrium constant K of the transition

$$N \overset{K}{\Leftrightarrow} U \qquad (17.4)$$

according to

$$\Delta H_{van't\ Hoff} = RT^2\, d(\ln K)/dT \qquad (17.5)$$

where R is the gas constant. The "two-state assumption" underlying ❯ Eq. 17.4 can be tested by comparing the results of both approaches. If only N and U and no intermediates are populated in the N → U transition, ΔH_{cal} equals $\Delta H_{van't\ Hoff}$; otherwise, $\Delta H_{cal}/\Delta H_{van't\ Hoff}$ is >1 (Privalov 1979; Jaenicke 1991b).

A further qualitative standard criterion to test the two-state assumption is to compare the denaturant-induced equilibrium transitions monitored by different spectral properties of a protein. If the unfolding profiles do not coincide, intermediates are present in significant amounts, and ❯ Eq. 17.4 does not adequately describe the denaturation process (Pace and Scholtz 1997). If ❯ Eq. 17.4 is sufficient to describe the reaction, the thermodynamic stability of the protein can be calculated from the apparent equilibrium constant K according to

$$\Delta G_{stab} = -RT \ln K \qquad (17.6)$$

Under physiological conditions, that is, in water at constant pH, pressure, and temperature (pH 7, 1 bar, 25 °C), the change in Gibbs free energy as 1 mole of substrate is converted to 1 mole of product ([substrate] = [product] = 1 mole/l) represents the standard Gibbs free energy change $\Delta G^{\circ\prime}$. Given the high molecular mass of proteins and their relatively high partial volume, molar concentrations are experimentally inaccessible. In the case of simple first-order reactions such as ❯ Eq. 17.4, this is irrelevant; in more complex reactions, to compare the stabilities of proteins, their free energies have to be normalized, for example, to millimolar or micromolar concentrations (Dams and Jaenicke 1999).

It is obvious that the above equilibrium constant K depends on the denaturation conditions, because in a given experiment, different variables may be superimposed in the destabilization of a protein. For example, sometimes neither low pH nor chaotropic agents (urea or guanidinium chloride) alone are able to denature a protein. This holds especially for ultrastable proteins from hyperthermophiles, where most proteins only unfold beyond the boiling point of water; thus, to shift the melting temperature (T_m) at which half of the protein is thermally unfolded down to a manageable range, low pH values or the addition of chaotropic agents is required. The measured ΔG_{stab} values at these nonphysiological solvent conditions have to be extrapolated to obtain ΔG_{stab} data at zero denaturant concentration; this allows the thermodynamic stabilities of different proteins, for example, from mesophiles and hyperthermophiles, to be compared (Privalov 1979; Pace 1986; Pace and Scholtz 1997; Pfeil 1998).

Because of the significant temperature dependence of ΔH_{stab} and ΔS_{stab}, ΔG_{stab} versus T profiles observed for the thermal unfolding of proteins exhibits parabolic characteristics (Privalov 1979; Schellman 1997; ❯ *Fig. 17.3a*); their maxima cluster in a narrow range between 30 kJ/mol and 80 kJ/mol

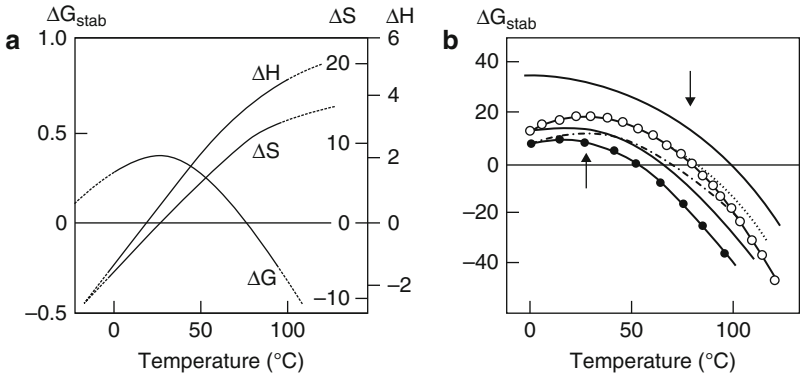


Fig. 17.3

Temperature dependence of ΔH, ΔS, and ΔG_{stab} of proteins. (a) Temperature dependence of the enthalpy, entropy, and free energy of stabilization (ΔG_{stab}) of sperm whale myoglobin, calculated per mole of amino acid residues (Privalov 1979; Privalov and Gill 1988). (b) ΔG_{stab} versus T profiles of structurally related proteins from mesophiles and hyperthermophiles. The various profiles illustrate that enhanced thermal stability may be accomplished either by flattening the parabola or by an upward shift (to a higher overall free energy), or by a shift to higher temperature; they belong to the β-barrel DNA-binding protein Sso7d from *Sulfolobus solfataricus* (—), all-β tyrosine kinases BtkSH3 (– – –) and Tec-SH3 (• – • –), α-spectrin (––), CspA from *Bacillus subtilis* (•), and Csp from *Thermotoga maritima* (o). The arrows at ∼30 and 80 °C refer to the physiological optimum temperatures of *B. subt* and *T. maritima*, respectively (Taken from Jaenicke (2000a))

(7–20 kcal/mol; *Fig. 17.3b*); other modes of denaturation such as guanidinium chloride, urea, or pH give the same result, provided they are corrected to standard conditions (Makhatadze and Privalov 1995; Pfeil 1998). Thus, in spite of the large number of noncovalent contacts maintaining the native structure of proteins, ΔG_{stab} is only marginal, no more than the equivalent of a few weak intermolecular interactions, even in the case of extremophilic proteins. Representing the minute difference between the large contributions of attractive and repulsive forces, ΔG_{stab} determines the close packing of the polypeptide chain and the minimization of the hydrophobic surface area that are typical for globular proteins in aqueous solution. The balance of attraction and repulsion corresponds exactly to the above-mentioned compromise between rigidity and flexibility.

In contrast to the two-state assumption, protein denaturation is rarely fully reversible, so that determining the thermodynamic stability in terms of ΔG_{stab} may be difficult or even impossible. In these cases, operational definitions of stability are used to characterize proteins, especially if homologs from different species or wild-type and mutant proteins are compared. To this end, most commonly apparent (nonequilibrium) T_m values or the denaturant concentration at which half of the protein is unfolded ($c_{1/2,urea}$ or $c_{1/2,GdmCl}$) is used. For multidomain and multisubunit proteins, this approach has often been the only means to obtain at least a qualitative estimate of stability.

Thermodynamics Versus Kinetics

Thermodynamics and kinetics are commonly considered as separate domains; the first, with emphasis on energy and mass action, ignores time as a physical parameter, while the second is focused on reaction coordinates and rates. However, describing chemical equilibrium as the situation defined by the balance of the formation and decay of the reaction product,

$$K = \frac{\vec{k}}{\overleftarrow{k}} \qquad (17.7)$$

with K as the equilibrium constant, and as rate constants of the forward and backward reactions, kinetics and thermodynamics are obviously connected. Combining Eqs. 17.6 and 17.7, evidently, kinetics can be used to define and measure stability: Higher ΔG_{stab} values, for example, of thermophilic proteins compared to homologous mesophilic proteins, could be due to an increase in the rate of folding or a decrease in the rate of unfolding, or a combination of the two. The rate of the unfolding reaction is limited by the highest activation barrier upon the N → U transition. Thus, protein stability may be governed, in terms of kinetic stabilization, by a high free energy of activation ($\Delta G_{N\rightarrow U}$) that separates N from the transition state. In a number of cases, the rate of unfolding of hyperthermophilic proteins has been shown to be drastically decelerated compared to their mesophilic counterparts, supporting the view that the increase in $\Delta G_{N\rightarrow U}$ is responsible for the enhanced ΔG_{stab} (Sterner and Liebl 2001). To give an example, the cold-shock proteins from *Bacillus subtilis* (equilibrium unfolding at T_m = 52 °C), *Bacillus caldolyticus* (T_m = 72 °C), and *Thermotoga maritima* (T_m = 90 °C) show a dramatic decrease in their unfolding rates with increasing T_m, whereas folding occurs at closely similar high rates (τ = 1.0 ± 0.2 ms; Perl et al. 1998, 2000; Perl and Schmid 2001). Evidently, from the ecological point of view, a high $\Delta G_{N\rightarrow U}$ may provide a significant advantage for the hyperthermophilic organism, because, owing to the turbulence in hydrothermal vents, exposure times to a lethal temperature range may be short compared to the half time of the N → U unfolding transition; on the other hand, the fast folding reaction allows the

rapid formation of the compact native structure, this way protecting the nascent protein during the folding process against irreversible damage by covalent modification or aggregation (Hensel et al. 1992; Jaenicke and Böhm 1998; Plaza del Pino et al. 2000).

Forces and General Mechanisms in Protein Stabilization

The observation that the Gibbs free energy of stabilization represents a minute difference between strong attractive and repulsive potentials means that proteins exist close to the borderline of denaturation. Comparing tabulated ΔG_{stab} values with the bond energies of the relevant weak interactions (Bernal 1939; Bernal 1958; Kauzmann 1959; Stillinger 1977; Pfeil 1998), it becomes clear that a few hydrogen bonds, or a hydrophobic patch, or just one ion pair may suffice to shift the optimum stability of proteins from the mesophilic to the thermophilic temperature regime (*Fig. 17.3b*). Evidently, evolution can choose between an astronomical number of ways to adapt to extreme conditions; therefore, no general rules of protein stabilization are to be expected. The following brief survey of the interatomic forces that contribute to macromolecular stability may provide some understanding of the many reasons why certain amino acid substitutions are essential for protein stability, while others are neutral.

Basically, the spatial structure of proteins is determined by electrostatic forces between polar and ionized groups and by hydrophobic interactions involving nonpolar residues (Dill 1990; Jaenicke 1991a; Jaenicke and Böhm 2001; Petsko 2001; Spyracopoulos and Sykes 2001). The electrostatic forces include ion pairs, hydrogen bonds, and van der Waals forces.

Single surface-exposed ion pairs have often been considered of secondary importance because they are normally present in small numbers and not highly conserved in proteins; thermodynamic data and evidence from protonation/deprotonation experiments seemed to confirm this assumption (Kauzmann 1959; Dill 1990). The relatively small contribution to the thermal stability of no more than ~4 kJ/mol could be easily explained because the gain in the free energy is practically compensated by the entropic cost of dehydration plus the reduction of the conformational freedom when the protein goes from the denatured to the native state (Jaenicke 1991b; Matthews 1996; Fersht 1998b). One would predict that at high temperature this effect becomes even more important. However, again referring to the above cold-shock proteins, about half of the difference in $\Delta G_{stab,70°C}$ between the mesophilic *B. caldolyticus* and the thermophilic *B. stearothermophilus* protein is due to electrostatic interactions between two exposed amino acid residues (Pace 2000; Perl et al. 2000; Mueller et al. 2000; Perl and Schmid 2001). Along these lines, an increase in the number of electrostatic interactions, which are often organized in clusters of ion pairs between charged amino acid side chains, has been frequently found in hyperthermophilic proteins compared to their mesophilic counterparts (Jaenicke and Böhm 1998; Szilagyi and Závodszky 2000; Sterner and Liebl 2001). These networks can increase protein stability by a number of mechanisms: (1) Each extra ion pair added to the network requires the desolvation and localization of only a single residue; (2) networks of charged groups are often located in cavities and at interfaces where their conformational freedom is restricted; as a consequence, part of the entropic cost has already been provided during the folding of the polypeptide chain; (3) hydration effects play a minor role at high temperature (Elcock 1998); and (4) the dielectric constant (in the denominator of the Coulomb equation) decreases with temperature, resulting in an increase in electrostatic energy upon ion-pair formation. Perutz's early hypothesis on the significance of ion pairs for the stability of thermophilic proteins may be rationalized on the basis of given arguments (Perutz and Raidt 1975).

In summarizing our present understanding of the contribution to ΔG_{stab} attributable to electrostatic potentials between charged groups, one important conclusion is that the key stabilizing feature is not so much the type and the number of groups but their structural context and optimum placement. The ion-pair network in the glutamate dehydrogenase family illustrates this conclusion: The comparison of the crystal structures of the mesophilic, moderately thermophilic, and hyperthermophilic enzymes and homology modeling clearly indicate that the decrease in T_m correlates with the reduction ("fragmentation") of ion-pair networks (Yip et al. 1995, 1998).

The significance of hydrogen bonds as the dominant stabilizing force in protein folding and stability has been controversial for more than 60 years (Mirsky and Pauling 1936; Bernal 1939; Bernal 1958; Kauzmann 1959; Fersht 1987; Dill 1990; Jaenicke 1991a, b; Makhatadze and Privalov 1995; Pace et al. 1996; Schellman 1997; Petsko 2001). Attempts to quantify their bond energy resulted in a range between 12 and 38 kJ/mol (3–9 kcal/mol), including 21 kJ/mol (5 kcal/mol) for the amide-amide NH···O bond (Fersht 1998b). Mutant studies focusing on the energy increment inherent to an intrachain H-bond relative to the H-bond with the aqueous solvent yielded 1.2 ± 0.6 kcal/mol (Fersht 1987; Matthews 1995, 1996; Pace et al. 1996; Jaenicke 1999). Using standard proteins such as ribonuclease T1 or phage T4 lysozyme, a large amount of experimental data corroborated this estimate. For the contribution of the H-bond to the stability of nucleic acids, also 1 kcal/mol was suggested by Crothers and Zimm (1965); their prediction was confirmed using polynucleotides as models (Freier et al. 1986).

Commonly, forming an H-bond, for example, in a helix or a β-strand, requires the transfer of polar groups from the periphery to the interior of the protein. This unfavorable process has been hypothesized to reduce the above H-bond energy to a value close to the thermal energy (kT); however, given the large number of H-bonds involved in secondary structure formation, it was assumed that even a marginal increase in the free energy of stabilization ΔG_{stab} value will accumulate to a significant net stabilization (Pace et al. 1996). Recent studies, comparing Asn → Ala, Leu → Ala, and Ile → Val mutants, have shown that the burial of an amide group contributes more to protein stability than the burial of an equivalent volume

of $-CH_2-$ groups. Obviously, this is in contrast to the above hypothesis because it clearly indicates that the desolvation penalty for peptide groups is much smaller than assumed so far; at the same time, it allows the conclusion that the hydrogen bonding and van der Waals interactions of peptide groups in the tightly packed interior of the folded protein are more favorable than similar interactions with water in the unfolded polypeptide chain (Pace 2001).

In summarizing the present state of theory and experiment regarding the role of hydrogen bonds in the stabilization of globular proteins and other biopolymers in aqueous solution, it is now well established that H-bonds exhibit a significant stabilizing effect over the whole biologically relevant temperature range.

The hydrophobic effect is a way of describing the tendency of nonpolar compounds such as hydrocarbons to transfer from water to an organic solvent. Its physical nature was previously considered to be entropic, attributable to the reorganization of the normal hydrogen-bonding network in water (called "iceberg formation") by the presence of a hydrophobic compound or nonpolar groups. In forming a hydrophobic interaction, the release of the icebergs from nonpolar groups or surfaces to the bulk phase of the aqueous solvent leads to an increase in entropy which exceeds the free energy terms due to losses of rotational or translational degrees of freedom (Kauzmann 1959; Tanford 1962, 1980; Lauffer 1975; Privalov 1979; Dill 1990; Privalov and Gill 1988). According to (Eq. 17.2), the entropic nature of hydrophobic interactions would suggest that their contribution to ΔG_{stab} increases with temperature. As a logical consequence, it has been claimed that the increased stability of proteins from thermophiles compared to mesophiles is attributable to an increase in hydrophobicity. A critical analysis proved the differences to be statistically insignificant (Böhm and Jaenicke 1994); this result has been recently confirmed for the much larger data base gained from the complete genomes of mesophilic, thermophilic, and hyperthermophilic bacteria and archaea (Jaenicke and Böhm 1998; Sterner and Liebl 2001). The results do not allow the conclusion that hydrophobic interactions do not contribute to ΔG_{stab}, for various reasons: (1) The result of determining the probabilities with which polar and nonpolar amino acids are localized in the core or in the exterior confirms the relative solubilities of all amino acids in water or other less polar solvents (Nozaki and Tanford 1971). However, detailed normalized distributions of the solvent accessibilities of all 20 amino acids, calculated from known 3D structures, clearly show that in the folding process, there are roughly equivalent decreases in the accessibility of both the polar and nonpolar groups (Richards 1977; Rose et al. 1985). Obviously, the relevant forces and the final structure of proteins require more careful definition than is implied by the common assumption that inside equals nonpolar and outside equals polar. (2) Using the relative solubilities, that is, the transfer of nonpolar substances into water, as a model reaction to quantify hydrophobic interactions, the temperature dependence is characterized by parabolic profiles (*Fig. 17.4*). At the minimum, the hydrophobic effect shows its maximum, $\Delta H_{transfer}$ equals zero, and $\Delta G_{transfer}$ is fully determined by $T\Delta S_{transfer}$ (Privalov 1988; Pace 1992).

At higher temperatures, the hydrophobic effect decreases and finally vanishes at ~120–140 °C (Sturtevant 1977; Baldwin 1986; Privalov and Gill 1988; Dill 1990; Makhatadze and Privalov 1995). Evidently, the hydrophobic effect is a highly complex phenomenon. (3) Recent thermodynamic data have shown that there is a significant enthalpic contribution to the hydrophobic effect, which can be attributed to van der Waals interactions (P.L. Privalov, personal communication, 1998). A sound thermodynamic treatment of the correlation of temperature, stability, and the hydrophobic interaction has been put forward by Schellman (1997).

In summarizing the forces and general mechanisms involved in the stabilization of proteins, it is important to note that, as a state function, the free energy of stabilization ΔG_{stab} is an additive quantity. According to the Gibbs-Helmholtz equation (Eq. 17.2), its increments are either enthalpic or entropic. Three major contributions are dominated by enthalpy: attractive forces between ion pairs, hydrogen bonds, and van der Waals interactions. Hydrophobic interactions have enthalpic and entropic increments. Apart from its significance in the hydrophobic effect, entropy clearly dominates three characteristics of thermophilic proteins: (1) the increase in proline or the decrease in glycine residues, (2) the reduced lengths of loops, and (3) anomalously high states of association. In the case of (1) and (2), stabilization comes from the destabilization of the denatured state, attributable to the decrease in the degrees of freedom of the unfolded polypeptide chain (Matthews et al. 1987; Suzuki et al. 1991; Watanabe et al. 1991, 1994, 1996, 1997). In the case of (3), both the burial of nonpolar sites in the inner core of the protein and the release of water (accompanying ion-pair formation) lead to an increase in entropy and a gain in free energy (Lauffer 1975; Jaenicke 1987; Jaenicke and Seckler 1997; Schellman 1997; Thompson and Eisenberg 1999).

Temperature Effects on Ligand Binding and Enzyme Function

Concerning thermal effects on enzymatic catalysis and regulation, ligand binding is of crucial importance. At this point, binding-site geometry, active-site fitting of the ligands, and conformational changes upon binding, transformation, and release of substrates, products, and effectors are expected to be temperature dependent. However, binding of substrates and/or coenzymes often exhibits entropy-enthalpy compensation (Lumry and Rajender 1970), so that the overall free energy of ligand binding seems to be more or less indifferent to temperature changes (*Fig. 17.5a*).

Michaelis-Menten constants (K_m) for homologous enzymes from mesophiles and thermophiles often cluster in a narrow range, when compared at optimum physiological temperatures (*Fig. 17.5b*). At a fixed temperature, enzymes from mesophiles show higher catalytic turnover numbers (k_{cat}) than their homologs from thermophiles; however, owing to the

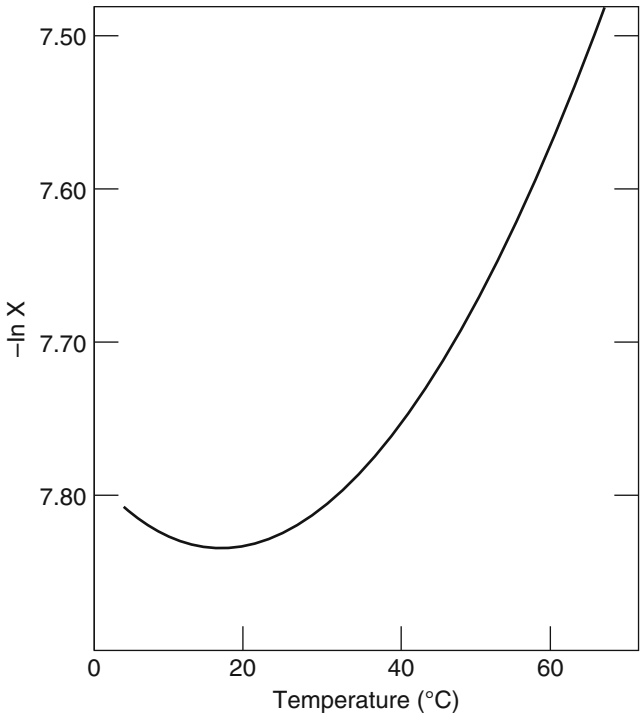


Fig. 17.4

The temperature dependence of the solubility (x) of benzene in water reflects hydrophobic interactions (Data taken from Franks et al. (1963)). The corresponding thermodynamic data for the transfer of benzene and ethylbenzene from the pure liquid phase to water at 25 °C are:

	Surface area (Å^2)	Solubility (mole fraction)	$\Delta H_{transfer}$ (kJ/mol)	$\Delta S_{transfer}$ (J/K·mol)	$\Delta G_{transfer}$ (kJ/mol)	ΔC_p (J/K·mol)
Benzene	240	4.01×10^{-4}	2.08	−58	19.4	225
Ethylbenzene	302	0.258×10^{-4}	2.02	−81	26.2	318

ΔC_p, the change in heat capacity, may be gained from a ΔH versus T plot according to the Kirchhoff equation $\Delta C_p = \partial(\Delta H)/\partial T$ (cf. Privalov and Gill 1988; Dill 1990; and textbooks of physical chemistry)

general enhancement of reaction rates with temperature, at physiological temperature, *kcat* also is conserved in accordance with the frequently observed unity in diversity observed in comparative physiology (Somero 2000).

Biochemical Limitations at High Temperature

The high-temperature regime of life is limited not only by the above-mentioned temperature coefficients of the relevant interatomic interactions in biomolecules but also by their hydrothermal decomposition (Dill 1990; Jaenicke 1991a, b; Blokzijl and Engberts 1993; Elcock 1998; Jaenicke and Böhm 1998; Sterner and Liebl 2001). In the cytosol's multicomponent system, amino acids, nucleic acid bases, carbohydrates, and lipids are prone to a wide variety of covalent chemical modifications, especially under various extreme physical conditions. In the case of proteins at elevated temperature, these modifications comprise deamidation of the amide side chains of Asn and Gln residues; succinimide formation at Glu and Asp; β-elimination; oxidation of His, Met, Cys, Trp, and Tyr; disulfide interchange; lanthionine formation; Maillard reactions; hydrolysis; and ring cleavage (Zale and Klibanov 1986; Ledl and Schleicher 1990; Creighton 1994; *Table 17.3*).

Since these reactions have high activation energies, they become increasingly important at high temperatures. On the other hand, their reaction rates have been shown to be higher for small peptides with high flexibility than for proteins, when comparing the same amino acid sequence (Wearne and Creighton 1989). Similarly, enhanced rates were observed for unfolded proteins compared to the same proteins in their native state (Hensel et al. 1992). Thus, the typical high packing density of thermophilic proteins will have a protecting effect, to the extent that degradative reactions may be assumed to play no significant role as long as the native conformation of a given protein is intact (Daniel et al. 1996). For this reason, hyperthermophiles and thermophiles must either inhibit unfolding and subsequent degradation of proteins or compensate for both processes. Possible strategies are the accumulation of compatible solutes or enhanced protein synthesis and repair. Little is known about the

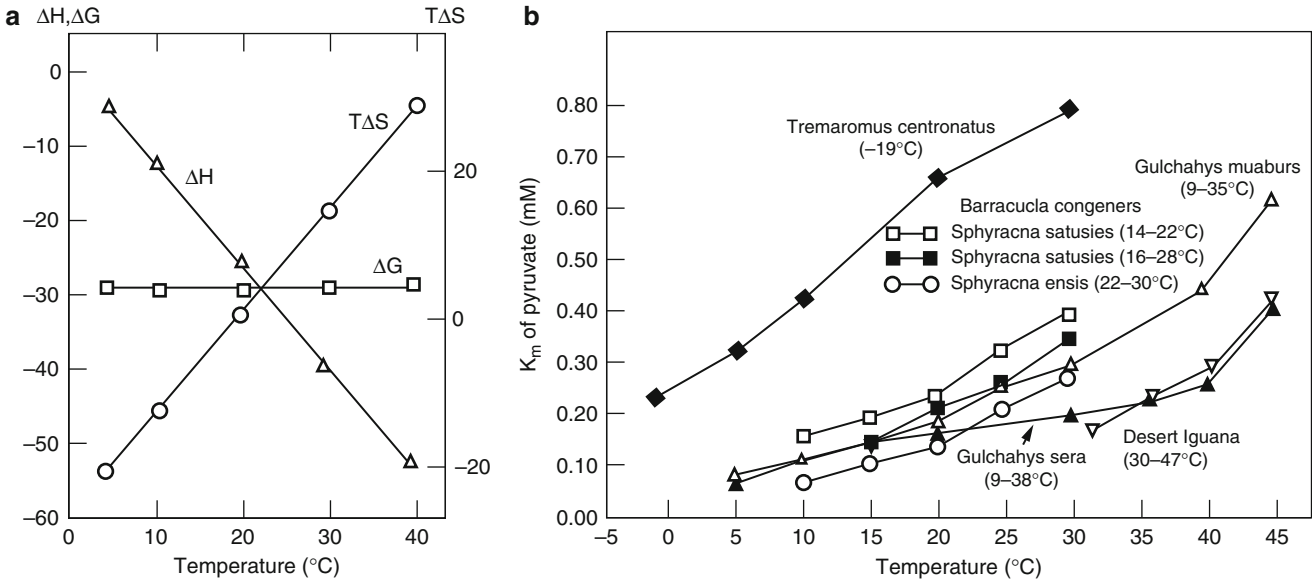


Fig. 17.5

Thermal adaptation and protein function. (a) Entropy-enthalpy compensation upon binary complex formation of NADH and lactate dehydrogenase from pig muscle, monitored by calorimetric analysis; ΔH, ΔS, and ΔG refer to ligand binding in kJ/mol (Data from Hinz and Schmid (1977)). (b) Temperature effect on the Michaelis-Menten constant (K_m) of pyruvate for the H4 isoenzyme of lactic dehydrogenase (LDH-H_4) of organisms adapted to different temperatures; physiological temperatures given in parentheses (Data taken from Somero (1995))

Table 17.3

Degradative chemical reactions and isomerization reactions important to irreversible protein denaturation, especially at elevated temperature

Reaction	Amino acids involved	Comments
Deamidation	Asn, Gln (especially in Asn-Gly and Asn-Ser sequences)	Independent of pH, product: *iso*-Asp as substrate of methyl transferase, leading to repair or clearance
Racemization	Asp	
Isomerization	Pro (*cis-trans* isomerization)	Catalyzed by peptidyl-prolyl *cis-trans* isomerases[a]
Glycation	Lys and other amino acids reacting with reducing sugars	Cross-linking by Maillard reactions, involved in in vivo degradation[b]
Oxidation	Cys ⇔ sulfenic → cysteic acid (oxidation or SH/SS exchange via mixed disulfides)	Thiolate mechanism catalyzed by Cu^{2+} or Fe^{2+} or protein disulfide isomerases (PDI, DsbA/DsbB, etc.)
	Met ⇔ sulfoxide → sulfone	Significant both in vivo and in vitro in the presence of oxygen radicals
Proteolysis	Polypeptides → amino acids	Caused either by proteases or autolysis, or by H^+-catalyzed peptide cleavage; nonenzymatic, between Asp and Pro and Asp and (C-terminal) Asn
Photodegradation	Trp → kynurenine → *N*-formyl kynurenine	Caused by nonionizing or ionizing radiation, depending on the local microenvironment of the amino acids
	Tyr → DOPA, dityrosine	
	Cystine → 2Cys	

[a]Schiene-Fischer and Fischer (2000)

[b]cf. Barrett (1985)

For further references and details, cf. Greenstein and Winitz (1961), Meister (1965), Cecil (1963), Gottschalk (1972), Friedman (1973), Barrett (1985), Stadtman (1990), Stadtman and Oliver (1991), Volkin et al. (1995), Berlett and Stadtman (1997), Jaenicke and Seckler (1997), Daniel and Cowan (2000), Jaenicke and Lilie (2000), Schiene-Fischer and Yu (2001), and Vieille and Zeikus (2001)

Asn asparagine, *Gln* glutamine, *Ser* serine, *Asp* aspartate, *Lys* lysine, *Pro* proline, *Cys* cysteine, *Met* methionine, *PDI* protein disulfide isomerase, *DsbA/DsbB* disulfide-bond-forming proteins, and *DOPA* dihydroxyphenylalanine

chemistry of thermal degradation or specific protection of proteins, and even less about repair. An exception is the L-*iso*aspartyl methyltransferase from *Thermotoga maritima*, a highly active repair enzyme that catalyzes the transfer of the methyl group from S-adenosylmethionine (SAM) to the α-carboxyl group of L-*iso*aspartyl residues, resulting from the deamidation of Asn and the isomerization of Asp (Ichikawa and Clarke 1998). Interestingly, the k_{cat} of the enzyme at 80 °C is ~20-fold higher than that of mesophilic homologs at 37 °C, supporting the view that it is specifically adapted to the high need of protein repair close to the boiling point of water.

The thermal stability of the different canonical natural amino acids at neutral pH decreases in the following series: (Val, Leu) > Ile > Tyr > Lys > His > Met > Thr > Ser > Trp > (Asp, Glu, Arg). Cysteine exhibits low stability: Depending on temperature and pH, it undergoes either oxidation (to form cystine) or elimination of sulfur (lanthionine formation). The lower limit at which degradation in aqueous buffer solutions was detectable was ca. 110 °C (Bernhardt et al. 1984). One may assume that up to this temperature range, biosynthesis can still balance the thermal decomposition. In the temperature regime of hydrothermal vents, for example, at 250 °C (265 bar), the half-lives of the amino acids, peptides, and proteins undergoing degradation were found to be too short to be offset by biosynthesis of these molecules (White 1984).

ATP and ADP hydrolysis become significant between 110 °C and 140 °C (Leibrock et al. 1995). This upper temperature limit coincides with the temperature range at which the hydrophobic hydration of nonpolar residues in aqueous solution vanishes (Sturtevant 1977; Privalov 1979; Baldwin 1986; Jaenicke 1991b, 2000a).

In summarizing the biochemical limitations of viability from the point of view of water-soluble proteins, temperatures beyond ca. 130–140 °C are not tolerable, for two reasons: (1) Natural amino acids are hydrothermally decomposed, and (2) the solvent properties of water are altered, blurring the difference between polar and nonpolar residues, thus interfering with the "hydrophobic collapse" (as the initial step of protein folding) and the formation of the densely packed hydrophobic core (as the prerequisite of protein stability). For nucleoproteins and lipoproteins or membranes, the same holds true because of the temperature limits of the intermolecular interactions between the polar and nonpolar components in the respective complexes. For both classes of proteins, extrinsic factors and compatible solutes may enhance the stability as well as the limits of growth (see below). The given upper temperature limit of viability has been confirmed for cells of the most extreme hyperthermophiles *Pyrolobus fumarii* and strain 121 with its temperature of maximal growth at 121 °C (Blöchl et al. 1997; Kashefi and Lovley 2003). Whether the protective action of compatible solutes and/or crowding induced by high levels of molecular chaperones contribute to this extreme thermotolerance needs further investigation (cf. Carpenter et al. 1993; Zimmerman and Minton 1993; Somero 1995; Trent et al. 1997; Minton 2000).

Clearly, the biochemical limit of viability depends not only on the intact organization of the cell's standard high molecular weight components but also on the low molecular weight compounds such as coenzymes and metabolites. Again, in general, extremophiles make use of the common repertoire of compounds known from the metabolism of mesophiles. Keeping in mind the high catalytic rate of most enzymes under physiological conditions, the majority of metabolites do not limit viability at temperatures close to 100 °C. The reported half-lives of ATP and ADP range from ~1 to 6 h at 100 °C, depending on the pH and the presence of metal ions (Ramirez et al. 1980; Leibrock et al. 1995; Daniel et al. 1996). However, the oxidized nicotinamide adenine dinucleotide (NAD^+) has a half-life at 100 °C of no more than 10 min. To cope with this instability, nature can make use of at least four strategies: (1) high catalytic turnover or (2) channeling of labile intermediates, (3) local stabilization in enzyme-ligand complexes, and (4) usage of an alternate metabolic pathway or a different, more stable compound. In the case of (3), the high affinity of ligands for their respective enzymes has frequently been shown to cause mutual stabilization (Danson 1988; Jaenicke et al. 1996; Dams and Jaenicke 1999).

Adaptive Stabilization Mechanisms of Nucleic Acids

The integrity of nucleic acids is threatened at high temperatures, which can induce either strand separation and chemical damage of the nucleotide constituents or, at the extreme, breakage of backbone phosphodiester bonds (Grogan 1998; Daniel and Cowan 2000).

Mechanisms to Avoid Strand Separation

An increased G + C content is known to increase the temperature T_m at which melting, that is, strand separation of DNA and RNA, occurs. Thus, a possible adaptation mechanism of nucleic acids to thermophilic and even more to hyperthermophilic conditions would be an increase in G + C. Indeed, a systematic study revealed a strong positive correlation between the G + C content of tRNAs and rRNAs with the optimum growth temperatures of prokaryotes (Galtier and Lobry 1997; ❯ *Fig. 17.6a*). The same study showed, however, that the G + C content of genomic DNA is not correlated with the growth temperature (❯ *Fig. 17.6b*).

Quite the contrary, the DNA of some of the most hyperthermophilic archaea has a strikingly low G + C content, with values as low as 31 mol%, for example, for *Acidianus fervidus* and *Methanococcus igneus* (T_{max} >90 °C), and an average of ca. 45 mol% for all presently known hyperthermophilic archaea and bacteria (Stetter 1996; Grogan 1998). These data clearly suggest that in these organisms, the DNA double helix must be stabilized either by extrinsic factors such as ions and small metabolites or by proteins. It has been known for a long time that the addition of salts or polyamines leads to an increase in T_m. Actually some, but not all, hyperthermophiles accumulate

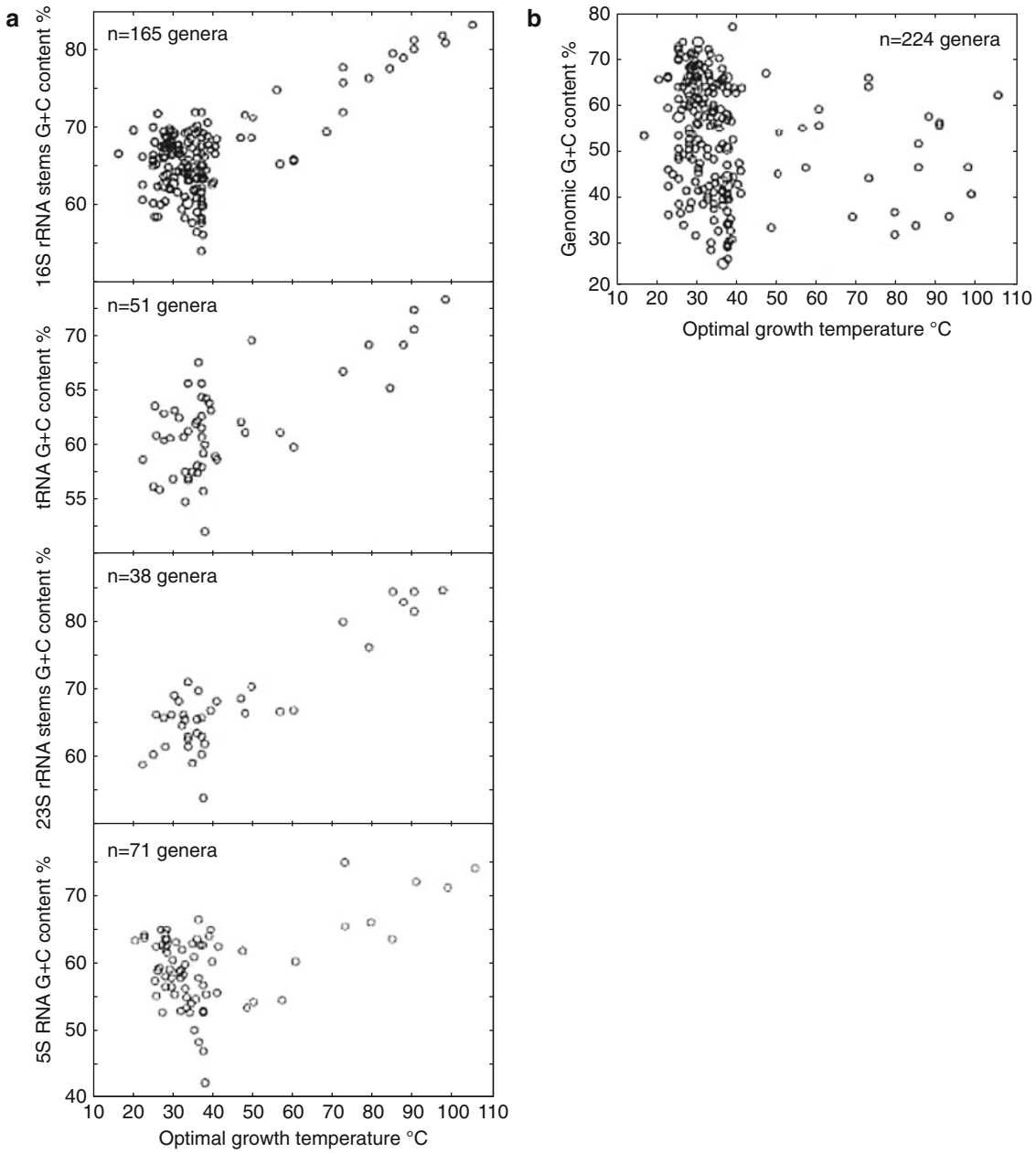


Fig. 17.6

G + C contents of (a) various RNAs and (b) genomic DNAs plotted against optimal growth temperatures (Data taken from Galtier and Lobry (1997))

high concentrations of putative ionic thermoprotectants such as potassium di-inositol-1′,1′-phosphate and tripotassium cyclic-2,3-diphosphoglycerate (Hensel and König 1988; Scholz et al. 1992). However, there is no clear correlation between the level of polycationic polyamines and growth temperature (Kneifel et al. 1986). In a number of archaeal hyperthermophiles, two unrelated groups of highly basic proteins were identified, which bind to DNA without marked sequence preference. Both the members of the HMf histone family, which are homologs of the eukaryal core histones, and the histone-like proteins from *Sulfolobus* species, for which no eukaryal homologs are known, increase the T_m of the DNA double helix significantly (McAfee et al. 1996; Soares et al. 1998). Thus, there is clear evidence that hyperthermophiles make use of different strategies to prevent DNA strand separation at their extreme growth temperatures. Certainly, the physiological interpretation of in vitro T_m data gained from topologically open molecules has to be taken with a grain of salt because cellular DNA is in a topologically closed conformation, and denaturation will not result in two independent single-stranded molecules, but in a random-coil structure with intertwined strands (Marguet and Forterre 2001). As a result, topologically closed DNA is undoubtedly more resistant to denaturation than open DNA. It was postulated that the introduction of positive supercoils into closed DNA, which is catalyzed

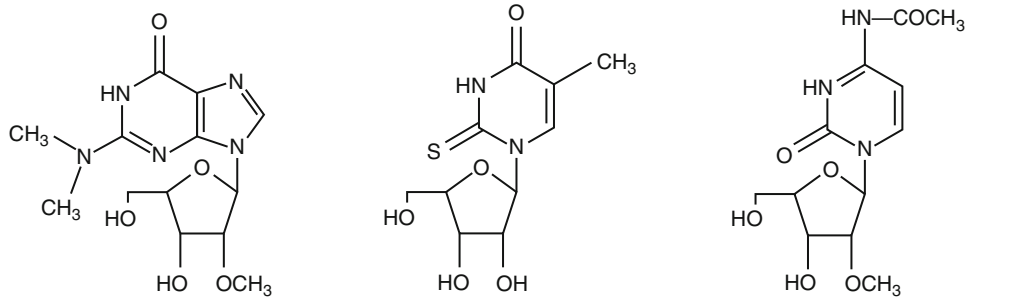


Fig. 17.7
Modified nucleosides implicated in the stabilization of hyperthermophile tRNA (From Daniel and Cowan (2000))

by reverse gyrases from hyperthermophiles, specifically stabilizes the double helix and keeps it in a functional state at high temperature (Forterre et al. 1996; López-Garcia and Forterre 1997, 2000). However, the hyperthermophile *Thermotoga maritima* contains both "normal" and reverse gyrases and propagates negatively supercoiled plasmid DNA (Guipaud et al. 1997).

tRNA molecules are not permanently integrated into larger macromolecular complexes. Therefore, in adapting to high temperatures, they must have developed mechanisms for intrinsic stabilization. Part of the stabilization energy may originate from an increased G + C content. However, unfractionated tRNA from the hyperthermophiles *Pyrococcus furiosus* and *Pyrodictium occultum* showed T_m values around 100 °C, too high to be attributable to the measured G + C content (Kowalak et al. 1994). An early investigation identified a broad variety of covalent posttranscriptional modifications in nucleosides from tRNA preparations of thermophiles and hyperthermophiles, six of which were structurally novel in showing alterations of their bases as well as methylation of their ribose moiety (Edmonds et al. 1991). Altogether, 23 modified nucleosides were identified in *Pyrococcus furiosus*; three of them (*Fig. 17.7*) not only exhibited enhanced relative abundance with increasing growth temperature but also higher stability, which they effected by (1) restricting the conformational flexibility of the ribose ring, (2) favoring the A-type helix, and (3) preventing phosphodiester-bond hydrolysis (Inoue et al. 1987; Kawai et al. 1992; Kowalak et al. 1994; Cummins et al. 1995).

Apparently, the protecting effect of posttranscriptional tRNA modification is not restricted to the archaea: Both the level of 5-methyl-2-thiouridine and the T_m value of tRNA from the bacterium *Thermus thermophilus* show a significant increase with increasing growth temperature (Watanabe et al. 1976). The effect becomes even more compelling if tRNAs from psychrophiles are included in the comparison. While the abundance and the variety of posttranscriptional tRNA modifications are more pronounced in thermophiles and hyperthermophiles than in mesophiles, significantly less modifications are found in tRNAs from psychrophiles (Dalluge et al. 1997). The most abundant one is dihydrouridine, whose nonplanar base resists stacking, this way decreasing stability. In addition, dihydrouridine favors the C-2′-endo sugar conformation, which is less rigid than the C-3′-endo conformer (Yokoyama et al. 1981). Obviously, enhanced flexibility is essential for optimal functioning at low temperature, whereas high intrinsic stability has lower priority.

In the case of rRNAs, significant stabilization is provided by their conjugation with proteins within the ribosomal complex. In accordance with this argument, the levels of posttranscriptional modifications of rRNAs are much lower than in tRNAs, both in mesophiles and in thermophiles. Still, rRNA modifications are much more abundant in *Sulfolobus solfataricus* than in *Escherichia coli*, and the level of stabilizing ribose O-2′ methylations significantly increases with the culture temperature of the hyperthermophile (Noon et al. 1998).

Mechanisms to Avoid and Repair Chemical Damage of Nucleotides

Chemical damage of nucleic acids by hydrolytic attack close to the boiling point of water is an enormous potential threat for hyperthermophiles. The most common damages to DNA are (1) base deamination, (2) loss of bases from one strand with apurinic or apyrimidinic sites as final products, and (3) hydrolytic cleavage of phosphodiester bonds. It was suggested that, above 100 °C, DNA would be subject to a ca. 3,000-fold increase in the levels of deamination and depurination compared with DNA at 37 °C (Lindahl 1993). Furthermore, it was estimated from in vitro stability data that under the physiological conditions of *S. solfataricus* (intracellular pH 6, 80 °C), two apurinic sites per gene per cell division would accumulate (Grogan 1998). The most severe damage of nucleic acids is the hydrolytic cleavage of the backbone phosphodiester bond. For this reaction, it was postulated that the preceding depurination at an adjacent site is the rate-limiting step (Marguet and Forterre 1998, 2001). In contrast, for RNA, hydrolytic strand breakage is not coupled to depurination; instead it occurs via the direct attack of the phosphodiester bond by the ribose 2′-OH oxygen. In vitro, at around 100 °C, single-strand breaks occur at a high rate (Marguet and Forterre 1994; Grogan 1998). The corresponding lesions could lead to lethal double-strand breaks, if not prevented or repaired in vivo. Therefore, it was suggested that

hyperthermophiles must have evolved highly efficient mechanisms to protect and/or repair their DNA (Grogan 1998). In support of this hypothesis, when *Pyrococcus furiosus* cells are exposed to 100 °C, their DNA is about 20 times more resistant to breakage than DNA from *Escherichia coli* at the same temperature (Peak et al. 1995). Furthermore, passive protection of DNA might be provided by similar mechanisms as used to increase the T_m of the DNA double helix, that is, high salt concentrations, and binding to proteins (see above). Indeed, it has been shown that the presence of Mg^{2+} and K^+ protects double-stranded DNA from depurination, probably by directly stabilizing the N-glycosidic bond between the deoxyribose and the base (Marguet and Forterre 1998). With respect to the formation of nucleoprotein complexes, archaeal histones are known to protect plasmid DNA against radiation (Isabelle et al. 1993).

In spite of these well-established protection mechanisms, DNA in hyperthermophiles will almost certainly be damaged to a larger extent than DNA in mesophiles. A model organism for comparative research in this context is the radiation-resistant bacterium *Deinococcus radiodurans*. Both γ-irradiation and heat have been shown to induce double-strand breakage of DNA, which can be repaired efficiently by *D. radiodurans*. This capacity derives from multiple copies of its chromosome providing intact copies for repair by a DNA recombinase (Minton and Daly 1995). In analogy, the chromosome of the archaeon *Pyrococcus furiosus*, after irradiation-induced fragmentation, was reassembled by the cells upon incubation at 95 °C (di Ruggiero et al. 1997). Open reading frames encoding homologs of RecA proteins involved in recombination repair in bacteria and eukarya were found in the archaeal genomes sequenced so far. Strong experimental evidence suggests that at least one of these homologs, FEN-1 from *P. furiosus*, is involved in double-strand breakage repair (di Ruggiero et al. 1999). Other than double-strand breakage repair, activities have been demonstrated in vitro for several other archaea. For example, *Methanobacterium thermoautotrophicum* is able to remove ultraviolet light-induced photoproducts, supposedly with a photolyase as catalyst (Kiener et al. 1989; Ögrünc et al. 1998). Furthermore, a T/G-selective DNA thymine N-glycosylase takes care of the mutagenic effect of hydrolytic 5-methylcytosine deamination (Horst and Fritz 1996), while uracil-DNA glycosylases seem to be involved in the repair of cytosine deamination (Koulis et al. 1996); in addition, O_6-alkylguanine-DNA transferase activities were also found in hyperthermophiles (Skorvaga et al. 1998). On the other hand, MutL and MutS, which are used in all bacterial and eukaryal mismatch-repair systems, have not been found in any of the archaeal genomes so far.

In summary, the present knowledge of the specific mechanisms by which hyperthermophilic microorganisms preserve the integrity of their genetic material is still incomplete. More information is needed about the intracellular salt concentrations and the DNA-binding and DNA-protecting proteins, to establish in vitro test systems that come as close as possible to the in vivo situation. Moreover, homologs of known bacterial and eukaryal repair enzymes from hyperthermophiles need to be characterized to identify their catalytic properties under physiological conditions. The ongoing genome-sequencing projects will help identify the most promising candidates for this approach.

Adaptive Stabilization Mechanisms of Lipids and Membranes

Living cells have a cytoplasmic membrane serving as a barrier between the cytoplasm and the environment. It consists of lipid layers with embedded proteins that generate specific and vital solute concentration gradients across the membrane. Penetration of small solutes through the lipid component of the membrane is caused either by active transport or passive diffusion. Being directly proportional to the thermal energy (kT), passive diffusion is accelerated with increasing temperature (Einstein 1905, 1906; van de Vossenberg et al. 1998). In hyperthermophiles, extreme temperature may lead to the breakdown of solute gradients. Therefore, their membranes need to be extremely thermostable, but they also require specific adaptive mechanisms to limit the permeability of ions. This holds especially for protons because of the essential role of proton gradients in energy-requiring processes such as ATP synthesis, active transport of specific solutes across the membrane, flagellar rotation, and maintenance of the intracellular pH and turgor (Albers et al. 2000).

Chemical Composition of Membrane Lipids

At physiological temperatures, membrane lipids are in a liquid-crystalline state (Melchior 1982), forming a suitable matrix for the attachment or integration of membrane proteins. The overall structure of the lipid membrane is conserved between eukarya, bacteria, and archaea. The inner and outer hydrophilic surfaces, which are composed of polar headgroups, enclose the hydrophobic interior consisting of long hydrophobic hydrocarbon chains. At this point, the chemical composition of archaeal membranes has been found to be significantly different from the chemical composition of bacterial and eukaryal membranes. Both bacterial and eukaryal lipids have esters between glycerol and fatty acid chains (glycerol fatty acyl diesters), whereas the lipids of archaeal membranes are formed by ethers between glycerol (or another alcohol such as nonitol) and branched C_{20}-hydrocarbon side chains (Langworthy and Pond 1986). The side chains consist of repeated saturated isoprenoid units containing a methyl side group at every fourth carbon atom in the backbone. These methyl side groups restrict the mobility of the chains, thereby stabilizing them and restricting ion permeability (see below). The two hydrocarbon chains can be ether linked to either one glycerol unit (forming a C_{20}, C_{20}-isopranyl glycerol diether = diphytanylglycerol diether = archaeol) or two glycerol units (forming a dibiphytanylglycerol tetraether = caldarchaeol; ❯ *Fig. 17.8a and b*). The archaeols are found in all archaea, whereas the

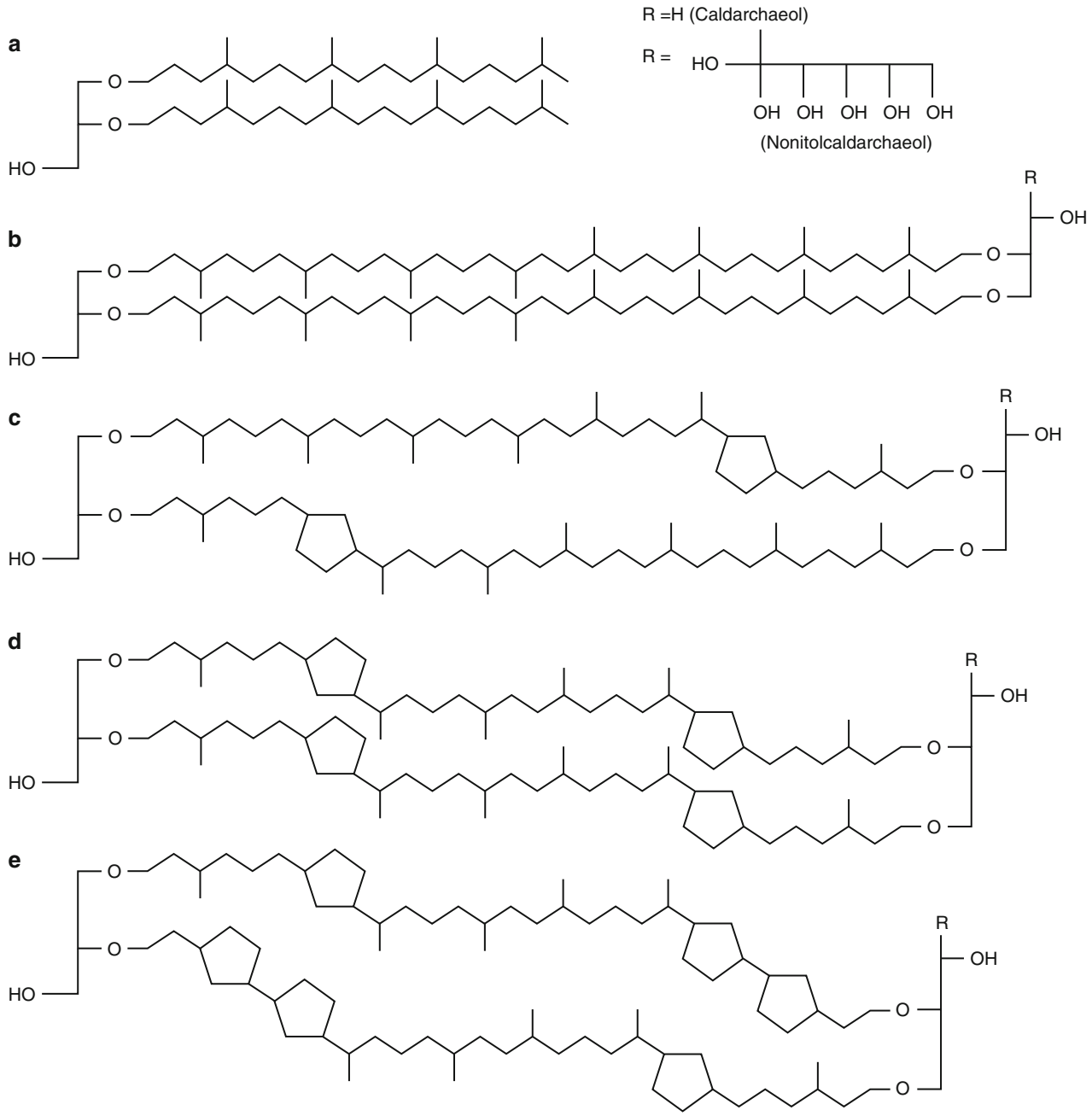


Fig. 17.8
Archaeal lipid architecture. (a) Diphytanyl glycerol diethers, (b) dibiphytanyl diglycerol tetraethers, and (c–e) internal cyclization in dibiphytanyl diglycerol tetraethers (From Daniel and Cowan (2000))

caldarchaeols (and nonitol-caldarchaeols) are only found in thermophilic archaea. The caldarchaeols can be further modified by cyclopentane rings in the biphytanyl side chains (*Fig. 17.8c–e*).

The caldarchaeols of thermophilic archaea are typically glycosylated at C_3 and C_6 of the glycerol and nonitol backbones, respectively. Probably, hydrogen bonds between the glycosyl headgroups stabilize the membrane structure by reducing lateral lipid mobility (van de Vossenberg et al. 1998; Daniel and Cowan 2000).

An unsaturated diether lipid was found in the archaeon *Methanopyrus kandleri* (Hafenbradl et al. 1993). This lipid, 2,3-di-*O*-geranylgeranyl-*sn*-glycerol, resembles terpenoids, but the consequences for membrane function are still unknown. Another type of unsaturated lipid was discovered in the psychrophilic archaeon *Methanococcoides burtonii* (Nichols and Franzmann 1992). This lipid contains a double bond that can distort the short-range order of the membrane, thus allowing the necessary fluidity of the membrane to be adapted to the physiological low temperature (Suutari and Laakso 1992).

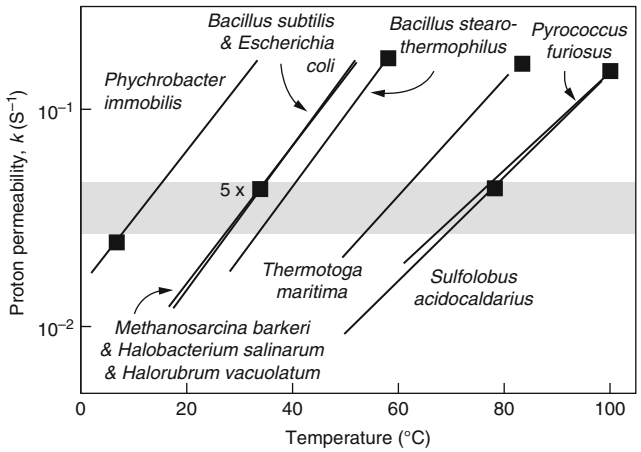


Fig. 17.9

The proton permeability of archaea and bacteria that live at different temperatures. At the respective growth temperatures, the proton permeability falls within a narrow range (*gray bar*). *Thermotoga maritima* and *Bacillus stearothermophilus* have higher permeabilities than those of other organisms. Both organisms overcome this problem differently (From Albers et al. (2000))

Topology, Stability, and Permeability of Membranes

The glycerol-diester lipids of bacteria and eukarya form bilayer membranes. The same holds for the archaeol lipids of halobacteria and most other archaea growing under moderate conditions (Kates et al. 1993; Upasani et al. 1994; Kates 1995). In contrast, the caldarchaeol lipids of the thermophilic and acidophilic archaea form monolayers spanning the entire membrane (de Rosa et al. 1991; Relini et al. 1996). In monolayers, two glycerol units are covalently linked by the phytanyl side chains, whereas in bilayers the glycerol units are noncovalently linked by hydrophobic interactions between the fatty acid side chains. As a consequence, monolayers have a diameter between 2.5 and 3.0 nm (Gliozzi et al. 1983), somewhat thinner than typical C_{18} glycerol-diester bilayers, but much more stable: Vesicles generated from *Thermoplasma acidophilum* ether lipids are more resistant to high temperature and surface-active agents than vesicles of bacterial dipalmitoyl-phosphatidylcholine (Ring et al. 1986). Moreover, liposomes prepared from tetraether lipids from a number of archaea were shown to be extremely stable toward high temperature, alkaline pH, and enzymatic degradation by phospholipases (Chang 1994; Choquet et al. 1994).

As has been mentioned, to guarantee energy production, membranes of all microorganisms, no matter whether they are psychro-, meso-, thermo-, or hyperthermophilic, must provide an efficient barrier against the flux of protons. Liposomes prepared from lipids derived from a variety of organisms with different growth temperatures were compared for their proton permeabilities (van de Vossenberg et al. 1995). This study showed that, at the respective growth temperature, proton permeability was closely similar for the various liposomes (*Fig. 17.9*).

This "homeoproton permeability adaptation" is reminiscent of the "corresponding states" observed for homologous pairs of enzymes from mesophiles and thermophiles, most of which were shown to exhibit comparable stabilities, flexibilities, and activities at their respective physiological temperatures (Jaenicke 1991b; Somero 1995; Jaenicke and Böhm 1998). As a logical consequence, at a given fixed temperature, the proton permeability of membranes is decreased with increasing temperature of adaptation, following the order: psychrophiles > mesophiles > thermophiles > hyperthermophiles. Various archaeol and caldarchaeol lipids were 6–120-fold less permeable to water, solutes, protons, and ammonia than bacterial diphytanyl-phosphatidylcholine liposomes (Mathai et al. 2001). It was shown that the crucial factor ensuring low permeability is cyclopentane rings in the phytanyl side chains, which limit the mobility in the midplane hydrocarbon region. The substitution of ether for ester bonds provides an additional barrier that specifically impairs the flux of protons.

Bacterial thermophiles have membrane lipids rich in saturated fatty acids, which make the membranes more rigid and stable at high temperatures because stronger hydrophobic interactions are formed between saturated fatty acids compared with unsaturated ones (Brock 2000). Other differences between membranes from mesophilic and thermophilic bacteria include alterations in acyl chain length, branching, and/or cyclization (Tolner et al. 1998). Interestingly, the extremely thermophilic *Thermodesulfobacterium* contains lipids combining bacterial and archaeal properties; here, glycerol is ether linked to a unique C_{17} hydrocarbon side chain along with some fatty acids instead of phytanyl side chains (Brock 2000).

Adaptation of Membrane Structure and Function to Temperature Fluctuations

Bacteria and archaea can grow over a wide range of temperatures. When facing environmental temperature shifts, most of them adapt the structure of their membranes to ensure constant stability and permeability. In archaea, as well as in mesophilic and psychrophilic bacteria, this adaptation is achieved by adjusting the chemical composition of the lipids. Archaea adapt to low temperatures by decreasing the degree of saturation of their hydrocarbon side chains (Nichols and Franzmann 1992), whereas they respond to high temperature by the cyclization of the side chains and by replacing diether to with tetraether lipids (de Rosa and Gambacorta 1988; de Rosa et al. 1991; Yamauchi and Kinoshita 1995): For *Sulfolobus solfataricus* and *Thermoplasma*, it was shown that the number of cyclopentane rings incorporated into the lipid diphytanyl side chains increases with growth temperature, this way rigidifying the membrane and limiting passive diffusion of small molecules (Mathai et al. 2001; *Fig. 17.10*).

In *Methanococcus jannaschii*, a different mechanism is observed: Here, increasing temperatures induce the change from diether lipids to the more thermostable tetraether lipids (Sprott et al. 1991).

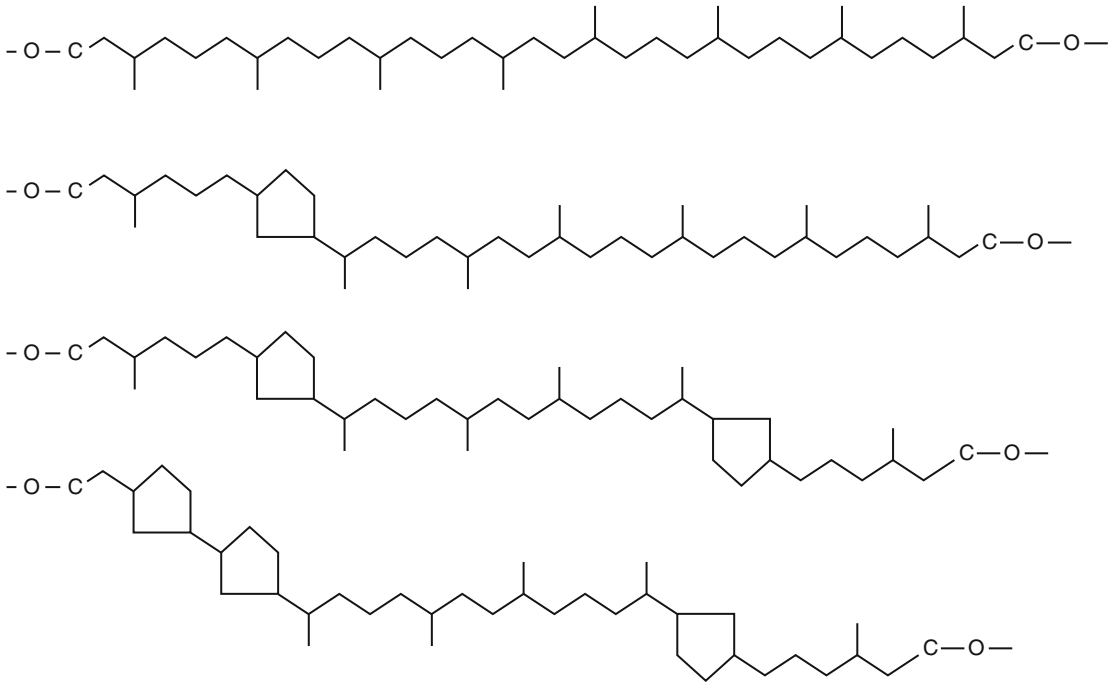


Fig. 17.10
Cyclization of the phytanyl chains of the *S. solfataricus* tetraether lipids. Only one of the phytanyl side chains is shown. The degree of cyclization increases from top to bottom (From Albers et al. (2000))

To investigate adaptive changes of membranes from bacteria, *Bacillus subtilis* was grown at the boundaries of its growth temperature (van de Vossenberg et al. 1999). The average lengths of lipid acyl side chains, the degree of saturation, and the ratio of *iso*- and *anteiso*-branched fatty acids increased with temperature. In accordance with the concept of homeoproton permeability adaptation, these modifications kept the proton permeability of the cytoplasmic membrane at a rather constant level. Likewise, in psychrophiles, the proton permeability is maintained at a constant level when the growth temperature is varied (van de Vossenberg et al. 1995). In contrast, in thermophilic bacteria such as *Bacillus stearothermophilus* and *Thermotoga maritima*, homeoproton permeability cannot be maintained, as their membranes become porous at high temperatures. Some moderately thermophilic bacteria can compensate for the high proton leakage by drastically increasing the respiration rate and, together with that, the rate of proton pumping (de Vrij et al. 1988). A different strategy is found in the moderate thermophile *Caloramator fervidus*, which, instead of the proton, uses the less permeable sodium ion as the main coupling component for energy transduction (Speelmans et al. 1993a, b).

In summary, a number of different mechanisms have been identified that keep membranes stable and functional at high temperatures. Archaea contain lipids with ether linkages between various alcohols and hydrocarbon side chains, in which cyclopentane rings are incorporated in a growth-temperature-dependent manner. Thermophilic bacteria, which contain less stable ester lipids prone to proton leakage, evolved alternative strategies to maintain vital chemiosmotic gradients under physiological conditions. As the number of novel lipid structures constantly grows, more variations on these themes are to be expected.

Adaptive Stabilization Mechanisms of Proteins

To fulfill their diverse functions, proteins from hyperthermophiles need to be in their native, folded state at temperatures around 100 °C. In contrast, most proteins from mesophiles are unfolded at ~50 °C (*Fig. 17.2*), often followed by irreversible aggregation and/or chemical damage (Jaenicke and Seckler 1997). What are the structural determinants that render proteins from hyperthermophiles much more thermostable than their homologs from mesophiles? As mentioned in the section on "Stability of Biomolecules," few additional favorable electrostatic or hydrophobic interactions suffice to shift ΔG_{stab} of a protein from the mesophilic to the thermophilic temperature regime (Jaenicke and Böhm 2001; *Fig. 17.3b*). In addition, proteins from hyperthermophiles are not only stabilized intrinsically but also by extrinsic factors such as compatible solutes or molecular chaperones. What follows briefly summarizes our current knowledge of the intrinsic and extrinsic stabilization of hyperthermophilic proteins. (For further details, see Jaenicke and Böhm 2001; Petsko 2001; Sterner and Liebl 2001; Vieille and Zeikus 2001).

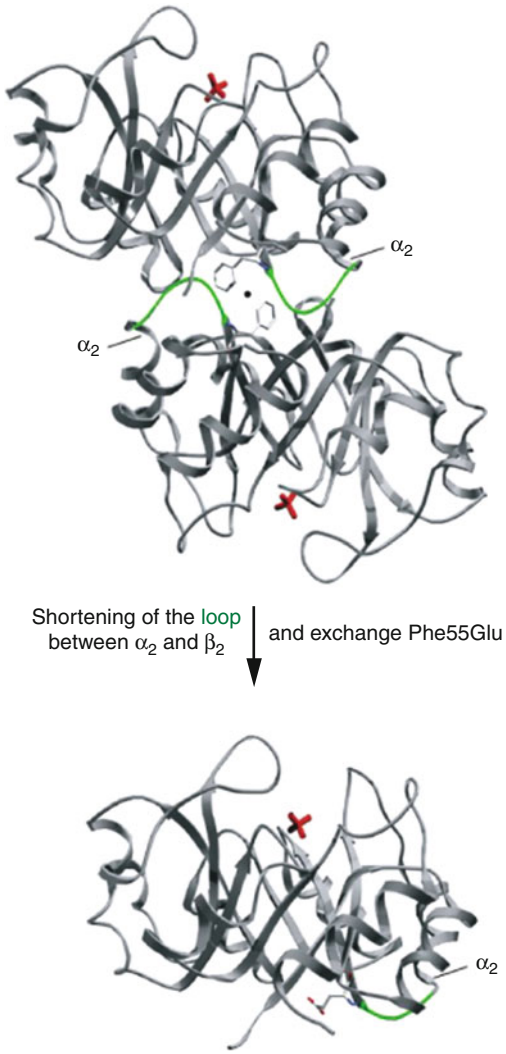


Fig. 17.11
Monomerization of the native homodimer of PRAI from *Thermotoga maritima* by rational design. Monomers were generated by shortening of the loops connecting helices α2 with strands β3 (in *green*) and by replacing the two Phe55 residues located close to the twofold symmetry axis (shown as a *black dot*) with glutamates (shown in *stick* format). The bound phosphate ions (*red* tetrahedrons) identify the active sites. The monomeric variants are catalytically as active as the dimer, but far more thermolabile (From Höcker et al. (2001), with permission)

Intrinsic Stabilization: There Are No General Rules

In the section on "Stability of Biomolecules," the electrostatic and hydrophobic interactions that stabilize proteins were discussed. Moreover, the contributions of enthalpy and entropy to the free energy gain caused by these interactions were pointed out. Pairwise comparisons of amino acid sequences and X-ray structures of homologous proteins from mesophiles, thermophiles, and hyperthermophiles showed that one or more of these stabilizing interactions were more frequent or more pronounced in the thermophilic and hyperthermophilic variants. These additional stabilizing interactions can in principle occur at all levels, from primary to the quaternary structure (Jaenicke and Böhm 1998; Daniel and Cowan 2000; Vieille and Zeikus 2001; Sterner and Liebl 2001; Yano and Poulos 2003).

A large number of mutational studies have been performed to identify stabilizing interactions, which were frequently detected in hyperthermophilic proteins. To this end, selected amino acid residues were substituted by site-directed mutagenesis, and the resulting changes in stability were measured. Instructive examples are the enzymes phosphoribosylanthranilate isomerase (PRAI) and indoleglycerol phosphate synthase (IGPS), which catalyze two successive reactions within tryptophan biosynthesis and adopt the frequently encountered $(\beta\alpha)_8$-barrel fold (Höcker et al. 2001; Wierenga 2001). PRAI is monomeric in most mesophiles but dimeric in *Thermotoga maritima* (Sterner et al. 1996). The two identical monomers of *Thermotoga maritima* PRAI are associated via intimate hydrophobic contacts at the N-terminal faces of their central β-barrels (Hennig et al. 1997). By replacing a Phe residue at the monomer-monomer interface of *T. maritima* PRAI by a Glu residue, the hydrophobic interactions are weakened. As a consequence, the enzyme becomes monomeric and thermolabile, without losing its catalytic activity (Thoma et al. 2000; *Fig. 17.11*).

The importance of increased association states for increased thermostability was also shown for ornithine carbamoyltransferase, which consists of four trimers in *Pyrococcus furiosus*, but only one in mesophiles. Gradual dissociation of dodecameric ornithine carbamoyltransferase from *Pyrococcus furiosus* into trimers, as induced by site-directed mutagenesis at subunit interfaces, led to a gradual decrease in thermal stability (Clantin et al. 2001). Indoleglycerol phosphate synthase is monomeric both in mesophiles and hyperthermophiles. However, IGPS from *Sulfolobus solfataricus* and *T. maritima* contain twice the number of potentially stabilizing ion pairs compared with *E. coli* (Hennig et al. 1995; Merz et al. 1999). Two *T. maritima* IGPS variants, which had one of these ion pairs disrupted by site-directed mutagenesis, showed significantly decreased thermostabilities (Merz et al. 1999). The stabilizing role of ion pairs was also proven by site-directed mutagenesis experiments performed with glyceralaldehyde-3-phosphate dehydrogenase (GAPDH) from *T. maritima*, glutamate dehydrogenases from both *Thermococcus litoralis* and *P. furiosus*, 3-isopropyl-malate dehydrogenase from *Thermus thermophilus*, rubredoxin from *P. furiosus*, and the archaeal histone from *Methanothermus fervidus* and citrate synthase from psychrophiles to hyperthermophiles. The latter represents a good example for the whole spectrum of adaptive changes, including intersubunit ionic networks and varying states of association (Wrba et al. 1990; Tomschy et al. 1994; Pappenberger et al. 1997; Vetriani et al. 1998; Li et al. 2000; Németh et al. 2000; Strop and Mayo 2000; Nordberg Karlsson et al. 2002, 2003; Bell et al. 2002, and references therein). The increased $\Delta G_{stab,70°C}$ of the hyperthermophilic cold-shock protein from *T. maritima* compared to its mesophilic counterpart from *B. subtilis* was shown to be largely due to Arg3, whose positive charge improves the global electrostatic potential of the protein (Perl and Schmid 2001; cf. the

Table 17.4

Change in amino acid composition going from proteins of mesophiles to proteins of thermophiles

Amino acid	Gains	Losses	Ratio	Net change	Change, %
Ile	842	658	1.28	184	9.5
Glu	739	562	1.31	177	9.1
Arg	383	214	1.79	169	16.5
Lys	789	620	1.27	169	8.3
Pro	167	96	1.74	71	7.0
Tyr	224	177	1.27	47	5.8
Ala	504	458	1.10	46	2.8
Trp	23	11	2.09	12	8.3
Leu	560	548	1.02	12	0.6
Cys	72	69	1.04	3	0.9
Phe	200	202	0.99	−2	−0.3
Asp	429	432	0.99	−3	−0.2
Val	666	670	0.99	−4	−0.2
His	80	92	0.87	−12	−2.8
Gly	201	264	0.76	−63	−3.4
Met	174	248	0.70	−74	−11.3
Gln	158	234	0.68	−76	−13.1
Thr	336	431	0.78	−95	−8.4
Asn	313	481	0.65	−168	−15.9
Ser	271	664	0.41	−393	−31.7

Data from Haney et al. (1999)

Ile isoleucine, *Glu* glutamic acid, *Arg* arginine, *Lys* lysine, *Pro* proline, *Tyr* tyrosine, *Ala* alanine, *Trp* tryptophan, *Leu* leucine, *Cys* cysteine, *Phe* phenylalanine, *Asp* aspartic acid, *Val* valine, *His* histidine, *Gly* glycine, *Met* methionine, *Gln* glutamine, *Asn* asparagine, *Ser* serine

section on "Stability of Biomolecules"). This result suggests that the optimum placement of charged groups on the surface of a protein is crucial for its thermostability (Xiao and Honig 1999), a hypothesis that is strengthened by a number of other experimental studies (Grimsley et al. 1999; Loladze et al. 1999; Spector et al. 2000).

In spite of these examples, in many cases, the predicted stabilizing interactions (as deduced from pairwise mesophile-thermophile comparisons of sequences and structures) could not be verified experimentally. Therefore, it is still not possible to deduce general mechanisms that would lead to high protein thermostability. The reason for this shortcoming is the large number of neutral changes of amino acid residues and 3D structures that have accumulated during evolution without affecting protein stability (Böhm and Jaenicke 1994; Arnold et al. 2001). Based on this argument, large-scale structural comparisons of amino acid sequences and 3D structures, which reduce the large "phylogenetic noise," are likely to provide more significant results. Such systematic comparisons are now possible owing to the growing number of complete genome sequences from mesophiles and hyperthermophiles and the fast rate with which new X-ray structures become available.

The amino acid compositions of a number of mesophiles and thermophiles were deduced from their genome sequences and compared in several systematic studies (*Table 17.4*).

These comparisons allow the following conclusions. Hyperthermophilic proteins (1) contain a decreased content of uncharged polar amino acids, this way avoiding deamidation of Gln and Asn catalyzed by Thr and Ser (Wright 1991; Haney et al. 1999; cf. the section on "Biochemical Limitations at High Temperature"); (2) show an increased content of the charged amino acids Glu and Asp, a significant fraction of which may be involved in stabilizing ion pairs at the protein surface (see above; Haney et al. 1999; Cambillau and Claverie 2000); and (3) are on average significantly smaller than their mesophilic homologs (Chakravarty and Varadarajan 2000), presumably owing to shorter solvent-exposed surface loops (Thompson and Eisenberg 1999) or extensions at the N- and/or C-terminal ends (*Fig. 17.12*).

Upon unfolding, small proteins show a smaller heat capacity change (ΔC_p) than large proteins (Murphy and Freire 1992; Myers et al. 1995); a decrease in ΔC_p flattens the ΔG_{stab} versus T profile and leads to an increase in T_m (*Fig. 17.3b*).

The three-dimensional structures of proteins from mesophiles and thermophiles were compared in a number of comprehensive studies. From a nonredundant dataset of high-quality X-ray structures of protein subunits from mesophiles, thermophiles, and hyperthermophiles, it revealed that the increase in intrinsic stability was paralleled by more ion pairs (apart from slight differences with respect to cavities),

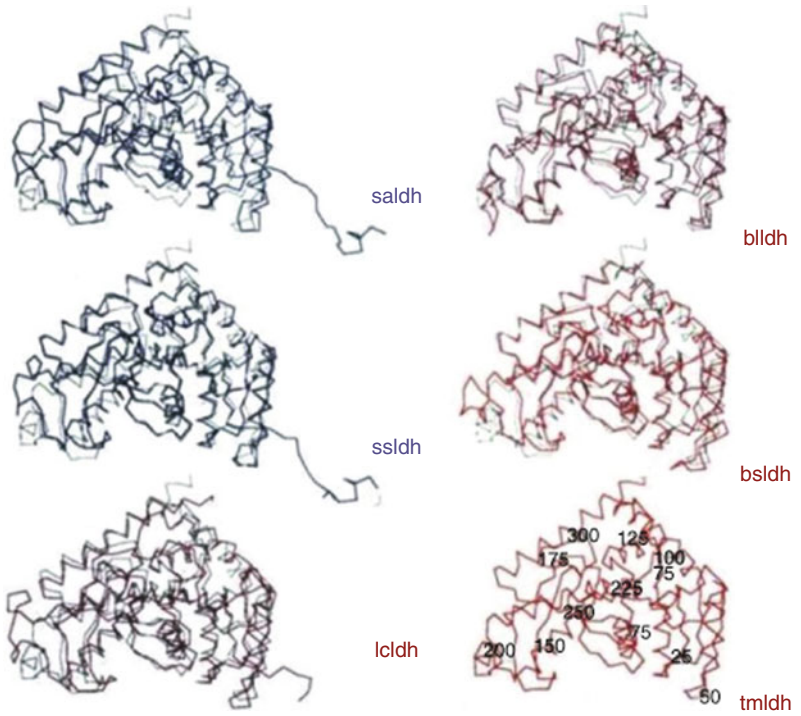


Fig. 17.12

The three-dimensional structures of lactate dehydrogenases (LDH) from hyperthermophiles, on the one hand, and mesophiles as well as a cold-blooded fish, on the other, are practically isomorphous, with root-mean-square (rms) differences below 2.4 Å. Comparisons of the 2–3 Å resolution crystal structures of the various homologs with the enzyme from *Thermotoga maritima* (as the reference; in gray) show that the increase in thermostability is paralleled (1) by a reduction in the length of the C-terminal extension, (2) by an increase in compactness of the tetrameric assembly, and (3) by the presence of an additional "thermohelix" (αT) in each of the subunits of the hyperthermophile enzyme. The shift from blue to red in the figure characterizes the temperature range of the organisms from which the various enzymes were isolated. The corresponding abbreviations and physiological T_{opt}-values refer to sa *Squalus acanthias* (dogfish, ~10 °C), ss *Sus scrofa* (pig, 37 °C), lc *Lactobacillus casei* (~30 °C), bl *Bifidobacterium longum* (~40 °C), bs *Bacillus stearothermophilus* (~65 °C), and tm *Thermotoga maritima* (~80 °C) (For details, see Auerbach et al. (1998))

hydrogen bonds, secondary structure content, and polarity of surfaces (Szilagyi and Závodszky 2000; *Table 17.5*).

A similar study suggested that ion pairs and side-chain-side-chain hydrogen bonds are more frequent in thermophilic than in mesophilic proteins (Kumar et al. 2000a, b). There was no evidence for significant differences with respect to compactness, hydrophobicity, polar and nonpolar surface area, protein size, and number of Pro residues in loops; however, thermophilic proteins appeared to have a higher fraction of residues in α-helices.

Two further investigations confirmed that the α-helices of thermophilic proteins show increased stability, mainly due to the higher intrinsic helical propensities of the amino acids involved (Petukhov et al. 1997; Facchiano et al. 1998). Two systematic comparisons of lactate dehydrogenases (LDH) and triosephosphate isomerases (TIM) from psychrophiles, mesophiles, and hyperthermophiles revealed positive correlations between thermostability and the number of intrasubunit (LDH) and intersubunit ion pairs (TIM), respectively (Auerbach et al. 1998; Maes et al. 1999).

The results of the cited mutational studies and those of the systematic and comprehensive comparisons between the amino acid sequences and 3D structures of psychrophilic, mesophilic, and thermophilic proteins can be summarized as follows: Owing to the small differences between ΔG_{stab} of hyperthermophilic and mesophilic proteins (Matthews 1993, 1996; Jaenicke and Böhm 1998), attempts to find a unifying set of rules of stabilization must fail. The structural features that characterize some of the known hyperthermophilic proteins are increased numbers of hydrogen bonds, higher packing densities and α-helical contents, improved hydrophobic interactions, optimized surface areas, decreased volumes, fewer cavities, and a shortening of the polypeptide chains. Attempts to define the relative significance of these many different factors by counting their frequency in comprehensive comparative studies led to four major contributions: (1) stabilized α-helices, (2) decreased entropy of the unfolded state by increased numbers of Pro and β-branched amino acid residues, (3) decreased content of chemically labile polar amino acid residues, and, in particular, (4) increase in the number of optimized ionic interactions (Sanchez-Ruiz and Makhatadze 2001). The latter finding is in accordance with theoretical work suggesting ion pairs are more stabilizing at high than at low temperatures and might therefore be crucial

Table 17.5

Systematic comparison of the structures of proteins from mesophiles, thermophiles, and extreme thermophiles

Property		Correlation with temperature	Change in proteins from moderate thermophiles	Change in proteins from extreme thermophiles
Cavities	Number	↓↓	0	↓↓↓
	Volume	↓	↑	↓
	Area	↓	↑	↓↓
Hydrogen bonds	Number	0	0	0
	Unsatisfied	↓	↓	↓
Ion pairs	<4.0 Å	↑↑	↑	↑↑↑
	<6.0 Å	↑↑	↑↑	↑↑↑
	<8.0 Å	↑↑↑	↑↑↑	↑↑↑
Secondary structure	α	0	↑	0
	β	↑	0	↑↑
	Irregular	↓	↓	↓
Polarity of surfaces		↓↓	↑↑↑	0
	Exposed			
	Buried	0	↑	↑

From Szilagyi and Závodszky (2000)

The number of arrows (1, 2 or 3) shows whether the represented correlation or change is considered insignificant, moderately significant, or highly significant

for the stability of hyperthermophilic proteins (Elcock and McCammon 1997; Elcock 1998; De Bakker et al. 1999). It is important to note that the stabilizing effect of a given ion pair depends on its structural context. Ion pairs that connect N- and C-termini in IGPS and GAPDH from *T. maritima* contribute significantly to thermostability, probably by preventing the fraying of the N- and C-termini, which might initiate thermal denaturation (Pappenberger et al. 1997; Merz et al. 1999). Also, for entropic reasons, clusters of ion pairs are likely to be more stabilizing than individual ion pairs (Yip et al. 1995, 1998).

Although our knowledge of the structural basis of high intrinsic protein thermostability is still incomplete, considerable operational progress has been achieved in the last years, especially in the first successful examples of rational or semiempirical improvements of protein thermostability (Malakauskas and Mayo 1998; van den Burg et al. 1998). An alternative approach to improve protein thermostability is "directed molecular evolution" (Wintrode and Arnold 2000; Arnold et al. 2001). It mimics the natural evolution process by applying iterative rounds of random mutagenesis and selection (or screening) of stabilized protein variants. Given an appropriate selection or screening system, this approach is generally applicable because it does not require specific knowledge of the structure of the protein to be stabilized. Moreover, directed evolution is instructive because it is unbiased and may provide stabilizing amino acid exchanges at positions in the protein that were not anticipated. Moreover, because wild-type and stabilized proteins differ only in few amino acids, the analysis of the observed effects is considerably simplified, compared with the analysis of the much more diverse homologous mesophilic-thermophilic protein pairs. Recent successful examples of stabilizing proteins by directed evolution were summarized in Arnold (2001) and Sterner and Liebl (2001).

Extrinsic Stabilization by Accessory Compounds

It has been known for some time that the intrinsic stability of some proteins from hyperthermophiles is too low to allow their function in vivo (Thomm et al. 1986; Fabry and Hensel 1987). These observations suggested that these proteins are stabilized by extrinsic factors such as metabolites or proteins. Many organisms accumulate high concentrations of organic solutes in response to various stress conditions. These solutes are called "compatible solutes" because they do not compromise cellular functions (Carpenter et al. 1993; da Costa et al. 1998). Low molecular mass solutes in the aqueous environment of proteins can have various effects on protein solubility and stability. At low concentrations, salts can increase protein solubility (i.e., have a salting-in effect), whereas at high concentrations they can lead to protein precipitation (i.e., a salting out of protein; cf. the section on ❯ "Water," subsection ❯ "Hydration"). Also, some solutes (e.g., urea or guanidinium chloride) destabilize proteins, whereas others (e.g., glycerol) have a stabilizing effect (Timasheff 1995; Timasheff and Arakawa 1997). Two conclusions follow from the fact that in all these cases, high concentrations (usually >1 M) of the additives are required: (1) The intermolecular interactions involved must be nonspecific and weak, and (2) water (i.e., hydration) must play an important role, since the effect of a particular compound depends on the differential affinities of the protein and the additive for water (Timasheff 1995). The precipitating and stabilizing compounds are preferentially excluded from the surface of the protein, that is, the protein has a higher affinity for water than for these agents. As a consequence, proteins are preferentially hydrated, which favors the native state and makes unfolding more unfavorable. In contrast, destabilizing

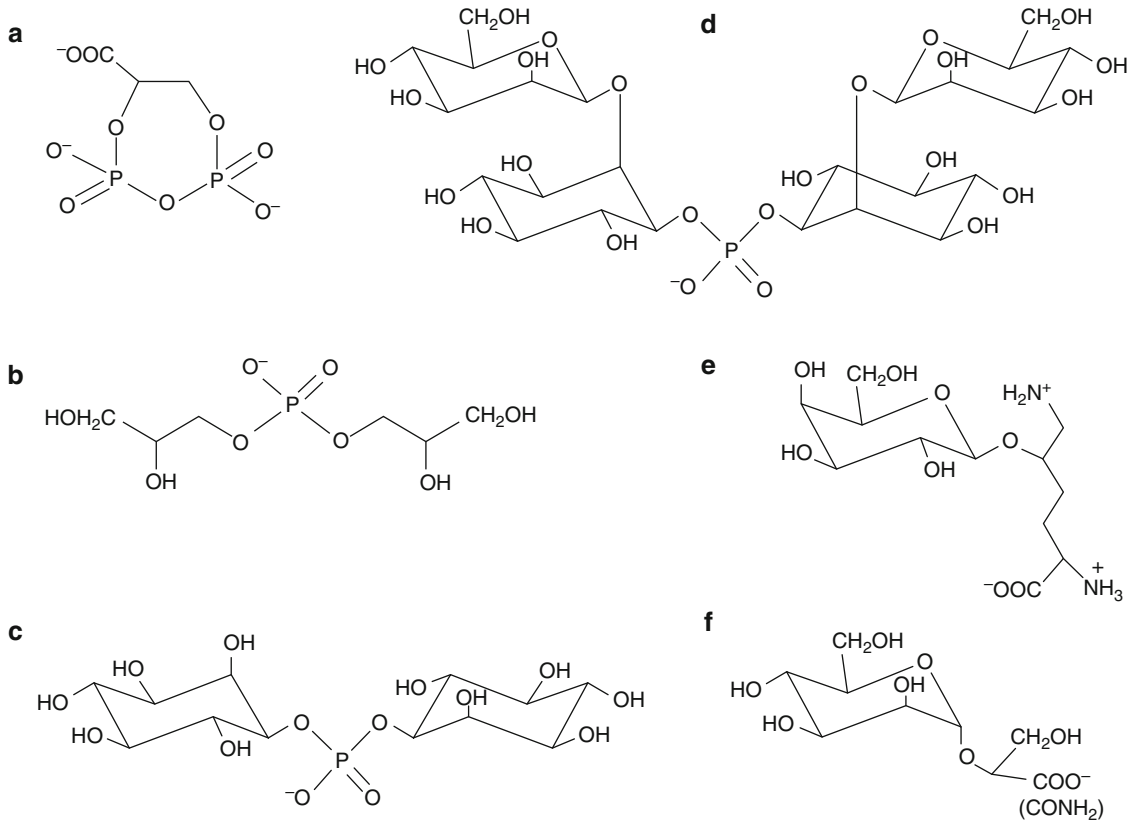


Fig. 17.13

Compatible solutes from hyperthermophiles. (a) Cyclic 2,3-diphosphoglycerate, (b) diglycerol phosphate, (c) di-*myo*-inositol-1,1′-phosphate, (d) di-2-*O*-β-mannosyl-di-*myo*-inositol-1,1′-phosphate, (e) β-galactopyranosyl-5-hydroxylysine, and (f) α-mannosylglycerate and α-mannosylglyceramide

agents bind more strongly than water to proteins. Stabilizing compounds include sugars and polyols (sucrose, trehalose, glycerol, mannitol, and sorbitol), amino acids (proline and glycine), methyl amines (sarcosine, trimethylamine-*N*-oxide, and glycine betaine), tetrahydropyrimidine derivatives (ectoins), and some salting-out salts (Timasheff 1995; Knapp et al. 1999).

In recent years, a number of compatible solutes have been found specifically in thermophiles and hyperthermophiles (Fig. 17.13). Some of them are likely to be adaptations to life at high temperatures: They are overproduced upon upshifting the growth temperature of a given microorganism and significantly increase the stability of a number of its proteins in in vitro measurements. For example, cyclic 2,3-diphosphoglycerate (cDPG) was discovered in hyperthermophilic methanogens (Hensel and König 1988; Martins et al. 1997), and increasing growth temperature was found to increase its concentration in *Methanothermus fervidus*. The addition of cDPG drastically increased the in vitro stability of GAPDH from *M. fervidus*, but not that of the homologous enzyme from rabbit (Hensel and König 1988). Various derivatives of *myo*-inositol phosphate were found in hyperthermophilic archaea and bacteria; a correlation between solute accumulation and growth temperature was detected in some cases (Ciulla et al. 1994; Martins and Santos 1995; Martins et al. 1996, 1997; Ramakrishnan et al. 1997; Lamosa et al. 1998). Di-*myo*-inositol phosphate was found to stabilize GAPDH from *Pyrococcus woesei*, but sodium citrate had a similar effect (Scholz et al. 1992). Clearly, more information is required about the stabilization of proteins by *myo*-inositol phosphate derivatives. It is remarkable that a number of hyperthermophiles with a low salt requirement, for example, *Thermotoga thermarum*, *Fervidobacterium islandicum*, *Pyrobaculum islandicum*, or *Thermococcus zilligii* AN1, do not produce significant amounts of compatible solutes, either at their optimum growth temperatures or after a temperature upshift (Martins et al. 1996; Lamosa et al. 1998). It was therefore postulated that some compatible solutes, which are produced by slightly halophilic thermophiles, might act when osmotic and temperature stresses occur simultaneously (Lamosa et al. 1998).

Molecular Chaperones

As mentioned earlier, molecular chaperones are ubiquitous in all living cells (cf. to the section "Stability of Biomolecules"). As heat-shock proteins (HSPs), they regulate the kinetic partitioning between folding → association and misfolding → aggregation of polypeptide chains at elevated temperature

(Bukau 1999; Jaenicke and Lilie 2000). "Thermosomes" in thermophilic and hyperthermophilic archaea are double-ring cages with eight- to ninefold symmetry consisting of HSP60 subunits (Archibald et al. 1999). As shown for *Pyrodictium occultum*, at the upper temperature limit of viability, they may be expressed to protein levels up to 80 % of the total cellular protein, indicating that the chaperone is essential for survival under stress conditions (Phipps et al. 1991). Similarly, the hyperthermophiles *Sulfolobus shibatae* and *Archaeoglobus fulgidus* display heat-induced synthesis of high chaperone levels (Kagawa et al. 1995; Emmerhoff et al. 1998). Apart from the correlation of heat stress and HSP expression, relatively little is known about the specific functions of thermosomes. In vitro experiments with the recombinant proteins clearly showed that the thermosomes from *Pyrodictium occultum* and *Methanopyrus kandleri* display chaperone-like activities, but only dead-end complexes with nonnative substrates bound to the reconstituted thermosomes were observed (Minuth et al. 1998, 1999). Because of the complexity of the systems, these experiments were performed with mesophilic substrate proteins below the optimum growth temperatures of the hyperthermophiles. To elucidate the in vivo function, further experiments under more physiological conditions are required.

Hsp70 (or DnaK) proteins have a multitude of functions; they are coupled to nucleotide binding and hydrolysis and modulated by the co-chaperones Hsp40 (DnaJ) and GrpE (Bukau and Horwich 1998). Some moderately thermophilic archaea such as *Methanobacterium thermoautotrophicum* possess Hsp70, while the most thermophilic ones have no *hsp*70-homolog encoding genes in their genomes (Gribaldo et al. 1999; Macario et al. 1999); it was therefore speculated that a structurally unrelated chaperone may take over its role in these archaea (Leroux et al. 1999; Siegert et al. 2000). In contrast, the hyperthermophilic bacteria belonging to the genera *Thermotoga* and *Aquifex* possess Hsp70 homologs (Macario et al. 1999).

An important function of chaperones, in addition to the inhibition of aggregation, is the unfolding of proteins, which either feeds misfolded proteins into the cellular degradation system or offers aggregated protein molecules another chance for proper folding by "iterative annealing" (Horwich et al. 1999; Shtilerman et al. 1999; Weber-Ban et al. 1999; Chen et al. 2001; Grantcharova et al. 2001). Members of the Hsp100/Clp family display significant unfoldase activity of misfolded proteins in yeast and *E. coli*, cooperating with Hsp70 (DnaK) in the subsequent refolding process (Glover and Lindquist 1998; Goloubinoff et al. 1999; Mogk et al. 1999; Weber-Ban et al. 1999). Protein aggregates are first bound by ClpB; an ATP-triggered structural change leads then to the presentation of hydrophobic regions of aggregated proteins, which are subsequently solubilized by DnaK. The cooperation of ClpB and DnaK was also demonstrated for ClpB and DnaK from the extreme thermophile *Thermus thermophilus*. In vitro, the DnaK system (DnaK, DnaJ, GrpE, and ATP) suppressed heat-induced aggregation of substrate proteins and ClpB-induced efficient refolding (Motohashi et al. 1999). It is not clear whether similar DnaK-ClpB systems exist in other hyperthermophiles. While the genomes of the hyperthermophilic bacteria *T. maritima* and *Aquifex aeolicus* contain *dnaK* and *clpB* gene homologs, no such homologs have been detected in the archaea so far.

In summarizing, the extrinsic stabilization of proteins by compatible solutes and molecular chaperones appears to be crucial for many hyperthermophiles, especially when they grow close to their upper temperature limits. Further experiments under these extreme conditions promise insights into the cellular mechanisms that set the upper temperature limit at which life is possible.

Conclusions

This survey is based on the assumption that the upper temperature limit of life is dictated by molecular instability at the level of both cytosolic low molecular weight compounds and biopolymers. As indicated by in vitro experiments, a number of metabolites, especially coenzymes, are unstable under optimum growth conditions of thermophiles and hyperthermophiles. Mechanisms to overcome this instability include (1) rapid catalytic turnover, (2) metabolic channeling, and (3) local stabilization by weak intermolecular interactions. In the case of biopolymers, the canonical building blocks are sufficiently stable to allow structural and functional integrity at temperatures close to or even beyond the boiling point of water. Depending on the structural context, significantly higher stabilities can be accomplished, for example, by evolutionary protein design or chemical modification. However, evolution in the biosphere optimizes for (multi)functionality rather than stability. Basically, the free energy of stabilization is accumulated from small increments involving electrostatic and hydrophobic interactions. Covalent modifications or extrinsic factors such as salts, specific ligands, and compatible solutes may significantly improve thermostability.

In the case of nucleic acids, chemical modification (e.g., RNA methylation) and binding of extrinsically stabilizing components (salts and histone-like basic proteins) are essential in maintaining replication, transcription, and translation at temperatures close to or even beyond the melting temperature of DNA and RNA. Only for RNAs, enhanced base-pairing has been observed, whereas thermophile and hyperthermophile DNA shows unexpectedly low G + C contents, even for archaea with optimal growth temperatures above 100 °C.

Considering the lipid constituents of mesophilic and thermophilic membranes, characteristic differences have been discovered: Thermophilic archaea contain highly caldarchaeol ether lipids, which form stable monolayers that span the entire membrane (Fig. 17.8), whereas the ester lipids of bacterial thermophiles are stabilized by a high content of saturated fatty acids. The necessary fluidity is regulated either by differences in the degree of saturation or by adjusting the chemical composition of the fatty-acid hydrocarbon side chains.

In the case of proteins, enhanced intrinsic stability in thermophiles compared to their mesophilic counterparts is the

Fig. 17.14
Universal phylogenetic tree based on rRNA sequences. The scale bar corresponds to 0.1 changes per nucleotide (From Pace (1997), with permission. Regarding the positioning of the Nanoarchaeota, see Huber et al. (2003))

cumulative effect of minute improvements of local interactions at the secondary-, tertiary-, and quaternary structural level, for example, higher packing efficiency, networks of ion pairs and/or hydrogen bonds, and reduction of conformational strain. Taken together, these increments suggest that thermostability corresponds to increased rigidity at low temperature and shifts to normal flexibility at physiological temperature; evidently, evolutionary adaptation to a physical parameter tends to maintain "corresponding states" with regard to conformational flexibility. At this point, it seems appropriate to stress that any generalization in considering the structure-function relationship of biopolymers, on the one hand, and their stability, on the other, needs careful controls. In the present case, this may be illustrated by a number of contradicting observations: (1) There are hyperthermophilic enzymes with high intrinsic thermostability that are more active than their mesophilic counterparts, even at room temperature, thus combining high catalytic efficiency with high overall rigidity (Sterner et al. 1996; Ichikawa and Clarke 1998; Merz et al. 1999). (2) In stressing overall rigidity, it is important to notice that there is not necessarily a single measure of flexibility: A given 3D structure of a protein molecule may provide a rigid scaffold, for example, a $(\beta\alpha)_8$-barrel, at the same time showing high catalytic efficiency due to the local flexibility of its active center (Shoichet et al. 1995). Along these lines, attempts have been reported to distinguish the "macro-" and "microstability" of proteins (Závodszky et al. 1998). (3) There is no fundamental reason for stability and rigidity to be strictly correlated because flexibility implies high conformational entropy of the folded state, which is favorable to thermodynamic stability. In addition, rigidity and flexibility may depend on the methods applied; one and the same protein may be rigid on a nanosecond time scale, but flexible on a millisecond time scale (Lazarides et al. 1997; Daniel and Cowan 2000). (4) Making use of the amide hydrogen-exchange rates in rubredoxin from *Pyrococcus furiosus* (the most thermostable protein presently known), it was shown that

conformational opening processes occur within milliseconds for all amide positions along the polypeptide chain; the corresponding distribution of amide protection factors is indistinguishable from data reported for typical mesophilic homologs (Hernández et al. 2000; Jaenicke 2000b). Obviously, these data are in contrast to the above generalization that enhanced conformational rigidity in the folded native state determines the increased thermal stability of thermophilic and hyperthermophilic proteins. At present, there is no way to resolve the apparent discrepancies; more experiments need to be done to combine the data to a new general view of protein stabilization.

Another open question refers to the phylogeny of microorganisms and their genes (cf. Doolittle 1998; Koonin et al. 1998). Considering the protein repertoire of mesophiles and thermophiles, a wealth of experimental data proved that the average stabilities of thermophilic proteins exceed the stabilities of the corresponding mesophilic proteins (*Fig. 17.2*). Roughly speaking, the mutative adaptation of a mesophile to a high-temperature environment requires the adaptation of the complete proteome to the higher temperature. For the reverse shift, a single temperature-sensitive mutation is sufficient. In spite of this simple argument, it is still unclear which of the two alternatives describes the direction of natural selection in the early evolution of the biosphere. The accumulation of extreme thermophiles close to the root of the (16 S rRNA) phylogenetic tree (*Fig. 17.14*) favored the hypothesis that the prebiotic soup was hot, suggesting that thermophiles preceded mesophiles in the early history of life (Woese et al. 1990).

However, the advent of complete genome sequences made it clear that the phylogenetic tree has more complex roots than expected from a single genetic marker molecule inasmuch as different genes may or may not agree with the tRNA tree. Even more perplexing, genomes may contain a mix of DNAs, some related to archaea, while others are close to bacteria, so that a given microorganism, depending on the marker gene, ends up at different phylogenetic placements. It looks as if each gene has its own history, possibly due to mechanisms such as horizontal gene transfer or "gene swapping" (Nelson et al. 1999; Ochman et al. 2000). Although the mechanism of gene swapping is still unknown, there seems to be no better explanation for the observation that 17 out of 34 families of eukaryotic proteins that date back to early cell evolution look as if they come from bacteria, while only 8 families show a greater similarity to archaea, the supposed ancestor of eukarya. In spite of these inconsistencies, presently available genome sequences still fit the three-kingdom hypothesis (Miller and Lazcano 1995; Woese 1998; Deckert et al. 1998; Doolittle 1998; Daniel and Cowan 2000). No thermophilic eukarya have been discovered so far (R. Rachel, personal communication, 2001); possible reasons for this observation have been mere speculation.

Concerning the geological time when hyperthermophilic microorganisms might have evolved, there are claims for the occurrence of a variety of early archaea around 4 billion years ago. Photosynthetic life (both anoxygenic and oxygenic) has been established as early as 3.5–3.7 billion years ago (Rosing 1999), molecular fossil evidence allowed the existence of cyanobacteria to be traced back 2.7 billion years (Brocks et al. 1999), and in addition, chemotrophic archaea have been spotted in 3.2 billion-year-old volcanogenic massive sulfide rocks (Rasmussen 2000). The latter finding extends the realms of thermophilic archaeal life into the extreme of deep-sea hot springs, in addition to the open ocean, mid-ocean ridges, lake communities, costal sediments, and coastal hydrothermal systems. It does not show that abyssal hydrothermal life came before photosynthesis; however, it may be taken to support the idea that steps in the early history of life took place around hydrothermal systems (Nisbet 2000).

References

Albers S-J, van de Vossenberg JL, Driessen AJ, Konings WN (2000) Adaptations of the archaeal cell membrane to heat stress. Front Biosci 5:D813–D820

Alexandrov VY (1969) Conformational flexibility of proteins, their resistance to proteinases and temperature conditions of life. Curr Med Biol 3:9–19

Andrä S, Frey G, Jaenicke R, Stetter KO (1998) The thermosome from Methanopyrus kandleri possesses a unique NH_4-dependent ATPase activity. Eur J Biochem 255:93–99

Archibald JM, Logsdon JM Jr, Doolittle W (1999) Recurrent paralogy in the evolution of archaeal chaperonins. Curr Biol 9:1053–1056

Arnold FH (2001) Evolutionary protein design. Adv Protein Chem 55:1–225

Arnold FH, Wintrode PL, Miyazaki K, Gershenson A (2001) How enzymes adapt: lessons from directed evolution. Trends Biochem Sci 26:100–106

Auerbach G, Ostendorp R, Prade L, Korndörfer I, Dams T, Huber R, Jaenicke R (1998) Lactate de-hydrogenase from the hyperthermophilic bacterium *Thermotoga maritima*: the crystal structure at 2.1 Å resolution reveals strategies for intrinsic protein stabilization. Structure 6:769–781

Auffinger P, Westhof E (2002) Melting of the solvent structure around an RNA duplex: a molecular dynamics simulation study. Biophys Chem 95:203–210

Baldwin RL (1986) Temperature dependence of the hydrophobic interaction in protein folding. Proc Natl Acad Sci USA 83:8069–8072

Baldwin RL (1996) How Hofmeister ion interactions affect protein stability. Biophys J 71:2056–2063

Barrett GC (1985) Reactions of amino acids. In: Chemistry and biochemistry of amino acids. Chapman and Hall, New York, pp 354–375

Beissinger M, Buchner J (1998) How chaperones fold proteins. Biol Chem 379:245–259

Bell GS, Russell RJM, Connaris H, Hough DW, Danson MJ, Taylor GL (2002) Stepwise adaptations of citrate synthase to survival at life's extremes. From psychrophile to hyperthermophile. Eur J Biochem 269:6250–6260

Benedek GB (1997) Cataract and protein condensation. Invest Ophthalmo Vis Sci 38:1911–1921

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316

Bernal JD (1939) Structure of proteins. Nature (London) 143:663–667

Bernal JD (1958) Structure arrangements of macromolecules. DiscussFaraday Soc 25:7–18

Bernhardt T, Lüdemann H-D, Jaenicke R, König H, Stetter KO (1984) Biomolecules are unstable under "Black smoker" conditions. Naturwissenschaften 71:583–586

Bieri A, Kiefhaber T (2000) Kinetic models in protein folding. In: Pain RH (ed) Mechanisms of protein folding, vol 32, 2nd edn, Frontiers in molecular biology. Oxford University Press, Oxford, pp 34–64

Blöchl E, Rachel R, Burggraf S, Hafenbradl D, Jannasch HW, Stetter KO (1997) Pyrolobus fumarii represents a novel group of archaea, extending the upper temperature limit for life to 113 °C. Extremophiles 1:14–21

Blokzijl W, Engberts JBFN (1993) Hydrophobe Effekte—Ansichten und Tatsachen. Angew Chem 105:1610–1648

Böhm G, Jaenicke R (1994) On the relevance of sequence statistics for the properties of extremophilic proteins. Int J Pept Protein Res 43:97–106

Bosch G, Baumeister W, Essen L-O (2000) Crystal structure of the β-apical domain of the thermosome reveals structural plasticity in the protrusion region. J Mol Biol 301:19–25

Branden C, Tooze J (1998) Introduction to protein structure, 2nd edn. Garland, New York, NY, pp 354–356

Brock TD (1978) Thermophilic microorganisms and life at high temperature. Springer, New York

Brock TD (ed) (1986) Thermophiles: general molecular and applied microbiology. Wiley, New York

Brock TD (2000) Biology of microorganisms, 9th edn. Prentice-Hall International, London

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

Bukau B (ed) (1999) Molecular chaperones and folding catalysts. Harwood Academic Publishers, Amsterdam, The Netherlands

Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. Cell 92:351–366

Burston SG, Saibil HR (1999) The relationship between chaperonin structure and function. In: Bukau B (ed) Molecular chaperones and folding catalysts. Harwood Academic, Amsterdam, The Netherlands, pp 523–553

Cambillau C, Claverie J-M (2000) Structural and genomic correlates of hyperthermostability. J Biol Chem 275:32383–32386

Careri G, Gratton E, Yang PH, Rupley JA (1980) Correlation of IR spectroscopic, heat capacity, diamagnetic susceptibility and enzymatic measurements on lysozyme powder. Nature (London) 284:572–573

Carpenter JF, Clegg JS, Crowe JH, Somero GN (eds) (1993) Compatible solutes and macromolecular stability. Cryobiology 30: 201–241

Cavicchioli R, Thomas T, Curmi PM (2000) Cold stress response in archaea. Extremophiles 4:321–331

Cecil R (1963) Intermolecular bonds in proteins. I: the role of sulfur in proteins. In: Neurath H (ed) The proteins: composition, structure, and function, vol 1, 2nd edn. Academic, New York, pp 380–476

Cecil R, McPhee JR (1963) The sulfur chemistry of proteins. Adv Protein Chem 14:255–389

Chakravarty S, Varadarajan R (2000) Elucidation of determinants of protein stability through genome sequence analysis. FEBS Lett 470:65–69

Chang EL (1994) Unusual thermal stability of liposomes made from bipolar tetraether lipids. Biochem Biophys Res Commun 202:673–679

Chen J, Walter S, Horwich AL, Smith DL (2001) Folding of malate dehydrogenase inside the GroEL-GroES cavity. Nat Struct Biol 8:721–728

Chiu HJ, Johnson E, Schroder I, Rees DC (2001) Crystal structure of a novel ferric reductase from the hyperthermophilic archaeon *Archaeoglobus fulgidus* and its complex with $NADP^+$. Structure 9:311–319

Choquet CG, Patel GB, Beveridge TJ, Sprott GD (1994) Stability of pressure extruded liposomes made from archaeobacterial ether lipids. Appl Microbiol Biotechnol 42:375–384

Ciulla RA, Burggraf S, Stetter KO, Roberts MF (1994) Occurrence and role of di-myo-inositol-1,1′-phosphate in *Methanococcus igneus*. Appl Environ Microbiol 60:3660–3664

Clantin B, Tricot C, Lonhienne T, Stalon V, Villeret V (2001) Probing the role of oligomerization in the high thermal stability of *Pyrococcus furiosus* ornithine carbamoyl-transferase by site-specific mutants. Eur J Biochem 268:3937–3942

Clegg JS (2001) Cryptobiosis—a peculiar state of biological organization. Comp Biochem Physiol B Biochem Mol Biol 128:613–624

Collins KD, Washabaugh MW (1985) The Hofmeister series and the behavior of water at interfaces. Q Rev Biophys 18:323–422

Conte MR, Conn GL, Brown T, Lane AN (1996) Hydration of the RNA duplex r (CGCAAAUUUGCG)2 determined by NMR. Nucleic Acids Res 24:3693–3699

Covan DA (2004) The upper temperature for life—where do we draw the line? Trends Microbiol 12:58–60

Creighton TE (1994) Proteins: structures and molecular properties. W. H. Freeman, New York

Crothers DM, Zimm BH (1965) Viscosity and sedimentation of the DNA from bacteriophages T2 and T7 and the relation to molecular weight. J Mol Biol 12:525–536

Crow JH, Clegg JS (eds) (1978) Dry biological systems. Academic, New York

Cummins LL, Owens SR, Risen LM, Lesnik EA, Freier SM, McGee D, Guinosso CJ, Cook PD (1995) Characterization of fully 2'-modified oligoribonucleotide hetero-and homoduplex hybridization and nuclease sensitivity. Nucleic Acids Res 23:2019–2024

da Costa MS, Santos H, Galinski EA (1998) An overview of the role and diversity of compatible solutes in Bacteria and Archaea. In: Antranikian G (ed) Biotechnology of extremophiles. Springer, New York, pp 118–153

Dahlhoff EP, Somero GN (1993) Kinetic and structural adaptation of cytosolic MDHs of eastern Pacific abalones (genus *Haliotis*) from different thermal habitats: biochemical correlates of biogeographical patterning. J Exp Biol 185:137–150

Dalluge JJ, Hamamoto T, Horikoshi K, Morita RY, Stetter KO, McCloskey JA (1997) Posttranscriptional modification of tRNA in psychrophilic bacteria. J Bacteriol 179:1918–1923

Dams T, Jaenicke R (1999) Stability and folding of DHFR from the hyperthermophilic bacterium Thermotoga maritima. Biochemistry 38:9169–9178

Daniel RM, Cowan DA (2000) Biomolecular stability and life at high temperatures. Cell Mol Life Sci 57:250–264

Daniel RM, Dines M, Petach HH (1996) The denaturation and degradation of stable enzymes at high temperatures. Biochem J 317:1–11

Daniel RM, Smith JC, Farrand M, Hery S, Finney JL (1998) Enzyme activity below the dynamical transition at 200 K. Biophys J 75:2504–2507

Daniel RM, Finney JL, Reat V, Dunn R, Ferrand M, Smith JC (1999) Enzyme dynamics and activity: time scale dependence of dynamical transitions in glutamate dehydrogenase solution. Biophys J 77:2184–2190

Danson MJ (1988) Archaebacteria: the comparative enzymology of their central metabolic pathways. Adv Microb Physiol 29:165–231

De Bakker PI, Hunenberger PH, McCammon JA (1999) Molecular dynamics simulations of the hyperthermophilic protein sac7d from *Sulfolobus acidocaldarius*: contribution of salt bridges to thermostability. J Mol Biol 285:1811–1830

De Decker BS, O´Brien R, Fleming PJ, Geiger JH, Jackson SP, Sigler PB (1996) The crystal structure of a hyperthermophilic archaeal TATA-box binding protein. J Mol Biol 264:1072–1084

de Grado WF (1988) Design of peptides and proteins. Adv Protein Chem 39:51–124

de Rosa M, Gambacorta A (1988) The lipids of archaebacteria. Prog Lipid Res 27:153–175

de Rosa M, Trincone A, Nicolaus B, Gambacorta A (1991) Archaebacteria: lipids, membrane structures and adaptations to environmental stress. In: di Presco G (ed) Life under extreme conditions. Springer, New York, pp 61–87

de Vrij W, Bulthuis RA, Konings WN (1988) Comparative study of energy-transducing properties of cytoplasmic membranes from mesophilic and thermophilic Bacillus species. J Bacteriol 170:2359–2366

Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Keller M, Aujay M, Huber R, Feldman RA, Short JM, Olsen CJ, Swanson RV (1998) The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. Nature (London) 392:353–358

di Ruggiero J, Santangelo N, Nackerdien Z, Ravel J, Robb FT (1997) Repair of extensive ionizing-radiation damage at 95 °C in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 179:4643–4645

di Ruggiero J, Brown JR, Bogert AP, Robb FT (1999) DNA repair systems in archaea: mementos from the last universal common ancestor? J Mol Evol 49:474–484

Dill KA (1990) Dominant forces in protein folding. Biochemistry 29:7133–7155

Doolittle RF (1998) Microbial genomes opened up. Nature (London) 392:339–342

Edmonds CG, Crain PF, Gupta R, Hashizume T, Hocart CH, Kowalak JA, Pomerantz SC, Stetter KO, McCloskey JA (1991) Posttranscriptional modification of tRNA in thermophilic archaea (*Archaebacteria*). J Bacteriol 173:3138–3148

Edsall JT, Wyman J (1958) Water and its biological significance biophysical chemistry. In: Thermodynamics, electrostatics, and the biological significance of the properties of matter, vol 1. Academic, New York, pp 27–46

Einstein A (1905) Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. Ann Phys 17:549–560

Einstein A (1906) Zur Theorie der Brownschen Bewegung. Ann Phys 19:371–381

Eisenberg H (1976) Biological macromolecules and polyelectrolyte solutions. Clarendon, Oxford

Elcock AH (1998) The stability of salt bridges at high temperature: implications for hyperthermophilic proteins. J Mol Biol 284:489–502

Elcock AH, McCammon JA (1997) Continuum solvation model for studying protein hydration thermodynamics at high temperatures. J Phys Chem 101:9624–9634

Emmerhoff OJ, Klenk HP, Birkeland NK (1998) Characterization and sequence comparison of temperature-regulated chaperonins from the hyperthermophilic archaeon *Archaeoglobus fulgidus*. Gene 215:431–438

Fabry S, Hensel R (1987) Purification and characterization of GAPDH from the thermophilic archaebacterium *Methanothermus fervidus*. Eur J Biochem 165:147–155

Facchiano AM, Colonna G, Ragone R (1998) Helix stabilizing factors and stabilisation of thermophilic proteins: an X-ray based study. Protein Eng 11:753–760

Fersht AR (1987) The hydrogen bond in molecular recognition. Trends Biochem Sci 12:321–325

Fersht A (1998a) Protein stability, disulfide crosslinks. In: Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding. W. H. Freeman, New York, pp 534–535

Fersht A (1998b) Forces between molecules, and binding energies. In: Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding. W. H. Freeman, New York, pp 324–348

Figueiredo L, Klunker D, Ang D, Naylor DJ, Kerner MJ, Georgopoulos C, Hartl FU, Hayer-Hartl M (2004) Functional characterization of an archaeal GroEL/GroES chaperonin system: significance of substrate encapsulation. J Biol Chem 279:1090–1099

Finegold L (1986) Molecular aspects of adaptation to extreme cold environments. Adv Space Res 6:257–264

Finegold L (1998) In: Fink AL, Goto Y (eds) Molecular chaperones in the life cycles of proteins: structure, function and mode of action. Marcel and Dekker, New York, p 626

Forterre P, Bergerat A, López-Garcia P (1996) The unique DNA topology and DNA topoisomerases of hyperthermophilic archaea. FEMS Microbiol Rev 18:237–248

Franks F (ed) (1975–1982) Water: a comprehensive treatise. Plenum, New York, pp 1–7

Franks F (1985a) Biophysics and biochemistry at low temperature. Cambridge University Press, New York

Franks F (ed) (1985–1990) Water science reviews. Cambridge University Press, New York 1 ff

Franks F, Eagland D (1975) The role of solvent interactions in protein conformation CRC. Crit Rev Biochem 3:165–219

Franks F, Grigera JR (1990) Solution properties of low molecular weight polyhydroxy compounds. In: Franks F (ed) Water science reviews, vol 5. Cambridge University Press, New York, pp 187–289

Franks F, Gent M, Johnson HH (1963) The solubility of benzene in water. J Chem Soc 8:2716–2723

Franks F, Mathias SF, Hatley RHM (1990) Water, temperature and life. Phil Trans R Soc Lond B 326:517–533

Freier SM, Sugimoto N, Sinclair A, Alkema D, Neilson T, Kierzek A, Caruthers MH, Turner DH (1986) Stability of XGCGCp and XGCGCYp helices: an empirical Estimate of the energetics of H-bonds in nucleic acids. Biochemistry 25:3214–3219

Frey AH (1993) Electromagnetic field interactions with biological systems. FASEB J 7:272–281

Friedman M (1973) The chemistry and biochemistry of the sulfhydryl group in amino acids peptides and proteins. Pergamon, New York

Galtier N, Lobry JR (1997) Relationships between genomic G + C content, RNA secondary structure, and optimal growth temperatures in prokaryotes. J Mol Evol 44:632–636

Gliozzi A, Rolandi R, de Rosa M, Gambacorta A (1983) Monolayer black membranes from bipolar lipids of archaebacteria and their temperature-induced structural changes. J Membr Biol 75:45–56

Glover JR, Lindquist S (1998) Hsp104, Hsp70 and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94:73–82

Goloubinoff P, Mogk A, Peres A, Ben Z, Tomoyasu T, Bukau B (1999) Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. Proc Natl Acad Sci USA 96:13732–13737

Goodfellow JM, Cruzeiro-Hansson L, Norberto de Souza O, Parker K, Sayle T, Umrania Y (1994) DNA structure, hydration and dynamics. Int J Radiat Biol 66:471–478

Gottschalk A (1972) Glycoproteins: composition, structure, and function, 2nd edn. Elsevier, Amsterdam, The Netherlands

Grantcharova V, Alm EJ, Baker D, Horwich AL (2001) Mechanisms of protein folding. Curr Opin Struct Biol 11:70–82

Graumann P, Marahiel MA (1996) Some like it cold: response of microorganisms to cold shock. Arch Microbiol 166:293–300

Greenstein JP, Winitz M (1961) Chemistry of the amino acids. Wiley, New York

Gribaldo S, Lumia V, Creti R, de Macario EC, Sanangelantoni A, Cammarano P (1999) Discontinuous occurrence of the hsp70 (dnaK) gene among *Archaea* and sequence features of HSP70 suggest a novel outlook on phylogenics. J Bacteriol 181:434–443

Grimsley GR, Shaw KL, Fee LR, Alston RW, Huyghues-Despointes BM, Thurlkill RL, Scholtz JM, Pace CN (1999) Increasing protein stability by altering long-range coulombic interactions. Protein Sci 8:1843–1849

Grogan DW (1998) Hyperthermophiles and the problem of DNA stability. Mol Microbiol 28:1043–1049

Groß M, Jaenicke R (1994) Proteins under pressure: the influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. Eur J Biochem 221:617–630

Guipaud O, Marguet E, Knoll KM, Boutier-de la Tour C, Forterre P (1997) Both DNA gyrase and reverse gyrase are present in the hyperthermophilic bacterium *Thermotoga maritima*. Proc Natl Acad Sci USA 94:10606–10611

Gutsche I, Essen L-O, Baumeister W (1999) Group II chaperonins: new TriC(k)s and turns of a protein folding machine. J Mol Biol 293:295–312

Gyi JI, Lane AN, Conn GL, Brown T (1998) The orientation and dynamics of the C2'-OH and hydration of RNA and DNA.RNA hybrids. Nucleic Acids Res 26:3104–3110

Hafenbradl D, Keller M, Stetter KO (1993) Lipid analysis of *Methanopyrus kandleri*. FEMS Microbiol Lett 136:199–202

Haney PJ, Badger JH, Buldak GL, Reich CL, Woese CR, Olsen GJ (1999) Thermal adaptation analyzed by comparison of protein sequences from mesophilic and extremely thermophilic *Methanococcus* species. Proc Natl Acad Sci USA 96:3578–3583

Hartl FU, Hayer-Hartl M (2002) Protein folding—Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858

Heller M, John M, Coles M, Bosch G, Baumeister W, Kessler H (2004) NMR studies on the substrate-binding domains of the thermosome: structural plasticity in the protrusion region. J Mol Biol 336:717–729

Hennig M, Darimont B, Sterner R, Kirschner K, Jansonius JN (1995) 2.0 Å Structure of indole-3-glycerol phosphate synthase from the hyperthermophile *Sulfolobus solfataricus*: possible determinants of protein stability. Structure 3:1295–1306

Hennig M, Sterner R, Kirschner K, Jansonius JN (1997) Crystal structure at 2.0 Å resolution of phosphoribosylanthranilate isomerase from the hyperthermophile *Thermotoga maritima*: possible determinants of protein stability. Biochemistry 36:6009–6016

Hensel R, König H (1988) Thermoadaptation of methanogenic bacteria by intracellular ion concentration. FEMS Microbiol Lett 49:75–79

Hensel R, Jakob I, Scheer H, Lottspeich R (1992) Proteins from hyperthermophilic archaea: stability towards covalent modification of the polypeptide chain. Biochem Soc Symp 58:127–133

Hernández G, Jenney FE Jr, Adams MWW, LeMaster DM (2000) Millisecond time scale conformational flexibility in a hyperthermophile protein at ambient temperature. Proc Natl Acad Sci USA 97:3166–3170

Hinz H-J, Schmid FX (1977) Thermodynamics of the LDH reaction. In: Sund H (ed) Pyridine nucleotide-dependent dehydrogenases. W de Gruyter, New York, pp 292–306

Hochachka PW, Somero GN (1973) Strategies in biochemical adaptation. W.B. Saunders, Philadelphia

Hochachka PW, Somero GN (1984) Temperature adaptation biochemical adaptation. Princeton University Press, Princeton, pp 355–449

Höcker B, Jürgens C, Wilmanns M, Sterner R (2001) Stability, catalytic versatility and evolution of the $(\beta\alpha)_8$-barrel fold. Curr Opin Biotechnol 12:376–381

Hofmeister F (1888) Zur Lehre von der Wirkung der Salze: 2. Mittheilung. Arch Exp Pathol Pharmakol 24:247–260

Horst J-P, Fritz H-J (1996) Counteracting the mutagenic effect of hydrolytic deamination of DNA 5-methylcytosine residues at high temperature: DNA mismatch N-glycosylase Mig.Mth of the thermophilic archaeon *Methanobacterium thermoautotrophicum* THF. EMBO J 15:5459–5469

Horwich AL, Weber-Ban EU, Finley D (1999) Chaperone rings in protein folding and degradation. Proc Natl Acad Sci USA 96:11033–11040

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

Huber H, Hohn MJ, Stetter KO, Rachel R (2003) The phylum Nanoarchaeota: present knowledge and future perspectives of a unique form of life. Res Microbiol 154:165–171

Ichikawa JK, Clarke S (1998) A highly active protein repair enzyme from an extreme thermophile: the isoaspartyl methyltransferase from *Thermotoga maritima*. Arch Biophys Biochem 358:222–231

Inoue H, Hayase Y, Imura A, Iwai S, Miura K, Ohtsuka E (1987) Synthesis and hybridization studies on two complementary nona(2'-O-methyl)ribonucleotides. Nucleic Acids Res 15:6131–6148

Isabelle V, Franchet-Beuzit J, Sabattier R, Laine B, Spotheim-Maurizot M, Charlier M (1993) Radioprotection of DNA by a DNA-binding protein: MC1 chromosomal protein from the archaeon *Methanosarcina CHTI55*. Int J Radiat Biol 63:2232–2236

Jacob U, Gaestel M, Katrin E, Buchner J (1993) sHSPs are molecular chaperones. J Biol Chem 268:1517–1520

Jaenicke R (1971) Volume changes in the isoelectric heat aggregation of serum albumin. Eur J Biochem 21:110–115

Jaenicke R (1981) Enzymes under extremes of physical conditions. Annu Rev Biophys Bioeng 10:1–67

Jaenicke R (1987) Folding and association of proteins. Progr Biophys Mol Biol 49:117–237

Jaenicke R (1990) Protein structure and function at low temperature. Proc Natl Acad Sci USA 326:535–553

Jaenicke R (1991a) Protein folding: local structures, domains, subunits and association. Biochemistry 30:3147–3161

Jaenicke R (1991b) Protein stability and molecular adaptation to extreme conditions. Eur J Biochem 202:715–728

Jaenicke R (1999) Stability and folding of domain proteins. Progr Biophys Mol Biol 71:155–241

Jaenicke R (2000a) Stability and stabilization of globular proteins in solution. J Biotechnol 79:193–203

Jaenicke R (2000b) Do ultrastable proteins from hyperthermophiles have high or low conformational rigidity? Proc Natl Acad Sci USA 97:2962–2964

Jaenicke R, Böhm G (1998) The stability opf proteins in extreme environments. Curr Opin Struct Biol 8:738–748

Jaenicke R, Böhm G (2001) Thermostability of proteins from *Thermotoga maritima*. Meth Enzymol 334:438–469

Jaenicke R, Creighton TE (1993) Junior chaperones: α-Crystallins of the vertebrate eye lens are members of the family of small heat shock proteins sharing with other family members the ability to "chaperone" protein folding. Curr Biol 3:234–235

Jaenicke R, Lilie H (2000) Folding and association of oligomeric and multimeric proteins. Adv Protein Chem 53:329–401

Jaenicke R, Seckler R (1997) Protein misassembly in vitro. Adv Protein Chem 50:1–59

Jaenicke R, Slingsby C (2001) Lens crystallins and their microbial homologs: structure, stability and function. Crit Rev Biochem Mol Biol 36:435–499

Jaenicke R, Schurig H, Beaucamp N, Ostendorp R (1996) Structure and stability of hyperstable proteins: glycolytic enzymes from hyperthermophilic bacterium *Thermotoga maritima*. Adv Protein Chem 48:181–269

Jiang Y, Nock S, Nesper M, Sprinzl M, Sigler PB (1996) Structure and importance of the dimerization domain in elongation factor Ts from *Thermus thermophilus*. Biochemistry 35:10269–10278

Jung A, Bamann C, Kremer W, Kalbitzer HR, Brunner E (2004) High-temperature solution NMR structure of TmCsp. Protein Sci 13:342–350

Kagawa HK, Osipiuk J, Maltsev N, Overbeek R, Quaite-Randall E, Joachimiak A, Trent JD (1995) The 60 kDa heat shock proteins in the hyperthermophilic archaeon *Sulfolobus shibatae*. J Mol Biol 253:712–725

Kashefi K, Lovley DR (2003) Extending the upper temperature limit for life. Science 301:934

Kates M (1995) Adventures with membrane lipids. Biochem Soc Trans 23:697–709

Kates M, Moldoveanu N, Stewart LC (1993) On the revised structure of the major phospholipid of *Halobacterium salinarium*. Biochim Biophys Acta 1169:46–53

Kauzmann W (1959) Some factors in the interpretation of protein denaturation. Adv Protein Chem 14:1–63

Kawai G, Yamamoto Y, Kamimura T, Masegi T, Sekine M, Hata T, Iimori T, Watanabe T, Miyazawa T, Yokoyama S (1992) Conformational rigidity of specific pyrimidine residues in tRNA arises from posttranscriptional modifications that enhance steric interaction between the base and the 2'-hydroxyl group. Biochemistry 31:1040–1046

Kelley DS, Karson JA, Blackman DK, Früh-Green GL, Butterfield DA, Lilley MD, Olson EJ, Schrenk MO, Roe KK, Lebon GT, Rivizzigno P, the AT3–60 Party (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30°N. Nature (London) 412:145–149

Kern G (2005) In: Kiefhaber T, Buchner J (eds) Protein folding handbook, vol 2. Wiley-VCH, New York, NY, p 2560

Kern G, Schülke N, Schmid FX, Jaenicke R (1992) Quaternary structure and stability of internal, external and core-glycosylated invertase from yeast. Protein Sci 1:120–131

Kern G, Kern D, Jaenicke R, Seckler R (1993) Kinetics of folding and association of differently glycosylated variants of invertase from *Saccharomyces cerevisiae*. Protein Sci 2:1862–1868

Kiener A, Husain I, Sancar A, Walsh C (1989) Purification and properties of *Methanobacterium thermoautotrophicum* DNA photolyase. J Biol Chem 264:13880–13887

Kim KK, Kim R, Kim SH (1998) Crystal structure of a small heat-shock protein. Nature 394:595–599

Kim R, Lai L, Lee H-H, Cheong G-W, Kim KK, Wu Z, Yokota H, Marqusee S, Kim SH (2003) On the mechanism of chaperone activity of the sHsp from *Methanococcus jannaschii*. Proc Natl Acad Sci USA 100:8151–8155

Klunker D, Haas B, Hirtreiter A, Figueiredo L, Naylor DJ, Pfeofer G, Müller V, Deppenmeier U, Gottschalk G, Hartl FU, Hayer-Hartl M (2003) Coexistence of group I and group II chaperonins in the archaeon *Methanosarcina Mazei*. J Biol Chem 278:33256–33267

Knapp S, Ladenstein R, Galinski EA (1999) Extrinsic protein stabilization by the naturally occurring osmolytes β-hydroxyectoine and betaine. Extremophiles 3:191–198

Kneifel H, Stetter KO, Andreesen JR, Weigel J, König H, Schoberth SM (1986) Distribution of polyamines in representative species of archaebacteria. Syst Appl Microbiol 7:241–245

Koonin EV, Tatusov RL, Galperin ML (1998) Beyond complete genomes: from sequence to structure and function. Curr Opin Struct Biol 8:355–363

Korber P, Stahl JM, Nierhaus KH, Bardwell JC (2000) Hsp15: a ribosome-associated heat shock protein. EMBO J 19:741–748

Koulis A, Cowan DA, Pearl LH, Savva R (1996) Uracil-DNA glycosylase activities in hyperthermophilic microorganisms. FEMS Microbiol Lett 143:267–271

Kowalak JA, Dalluge JJ, McCloskey JA, Stetter KO (1994) The role of posttranscriptional modification in the stabilization of transfer RNA from hyperthermophiles. Biochemistry 33:1869–1876

Kumar A, Ma B, Tsai CJ, Nussinov R (2000a) Electrostatic strengths of salt bridges in thermophilic and mesophilic glutamate dehydrogenase monomers. Proteins 38:368–383

Kumar S, Tsai CJ, Nussinov R (2000b) Factors enhancing protein thermostability. Protein Eng 13:179–191
Kuntz ID (1971) Hydration of macromolecules IV: polypeptide conformation in frozen solution. J Am Chem Soc 93:516–518
Kuntz ID, Kauzmann W (1974) Hydration of proteins and polypeptides. Adv Protein Chem 28:239–345
Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157:105–132
Laksanalamai P, Robb FT (2004) Small heat shock proteins from extremophiles. Extremophiles 8:1–11
Lamosa P, Martins LO, da Costa MS, Santos H (1998) Effects of temperature, salinity, and medium composition on compatible solute accumulation by *Thermococcus* spp. Appl Environ Microbiol 64:3591–3598
Langworthy TA, Pond JL (1986) Membranes and lipids of thermophiles. In: Brock TD (ed) Thermophiles: general, molecular and applied microbiology. Wiley, New York, NY, pp 107–135
Lauffer MA (1975) Entropy-driven processes in biology. Springer, New York, NY
Laws RM, Franks F (eds) (1990) Life at low temperature. Phil Trans R Soc Lond B 326:517–692
Lazarides T, Lee I, Kaplan M (1997) Dynamics and unfolding pathways of a hyperthermophilic and a mesophilic rubredoxin. Protein Sci 6:2589–2605
Ledl F, Schleicher E (1990) Die Maillard Reaktion in Lebensmitteln und im menschlichen Körper. Angew Chem 102:597–626
Leibrock E, Bayer P, Lüdemann H-D (1995) Non-enzymatic hydrolysis of ATP at high temperatures and high pressures. Biophys Chem 54:175–180
Leroux MP, Fändrich M, Klunker D, Siegers K, Lupas AN, Brown JR, Schiebel E, Dobson CM, Hartl FU (1999) MtGimC, a novel archaeal chaperone related to the eukaryotic chaperonin cofactor GimC/prefoldin. EMBO J 18:6730–6743
Li WT, Shriver JW, Reeve JN (2000) Mutational analysis of differences in thermostability between histones from mesophilic and hyperthermophilic archaea. J Bacteriol 182:812–817
Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature (London) 362:709–715
Loladze VV, Ibarra-Molero B, Sanchez-Ruiz JM, Makhatadze GI (1999) Engineering a thermostable protein via optimization of charge-charge interactions on the protein surface. Biochemistry 38:16419–16423
López-Garcia P, Forterre P (1997) DNA topology in hyperthermophilic archaea: Reference states and their variation with growth phase, growth temperature and temperature stresses. Mol Microbiol 23:1267–1279
López-Garcia P, Forterre P (2000) DNA topology and the thermal stress response, a tale from mesophiles and hyperthermophiles. Bioessays 22:738–746
Lorimer G (1996) A quantitative assessment of the role of the chaperonin proteins in protein folding in vivo. FASEB J 10:5–9
Lumry R, Rajender S (1970) Enthalpy-entropy compensation phenomena in water solutions of proteins and small molecules: a ubiquitous property of water. Biopolymers 9:1125–1227
Macario AJL, Lange M, Ahring BK, Conway de Macario E (1999) Stress genes and proteins in the archaea. Microbiol Mol Biol Rev 63:923–967
Maes D, Zeelen JP, Thanki N, Beaucamp N, Alvarez M, Thi MH, Backmann J, Martial JA, Wyns L, Jaenicke R, Wierenga RK (1999) The crystal structure of triosephosphate isomerase (TIM) from *Thermotoga maritima*: a comparative thermostability structural analysis of ten different TIM structures. Proteins 37:441–453
Makhatadze GI, Privalov PL (1995) Energetics of protein structure. Adv Protein Chem 47:307–345
Malakauskas SM, Mayo SL (1998) Design, structure and stability of a hyperthermophilic protein variant. Nat Struct Biol 5:470–475
Mallick R, Boutz DR, Eisenberg D, Yeates TO (2002) Genomic evidence that the intracellular proteins of archaeal microbes contain disulfide bonds. Proc Natl Acad Sci USA 99:9679–9684
Marguet E, Forterre P (1994) DNA stability at temperatures typical for hyperthermophiles. Nucleic Acids Res 22:1681–1686
Marguet E, Forterre P (1998) Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles. Extremophiles 2:115–122
Marguet E, Forterre P (2001) Stability and manipulation of DNA at extreme temperatures. Meth Enzymol 334:205–215
Marshall CJ (1997) Cold-adapted enzymes. Trends Biotechnol 15:359–364
Martins LO, Santos H (1995) Accumulation of mannosylglycerate and di-myo-inositol-phosphate by *Pyrococcus furiosus* in response to salinity and temperature. Appl Environ Microbiol 61:3299–3303
Martins LO, Carreto LS, da Costa MS, Santos H (1996) New compatible solutes related to di-myo-inositol-phosphate in members of the order Thermotogales. J Bacteriol 178:5644–5651
Martins LO, Huber R, Huber H, Stetter KO, da Costa MS, Santos H (1997) Organic solutes in hyperthermophilic archaea. Appl Environ Microbiol 63:896–902
Mathai G, Sprott GD, Zeidel ML (2001) Molecular mechanism of water and solute transport across archaebacterial lipid membranes. J Biol Chem 276:27266–27271
Matthews BW (1993) Structural and genetic analysis of protein stability. Annu Rev Biochem 62:139–160
Matthews BW (1995) Studies on protein stability with T4 lysozyme. Adv Protein Chem 46:249–278
Matthews BW (1996) Structural and genetic analysis of the folding and function of T4 lysozyme. FASEB J 10:35–41
Matthews BW, Nicholson H, Becktel WJ (1987) Enhanced protein thermostability from site-directed mutations that decrease the entropy of unfolding. Proc Natl Acad Sci USA 84:6663–6667
McAfee JG, Edmondson SP, Zegar I, Shriver JW (1996) Equilibrium DNA binding of Sac7d protein from the hyperthermophile Sulfolobus acidocaldarius: fluorescence and circular dichroism studies. Biochemistry 35:4034–4045
McFall-Ngai M, Horwith J (1990) A comparative study of the thermal stability of the vertebrate eye lens: Antarctic fish to the desert iguana. Exp Eye Res 50:703–709
Meister A (1965) Biochemistry of the amino acids, 2nd edn. Academic, New York
Melchior DL (1982) Lipid phase transitions and regulation of membrane fluidity in prokaryotes. Curr Top Membr Transp 17:263–316
Merz A, Knöchel T, Jansonius JN, Kirschner K (1999) The hyperthermostable indole-glycerol phosphate synthase from *Thermotoga maritima* is destabilised by mutational: disruption of two solvent-exposed salt bridges. J Mol Biol 288:753–763
Meyer J, Clay MD, Johnson MK, Stubna A, Münck E, Higgins C, Wittung-Stafshede P (2002) A hyperthermophilic plant-type [2Fe-2 S] ferredoxin from Aquifex aeolicus is stabilized by a disulfide bond. Biochemistry 41:3096–3108
Miller SL, Lazcano A (1995) The origin of life—did it occur at high temperature? J Mol Evol 41:689–692
Minton AP (2000) Implications of macromolecular crowding for protein assembly. Cur Opin Struct Biol 10:34–39
Minton KW, Daly MJ (1995) A model for repair of radiation-induced DNA double-strand breaks in the extreme radiophile *Deinococcus radiodurans*. Bioessays 17:457–464
Minuth T, Frey G, Lindner P, Rachel R, Stetter KO, Jaenicke R (1998) Recombinant homo-and hetero-oligomers of an ultrastable chaperonin from the archaeon *Pyrodictium occultum* show chaperone activity in vitro. Eur J Biochem 258:837–845
Minuth T, Henn M, Rutkat K, Andrä S, Frey G, Rachel R, Stetter KO, Jaenicke R (1999) The recombinant thermosome from the archaeon *Methanopyrus kandleri*: in vitro analysis of its chaperone activity. Biol Chem 380:55–62
Mirsky AE, Pauling L (1936) On the structure of native, denatured and coagulated proteins. Proc Natl Acad Sci USA 22:439–447
Mogk A, Tomoyasu T, Goloubinoff P, Rüdiger S, Röder D, Langen H, Bukau B (1999) Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB. EMBO J 18:6934–6949
Motohashi K, Watanabe Y, Yohda M, Yoshida M (1999) Heat-inactivated proteins are rescued by the DnaK.J-GrpE set and ClpB chaperones. Proc Natl Acad Sci USA 96:7184–7189
Mueller U, Perl D, Schmid FX, Heinemann U (2000) Thermal stability and atomic-resolution crystal structure of the *Bacillus caldolyticus* cold shock protein. J Mol Biol 297:975–988
Murphy KP, Freire E (1992) Thermodynamics of structural stability and cooperative folding behavior in proteins. Adv Protein Chem 43:313–361

Myers JK, Pace CN, Scholtz JM (1995) Denaturant m values and heat capacity changes: relation to changes in accessible surface areas of protein unfolding. Protein Sci 4:2138–2148

Nelson KE, Clayton RA, Gill SR, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Nelson WC, Ketchum KA, McDonald L, Utterback TR, Malek JA, Linher KD, Garrett MM, Stewart AM, Cotton MD, Pratt MS, Phillips CA, Richardson D, Heidelberg J, Sutton GG, Fleischmann RD, Eisen JA, White O, Salzberg SL, Smith HO, Fraser CM (1999) Evidence for lateral gene transfer between archaea and bacteria from gene sequence of *Thermotoga maritima*. Nature (London) 399:323–329

Németh A, Svingor A, Pocsik M, Dobo J, Magyar C, Szilagyi A, Gal P, Závodszky P (2000) Mirror image mutations reveal the significance of an intersubunit ion cluster in the stability of 3-isopropylmalate dehydrogenase. FEBS Lett 468:48–52

Nichols PD, Franzmann PD (1992) Unsaturated diether phospholipids in the Antarctic methanogen *Methanococcoides burtonii*. FEMS Microbiol Lett 98:205–208

Nisbet E (2000) The realms of archaean life. Nature (London) 405:625–626

Noon KR, Bruenger E, McCloskey JA (1998) Posttranscriptional modifications in 16 S rRNA and 23 S rRNA of the archaeal hyperthermophile *Sulfolobus solfataricus*. J Bacteriol 180:2883–2888

Nordberg Karlsson E, Abou-Hachem M, Holst O, Danson MJ, Hough DW (2002) *Rhodothermus marinus*: a thermophilic bacterium producing dimeric and hexameric citrate synthase isoenzymes. Extremophiles 6:51–56

Nordberg Karlsson E, Crennell SJ, Higgins C, Nawaz S, Yeoh L, Hough DW, Danson MJ (2003) Citrate synthase from *Thermus aquaticus*: a thermostable bacterial enzyme with a five-membered inter-subunit ionic network. Extremophiles 7:9–16

Nozaki Y, Tanford C (1971) The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. Establishment of a hydrophobic scale. J Biol Chem 246:2211–2217

Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature (London) 405:298–304

Ögrünc M, Becker DF, Ragsdale SW, Sancar A (1998) Nucleotide excision repair in the third kingdom. J Bacteriol 180:5796–5798

Otting G, Liepinsh E, Wüthrich K (1991) Protein hydration in aqueous solution. Science 254:974–980

Pace CN (1986) Determination and analysis of urea and guanidine hydrochloride denaturation curves. Meth Enzymol 131:266–280

Pace CN (1992) Contribution of the hydrophobic effect to globular protein stability. J Mol Biol 226:29–35

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276:734–740

Pace CN (2000) Single surface stabilizer. Nat Struct Biol 7:345–346

Pace CN (2001) Polar group burial contributes more to protein stability than non-polar group burial. Biochemistry 40:310–313

Pace CN, Scholtz JM (1997) Measuring the conformational stability of a protein. In: Creighton TE (ed) Protein structure: a practical approach. IRL Press, Oxford, UK, pp 299–321

Pace CN, Shirley BA, McNutt M, Gajiwala K (1996) Forces contributing to the conformational stability of proteins. FASEB J 10:75–83

Pappenberger G, Schurig H, Jaenicke R (1997) Disruption of an ionic network leads to accelerated thermal denaturation of GAPDH from the hyperthermophilic bacterium *Thermotoga maritima*. J Mol Biol 274:676–683

Pauling L (1940) The nature of the chemical bond. Cornell University Press, Ithaca, NY

Pauling L, Hayward R (1964) The architecture of molecules. W. H. Freeman, San Francisco and London, US/UK, pp 1–116

Peak MJ, Robb FT, Peak JG (1995) Extreme resistance to thermally induced DNA backbone breaks in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 177:6316–6318

Perl D, Schmid FX (2001) Some like it hot: the molecular determinants of protein thermostability. Chem BioChem 3:39–44

Perl D, Welker C, Schindler T, Schröder K, Marahiel MA, Jaenicke R, Schmid FX (1998) Conservation of rapid two-state folding in mesophilic, thermophilic and hyperthermophilic cold-shock proteins. Nature Struct Biol 5:229–235

Perl D, Mueller U, Heinemann U, Schmid FX (2000) Two exposed amino acid residues confer thermostability on a cold shock protein. Nature Struct Biol 7:380–383

Perutz MF, Raidt H (1975) Stereochemical basis of heat stability in bacterial ferredoxins and in hemoglobin A2. Nature (London) 255:256–259

Peterson ME, Eisenthal R, Danson MJ, Spence A, Daniel RM (2004) A new, intrinsic, thermal parameter for enzymes reveals true temperature optima. J Biol Chem 279(20):20717–20722

Petsko GA (2001) Structural basis of thermostability in hyperthermophilic proteins, or "There's more than one way to skin a cat. Meth Enzymol 334:469–478

Petukhov M, Kil Y, Kuramitsu S, Lanzov V (1997) Insights into thermal resistance of proteins from the intrinsic stability of their α-helices. Proteins 29:309–320

Pfeil W (1998) Protein stability and folding: a collection of thermodynamic data. Springer, New York, NY

Phadtare S, Alsina J, Inouye M (1999) Cold-shock response and cold-shock proteins. Curr Opin Microbiol 2:175–180

Phipps BM, Hoffmann A, Stetter KO, Baumeister W (1991) A novel ATPase complex selectively accumulated upon heat shock is a major cellular component of thermophilic archaebacteria. EMBO J 10:1711–1722

Plaza del Pino IM, Ibarra-Molero B, Sanchez-Ruiz JM (2000) Lower kinetic limit to protein thermal stability: a proposal regarding protein stability in vivo and its relation with misfolding diseases. Prot Struct Funct Genet 40:58–70

Privalov PL (1979) Stability of proteins. Adv Protein Chem 33:167–241

Privalov PL (1988) Hydrophobic interactions in proteins. In: Winnacker E-L, Huber R (eds) Protein structure and protein design. Springer, New York, NY, pp 6–15

Privalov PL, Gill SJ (1988) Stability of protein structure and hydrophobic interaction. Adv Protein Chem 39:193–231

Ramakrishnan V, Verhagen MFJM, Adams MWW (1997) Characterization of di-myo-inositol-1,1′-phosphate in the hyperthermophilic bacterium *Thermotoga maritima*. Appl Environ Microbiol 63:347–350

Ramirez F, Marecek JF, Szamosi J (1980) Magnesium and calcium ion effects on hydrolysis rates of ATP. J Org Chem 45:4748–4752

Rasmussen B (2000) Filamentous microfossils in a 3,235-million-year-old volcanogenic sulphide deposit. Nature (London) 405:676–679

Raviv U, Laurat P, Klein J (2001) Fluidity of water confined to subnanometre films. Nature (London) 413:51–54

Relini A, Cassinadri D, Fan Q, Gulik A, Mirghani Z, de Rosa M, Gliozzi A (1996) Effect of physical constraints on the mechanism of membrane fusion: bolaform lipid vesicles as model systems. Biophys J 71:1789–1795

Richards FM (1977) Areas, volumes, packing and protein structure. Ann Rev Biophys Bioeng 6:151–176

Ring K, Henkel B, Valenteijn A, Gutermann R (1986) Studies on the permeability and stability of liposomes derived from a membrane spanning bipolar archaebacterial tetraether lipid. In: Schmidt KH (ed) Liposomes as drug carriers. Thieme Verlag, Stuttgart, Germany, pp 101–123

Rose GD, Geselowitz AR, Lesser G-J, Lee RH, Zehfuss MH (1985) Hydrophobicity of amino acid residues in globular proteins. Science 229:834–838

Rosing MT (1999) ^{13}C-depleted carbon microparticles in >3700-Ma sea-floor sedimentary rocks from West Greenland. Science 283:674–676

Saenger W (1984) Water and nucleic acids principles of nucleic acid structure. Springer, New York, NY, pp 368–384

Sanchez-Ruiz JM, Makhatadze GI (2001) To charge or not to charge? Trends Biotechnol 19:132–135

Schellman JA (1997) Temperature, stability, and the hydrophobic interaction. Biophys J 73:2960–2964

Schiene-Fischer C, Fischer G (2000) Enzymes that catalyse the restructuring of proteins. Curr Opin Struct Biol 10:40–45

Schiene-Fischer C, Yu C (2001) Receptor accessory folding helper enzymes: The functional role of peptidyl prolyl cis-trans isomerases. FEBS Lett 495:1–6

Scholz S, Sonnenbichler J, Schäfer W, Hensel R (1992) Di-myo-inositol-1,1'-phosphate: a new inositol phosphate isolated from *Pyrococcus woesei*. FEBS Lett 306:239–242

Schuler B, Kremer W, Kalbitzer HR, Jaenicke R (2002) Role of entropy in protein thermostability: folding kinetics of a hyperthermophilic Csp at high temperatures using 19 F NMR. Biochemistry 41:11670–11680

Schulz GE, Schirmer H (1979) Principles of protein structure. Springer, New York, NY, p 160

Shoichet BK, Baase WA, Kuroki R, Matthews BW (1995) A relationship between protein stability and protein function. Proc Natl Acad Sci USA 92:452–456

Shtilerman M, Lorimer GH, Englander SW (1999) Chaperonin function: Folding by forced unfolding. Science 284:822–825

Siegert R, Leroux MR, Scheuffler C, Hartl FU, Moarefi I (2000) Structure of the molecular chaperone prefoldin: Unique interaction of multiple coiled coil tentacles with unfolded proteins. Cell 103:621–623

Sigler PB, Xu ZH, Rye HS, Burston SG, Fenton WA, Horvich AL (1998) Structure and function in GroEL-mediated protein folding. Annu Rev Biochem 67:581–608

Skorvaga M, Raven ND, Margison GP (1998) Thermostable archaeal O6-alkylguanine-DNA alkyltransferases. Proc Natl Acad Sci USA 95:6711–6715

Soares D, Dahlke I, Li W-T, Sandman K, Hethke C, Thomm M, Reeve JN (1998) Archaeal histone stability, DNA binding, and transcription inhibition above 90 °C. Extremophiles 2:75–81

Somero GN (1992) Adaptations to high hydrostatic pressure. Ann Rev Physiol 54:557–577

Somero GN (1995) Proteins and temperature. Ann Rev Physiol 57:43–68

Somero GN (2000) Unity in diversity: a perspective on the methods, contributions and future of comparative physiology. Ann Rev Physiol 62:927–937

Spector S, Wang M, Carp SA, Robblee J, Hendsch ZS, Fairman R, Tidor B, Raleigh DP (2000) Rational modification of protein stability by the mutation of charged surface residues. Biochemistry 39:872–879

Speelmans G, Poolman B, Abee T, Konings WN (1993a) Energy transduction in the thermophilic anaerobic bacterium *Clostridium fervidus* is exclusively coupled to sodium ions. Proc Natl Acad Sci USA 90:7579–7979

Speelmans G, Poolman B, Konings WN (1993b) Amino acid transport in the thermophilic anaerobe *Clostridium fervidus* is driven by an electrochemical sodium gradient. J Bacteriol 175:2060–2066

Sprott GD, Meloche M, Richards JC (1991) Proportions of diether and tetraether lipids in *Methanococcus jannaschii* grown at different temperatures. J Bacteriol 173:3907–3910

Spyracopoulos L, Sykes BD (2001) Thermodynamic insights into proteins from NMR spin relaxation studies. Curr Opin Struct Biol 11:555–559

Stadtman ER (1990) Covalent modification reactions are marketing steps in protein turnover. Biochemistry 29:6323–6331

Stadtman ER, Oliver CN (1991) Metal-catalyzed oxidation of proteins. J Biol Chem 266:2005–2008

Sterner R, Liebl W (2001) Thermophilic adaptation of proteins. Crit Rev Biochem Mol Biol 36:39–106

Sterner R, Kleemann GR, Szadkowski H, Lustig A, Hennig M, Kirschner K (1996) Phosphoribosyl anthranilate isomerase from *Thermotoga maritima* is an extremely stable and active homodimer. Protein Sci 5:2000–2008

Stetter KO (1996) Hyperthermophilic prokaryotes. FEMS Microbiol Rev 18:149–158

Stetter KO (1998) Hyperthermophiles: isolation, classification and properties. In: Horikoshi K (ed) Grant extremophiles: microbial life in extreme environments. Wiley-Liss, New York, NY, pp 1–24

Stetter KO (1999) Extremophiles and their adaptation to hot environments. FEBS Lett 452:22–25

Stillinger FH (1977) Theoretical approaches to the intermolecular nature of water. Phil Trans R Soc London Ser B Biol Sci 278:97–112

Strop P, Mayo SL (2000) Contribution of surface salt bridges to protein stability. Biochemistry 39:1251–1255

Sturtevant JM (1977) Heat capacity and entropy changes in processes involving proteins. Proc Natl Acad Sci USA 74:2236–2240

Suggett A (1975) Polysaccharides. In: Franks F (ed) Water: a comprehensive treatise, vol 4. Plenum Press, New York, NY, pp 519–567

Suutari M, Laakso S (1992) Unsaturated and branched-chain fatty acids in temperature adaptation of *B. subtilis* and *B. megaterium*. Biochim Biophys Acta 1126:119–124

Suzuki Y, Hatagaki K, Oda H (1991) A hyperthermostable pullulanase produced by an extreme thermophile, B. flavocaldarius KP1228 and evidence for the proline theory of increasing protein thermostability. Appl Microbiol Biotechnol 34:707–714

Szilagyi A, Závodszky P (2000) Structural differences between mesophilic, moderately thermophilic and extremely thermophilic protein subunits: Results of a comprehensive survey. Struct Fold Des 8:493–504

Tanford C (1961) Physical chemistry of macromolecules. Wiley, New York, NY

Tanford C (1962) Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. J Am Chem Soc 84:4240–4247

Tanford C (1968) Protein denaturation, Part A and B. Adv Protein Chem 23:121–282

Tanford C (1970) Protein denaturation. Part C Adv Protein Chem 24:1–95

Tanford C (1980) The hydrophobic effect, 2nd edn. Wiley, New York, NY

Tardieu A, Le Verge A, Malfois M, Bonneté F, Finet S, Riès-Kautt M, Belloni L (1999) Proteins in solution: from X-ray scattering intensities to interaction potentials. J Crystal Growth 196:193–203

Thieringer HA, Jones PG, Inouye M (1998) Cold shock and adaptation. Bioessays 20:49–57

Thoma R, Hennig M, Sterner R, Kirschner K (2000) Structure and function of mutationally generated monomers of dimeric phosphoribosylanthranilate isomerase from *Thermotoga maritima*. Struct Fold Des 8:265–276

Thomm M, Madon J, Stetter KO (1986) DNA-dependent RNA polymerases of the three orders of methanogens. Biol Chem Hoppe Seyler 367:473–481

Thompson MJ, Eisenberg D (1999) Transproteomic evidence of a loop-deletion mechanism for enhancing protein thermostability. J Mol Biol 290:595–604, errata 292 and 946

Timasheff SN (1995) Solvent stabilization of protein structure. In: Shirley BA (ed) Protein stability and folding, vol 40, Methods in molecular biology. Humana Press, Totowa, NJ, pp 253–269

Timasheff SN, Arakawa T (1997) Stabilization of protein structure by solvents. In: Creighton TE (ed) Protein structure: a practical approach, 2nd edn. IRL Press, Oxford, UK, pp 331–345

Tolner B, Poolman B, Konings WN (1998) Adaptation of microorganisms and their transport systems to high temperature. Comp Biochem Physiol 118A:423–428

Tomschy A, Böhm G, Jaenicke R (1994) Effect of central and peripheral ion pairs on the thermal stability of GAPDH from the hyperthermophilic bacterium *Thermotoga maritima*. Protein Eng 7:1471–1478

Toth EA, Worby C, Dixon JE, Goedken ER, Marqusee S, Yeates TO (2000) The crystal structure of adenylosuccinate lyase from *Pyrobaculum aerophilum* reveals a intracellular protein with three disulfide bonds. J Mol Biol 301:433–450

Trent JD (1996) A review of acquired thermotolerance, heat-shock proteins and molecular chaperones in archaea. FEMS Microbiol Rev 18:249–258

Trent JD, Nimmesgern E, Wall JS, Hartl U, Horvich AL (1991) A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein Tcp1. Nature 354:490–493

Trent JD, Kagawa HK, Yaoi T, Olle E, Zaluze NJ (1997) Chaperonin filaments: the archaeal cytoskeleton. Proc Natl Acad Sci USA 94:5383–5388

Upasani VN, Desai SG, Moldoveanu N, Kates M (1994) Lipids of extremely halophilic archaebacteria from saline environments in India: a novel glycolipid in Natronobacterium strains. Microbiology 40:1959–1966

van de Vossenberg JL, Ubbink-Kok T, Elferink MG, Konings WN (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Mol Microbiol 18:925–932

van de Vossenberg JL, Driessen AJ, Konings WN (1998) The essence of being extremophilic: the role of the unique archaeal membrane lipids. Extremophiles 2:163–170

van de Vossenberg JL, Driessen AJ, da Costa MS, Konings WN (1999) Homeostasis of the membrane proton permeability in Bac. subtilis grown at different temperatures. Biochim Biophys Acta 1419:97–104

van den Burg B, Vriend G, Veltman OR, Venema G, Eijsink VGH (1998) Engineering an enzyme to resist boiling. Proc Natl Acad Sci USA 95:2056–2060

van Holde KE (1985) Physical biochemistry, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ

van Montfort RL, Slingsby C, Vierling E (2002) Structure and function of the small heat shock protein/a-crystallin family of molecular chaperones. Adv Protein Chem 59:105–156

Vetriani C, Maeder DL, Tolliday N, Yip KS, Stillman TJ, Britton KL, Rice DW, Klump HH, Robb FT (1998) Protein thermostability above 100 °C: a key role for ionic interactions. Proc Natl Acad Sci USA 95:12300–12305

Vieille C, Zeikus GJ (2001) Hyperthermophilic enzymes: Sources, uses, and molecular mechanisms for thermostability. Microbiol Mol Biol Rev 65:1–43

Volkin DB, Mach H, Middaugh CR (1995) Degradative covalent reactions important to protein stability. In: Shirley BA (ed) Protein stability and folding, Methods in molecular biology. Humana Press, Totowa, NJ, pp 35–63

von Hippel PH, Schleich T (1969) The effects of neutral salts on the structure and conformational stability of macromolecules in solution. In: Timasheff SN, Fasman GD (eds) Structure and stability of biological macromolecules, vol 2, Biological macromolecules. M. Dekker, New York, NY, pp 417–574

Walter S, Buchner J (2002) Molecular chaperones—Cellular machines for protein folding. Angew Chem Int Ed 41:1098–1113

Watanabe K, Shima M, Oshima T, Nishimura S (1976) Heat-induced stability of tRNA from an extreme thermophile, *Thermus thermophilus*. Biochem Biophys Res Commun 72:1137–1144

Watanabe K, Chishiro K, Kitamura K, Suzuki Y (1991) Proline residues responsible for thermostability occur with high frequency in the loop regions of an extremely thermostable oligo-1,6-glucosidase from Bac. thermoglucosidasius KP1006. J Biol Chem 266:24287–24294

Watanabe K, Masuda T, Ohashi H, Mihara H, Suzuki Y (1994) Multiple proline substitutions cumulatively thermostabilize B. cereus ATCC7064 oligo-1,6-glucosidase irrefragable proof supporting the proline rule. Eur J Biochem 226:277–283

Watanabe K, Kitamura K, Suzuki Y (1996) Analysis of the critical sites for protein thermostabilization by proline substitution in oligo-1,6-glucosidase from Bac. coagulans ATCC7050 and the evolutionary consideration of proline residues. Arch Environ Microbiol 62:2066–2073

Watanabe K, Hata Y, Kizaki H, Katsube Y, Suzuki Y (1997) The refined crystal structure of Bac. stereus oligo-1,6-glucosidase at 2.0 Å resolution: structural characterization of proline-substitution sites for protein thermostabilization. J Mol Biol 269:142–153

Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, Beeson KY, Bibbs L, Bolanos R, Keller M, Kretz K, Lin X, Mathur E, Ni J, Podar M, Richardson T, Sutton GG, Simon M, Söll D, Stetter KO, Short JM, Noordewier M (2003) The genome of Nanoarchaeum equitans: insights into early archaeal evolution and derived parasitism. Proc Natl Acad Sci USA 100:12984–12988

Wearne SJ, Creighton TE (1989) Effect of protein conformation on rate of deamidation: Ribonuclease A. Proteins 5:8–12

Weber-Ban EU, Reid BG, Miranker AD, Horwich AL (1999) Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. Nature (London) 401:90–93

Westhof E, Beveridge DL (1990) Hydration of nucleic acids. In: Franks F (ed) Water science reviews, vol. 5: the modes of life, 5. Cambridge University Press, New York, NY, pp 24–136

Wetlaufer DB (1980) Practical consequences of protein folding mechanisms. In: Jaenicke R (ed) Protein folding. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands, pp 323–329

White RH (1984) Hydrolytic stability of biomolecules at high temperatures and its implication for life at 250 °C. Nature (London) 310:430–432

Wierenga RK (2001) The TIM-barrel fold: a versatile framework for efficient enzymes. FEBS Lett 492:193–198

Wintrode PL, Arnold FH (2000) Temperature adaptation of enzymes: lessons from directed evolution. Adv Prot Chem 55:161–225

Woese CR (1998) The universal ancestor. Proc Natl Acad Sci USA 95:6854–6859

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains of archaea, bacteria and eucarya. Proc Natl Acad Sci USA 87:4576–4579

Wrba A, Schwaiger A, Schultes V, Jaenicke R, Závodszky P (1990) GAPDH from the extreme thermophilic eubacterium *Thermotoga maritima*. Biochemistry 29:7585–7592

Wright HT (1991) Nonenzymatic deamidation of asparaginyl and glutaminyl residues in proteins. Crit Rev Biochem Mol Biol 26:1–52

Wright PE, Baldwin RL (2000) The folding process of apomyoglobin. In: Pain RH (ed) Mechanisms of protein folding, vol 32, 2nd edn, Frontiers in molecular biology. Oxford University Press, Oxford, UK, pp 309–329

Xiao L, Honig B (1999) Electrostatic contributions to the stability of hyperthermophilic proteins. J Mol Biol 289:1435–1444

Yamauchi K, Kinoshita M (1995) Highly stable lipid membranes from archaeabacterial extremophiles. Prog Polymer Sci 18:763–804

Yano JK, Poulos TL (2003) New understandings of thermostable and peizostable enzymes. Curr Opin Biotechnol 14:360–365

Yip KS, Stillman TJ, Britton KL, Atrymiuk PJ, Baker PJ, Sedelnikova SE, Engel PC, Pasquo A, Chiaraluce R, Consalvi V, Scandurra R, Rice DW (1995) The structure of Pyrococcus furiosus glutamate dehydrogenase reveals a key role for ion-pair networks in maintaining enzyme stability at extreme temperatures. Structure 3:1147–1158

Yip KS, Britton KL, Stillman TJ, Lebbink J, de Vos WM, Robb FT, Vetriani C, Maeder D, Rice DW (1998) Insights into the molecular basis of thermal stability from the analysis of ion-pair networks in the glutamate dehydrogenase family. Eur J Biochem 253:336–346

Yokoyama S, Inagaki F, Miyazawa T (1981) Advanced nuclear magnetic resonance lanthanide probe analyses of short-range conformational interrelations controlling ribonucleic acid structures. Biochemistry 20:2981–2988

Zachariassen KE, Kristiansen E (2000) Ice nucleation and antinucleation in nature. Cryobiology 41:257–279

Zale SE, Klibanov AM (1986) What does ribonuclease irreversibly inactivate at high temperatures? Biochemistry 25:5432–5444

Závodszky P, Kardos J, Svingor R, Petsko GA (1998) Adjustment of conformational flexibility is a key event in the thermal adaptation of proteins. Proc Natl Acad Sci USA 95:7406–7411

Zhang XJ, Matthews BW (1994) Conservation of solvent-binding sites in 10 crystal forms of lysozyme. Protein Sci 3:1031–1039

Zimmerman SB, Minton AP (1993) Macromolecular crowding: biochemical, biophysical and physiological consequences Ann. Rev Biophys Biomol Struct 22:27–65

18 Life at Low Temperatures

Thilo M. Fuchs · Klaus Neuhaus · Siegfried Scherer
Lehrstuhl für Mikrobielle Ökologie, Department für Biowissenschaftliche Grundlagen, Wissenschaftszentrum Weihenstephan, Technische Universität München, Freising, Germany
Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL), Abteilung Mikrobiologie, Technische Universität München, Freising, Germany

Abbreviations *BCAA,* Branched-chain amino acids; *CSP,* Cold shock protein; *EPS,* Exopolysaccharides; *IF,* Initiation factor; *[p]ppGpp,* 5′-diphosphate-3′-diphosphate; *LAB,* Lactic acid bacteria; *MCSP,* Major cold shock protein; *PNPase,* Polynucleotide phosphorylase; *PTS PEP-dependent,* Phosphotransferase system; *TKS,* Two-component system; *UFA,* Unsaturated fatty acid

Introduction

Most habitats on our planet are permanently cold. By volume, 90% of the world's oceans have a temperature of 5°C or less, supporting cold-adapted microorganisms. When terrestrial habitats are included, over 80% of the earth's biosphere is permanently cold (Russell 1990). Both Bacteria and Eukarya comprise cold-adapted organisms (Margesin et al. 2007), and also archaebacteria contribute significantly to biomass in cold environments (Cavicchioli 2011).

Microorganisms that are adapted to thrive at low or even subzero temperatures have been termed "psychrophilic" (or "obligate psychrophiles"), "psychrotolerant" (or "facultative psychrophiles"/"psychrotrophs") or even "psychroactive" (Russell 1990; Nozhevnikova et al. 2001b). The terms "stenothermal" and "eurythermal" have also been suggested to characterize organisms that grow within narrow or broad temperature

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_56,

ranges, respectively. "Piezo-psychrophiles" are exposed to high pressures in the ocean depth and "halo-psychrophiles" to high salt concentration. Morita (1975) has defined psychrophiles "as organisms having an optimal temperature for growth at about 15°C or lower, a maximal temperature for growth at about 20°C, and a minimal temperature for growth at about 0°C or lower." The so-called optimal temperature at which generation times are shortest is not identical with the temperature of highest growth yield, which is often higher at lower temperature (Margesin 2009). Microorganisms are adapted to low temperature to very different degrees. For instance, strains of typical psychrophiles within the same species may have quite different doubling times at the same low temperature. As an approximate guideline, mesophilic bacteria exhibit optimal growth at a temperature above 25°C, and psychrophilic bacteria below 15°C. In this review, the term "psychrotolerant" denotes pathogens and other bacteria with a minimal growth temperature below 7°C and an optimal growth temperature above 20°C.

Forster (1887) was one of the first to report on the growth of a bacterium at low temperature. He described the physiology of luminescent bacteria from salt water as follows: "... they grow almost as well in the ice box as at the usual room temperature." Cold-adapted species are found throughout the entire taxonomic range of bacteria and archaea as well as in many climates. Within the last decade, the number of original papers and reviews dealing with cold adaptation has strongly increased. This is true both for the analysis of cold adaptation mechanisms as well as for the description of new psychrophilic and psychrotolerant species, especially from permanently cold habitats. *Table 18.1* shows an arbitrary list of psychrotolerant and psychrophilic species described in the literature between 2004 and 2011. The emerging interest in this field of research is fueled not only by the expectation to exploit cold tolerant organisms for biotechnological uses (Margesin and Schinner 1999) but also by the possible application of such microorganisms in bioremediation of sea or alpine areas (Brakstad and Lodeng 2005; Margesin and Schinner 2001) and by the putative function of icy environments in life origin scenarios (Price 2009). The possibility of extraterrestrial life as well as long-term carbon storage via microbial activity in the ocean are other examples for emerging topics in which cold-adapted bacteria have a central role (Rivkina et al. 2004; Cavicchioli 2002).

Growth of bacteria at low temperature requires a broad spectrum of adaptive mechanisms. These include regulation of membrane fluidity, maintenance of protein biosynthesis and metabolism, structural adaptation of enzymes, and the expression of cold shock proteins and cryoprotectants (see *Table 18.2* for review articles). The application of genomic, transcriptomic, proteomic, and metagenomic approaches on psychrophilic and psychrotolerant bacteria has shed further light on these aspects (*Table 18.3*). An increased flexibility in cellular structures including membranes, proteins, or RNAs appeared as a general concept of bacteria for coping with low temperatures. This chapter will introduce environmental aspects and then highlight mechanisms of bacterial cold-adaptation including cold sensing, membrane composition, enzymatic activity, metabolism, and the cold shock response. Finally, we will address biotechnological applications, the influence of low temperature on pathogens, and novel aspects of their ecology.

Biodiversity and Environments

Martinus Beijerinck (1851–1931) stated, "Everything is everywhere; the environment selects," which also refers to microbial thermotypes (O'Malley 2008). Indeed, in nearly any cold environment, be it a cold sulfurous spring (Rudolph et al. 2001), the atmosphere (Pearce et al. 2009), alpine meadows (von Stetten et al. 1999), Siberian permafrost (Hinsa-Leasure et al. 2010), the West Antarctic Ice Sheet (Lanoil et al. 2009), or Greenland glacier ice (Miteva and Brenchley 2005), microorganisms adapted to low temperature were isolated. All three domains are represented in cold ecosystems with a high biodiversity spanning mainly bacteria, archaea, fungi, and algae. Gram-negative α-, β-, and γ-proteobacteria such as *Pseudomonas* spp. and *Vibrio* spp. and the phylum Cytophaga-Flavobacterium-Bacteroides, together with the gram-positive corynebacteria, *Arthrobacter* sp. and *Micrococcus* sp., dominate in number and diversity over archaea in polar environments, while archaebacteria such as *Methanogenium* and *Methanococcus* are widespread in deep ocean water (Miteva et al. 2004; D'Amico et al. 2006; Cavicchioli 2006).

Current research activities on cold-adapted microorganisms are driven by a limited understanding of geo-microbiological processes and therefore aim to answer three fundamental questions: Which microorganisms are found in which low-temperature environment? Which adaptational mechanisms do they use? And how do low temperature and climate change influence microbial communities, and vice versa (Bidle et al. 2002; Kirchman et al. 2009)? Investigating the ecology of bacteria and archaea is also vital to the understanding of global biochemical cycles. Sulfate-reducing bacteria and methanogenic archaebacteria are important terminal oxidizers in the anaerobic mineralization of organic matter and can be seen as ecological equivalents, mineralizing organic matter to CO_2 or to CO_2 and CH_4 in high- or low-sulfate environments (Purdy et al. 2003). In low-temperature sediments in the Antarctic, *Desulfotalea-Desulforhopalus fulforhopalus* and *Methanosaeta* appear to be the most abundant species of sulfate-reducing bacteria and methanogenic archaea, respectively (Purdy et al. 2003). Methanogenesis is important as a possible climate influence as methane contributes greatly to the greenhouse effect in the atmosphere. Consequently, there is a research focus on methanogenesis that occurs in low-temperature environments such as the sea, the permafrost regions, and deep lakes (Rivkina et al. 2007; Simankova et al. 2003).

Those ecophysiological processes may differ under extreme conditions such as freezing. In low-sulfate sediments, H_2-driven methanogenesis was found to be mediated by sulfate reduction. After freezing, both methanogenesis and sulfate reduction decreased. In high-sulfate sediments, sulfate reduction was

Table 18.1
New cold-adapted genera and species described between 2004 and 2010

Species	TT	Isolated from	References
Arthrobacter alpinus	pp	Alpine soil	Zhang et al. (2010f)
Chromohalobacter sarecensis	pt	Saline Andean region	Quillaguaman et al. (2004)
Clostridium schirmacherense	pp	Lake sediment of Antarctic	Alam et al. (2006)
Cryobacterium roopkundense	pp	Glacial soil	Reddy et al. (2010)
Clostridium tagluense	pt	Permafrost	Suetin et al. (2009)
Desulfuromonas svalbardensis	pp	Arctic sediments	Vandieken et al. (2006)
Dyadobacter psychrophilus	pp	Hydrocarbon-contaminated soil	Zhang et al. (2010b)
Exiguobacterium indicum	pp	Himalayan glacier	Chaturvedi and Shivaji (2006)
Exiguobacterium soli	pp	Antarctic McMurdo dry valleys	Chaturvedi et al. (2008)
Flavobacterium glaciei	pp	Chinese glacier	Zhang et al. (2006a)
Flavobacterium weaverense	pp	King George Island, Antarctic	Yi and Chun (2006)
Glaciecola psychrophila	pp	Arctic locations	Zhang et al. (2006b)
Glaciibacter superstes	pp	Permafrost ice wedge	Katayama et al. (2009)
Glaciimonas immobilis	pp	Alpine glacier cryoconite	Zhang et al. (2010e)
Hymenobacter psychrophilus	pp	Hydrocarbon-contaminated soil	Zhang et al. (2011)
Luteimonas terricola	pp	Hydrocarbon-contaminated soil	Zhang et al. (2010c)
Marinobacter psychrophilus	pp	Canadian basin, Arctic	Zhang et al. (2008a)
Maribacter antarcticus	pp	Antarctic green alga	Zhang et al. (2009)
Methanogenium boonei	pt	Marine sediments, Alaska	Kendall et al. (2007)
Moritella dasanensis	pp	Arctic glacier	Kim et al. (2008)
Mrakiella cryoconiti	pp	Alpine and Arctic habitats	Margesin and Fell (2008)
Oceaniserpentilla haliotis	pp	Hemolymph serum of blacklip abalone	Schlösser et al. (2008)
Phaeobacter arcticus	pp	Canadian basin, Arctic	Zhang et al. (2008b)
Psychromonas boydii	pp	Arctic sea-ice core, Alaska	Auman et al. (2010)
Psychromonas ingrahamii	pp	Arctic sea-ice core, Alaska	Auman et al. (2006)
Psychromonas spp.	pp	Sperm whale carcasses	Miyazaki et al. (2008)
Rhodonellum psychrophilum	pp	Greenland	Schmidt et al. (2006)
Salinibacterium xinjiangense	pp	Chinese glacier	Zhang et al. (2008c)
Shewanella canadensis	pp	Atlantic ocean, Canada	Zhao et al. (2007)
Shewanella donghaensis	ps, pp	Deep-sea sediment, Sea of Japan	Yang et al. (2007)
Shewanella spongiae	pp	Marine sponge, Sea of Japan	Yang et al. (2006)
Sphingomonas glacialis	pp	Alpine glacier cryoconite	Zhang et al. (2010a)
Sphingopyxis bauzanensis	pp	Hydrocarbon-contaminated soil	Zhang et al. (2010d)
Sporosarcina antarctica	pp	King George Island, Antarctic	Yu et al. (2008)
Tomitella biformata	pp	Permafrost, Alaska	Katayama et al. (2010)

TT thermal type, *pt* psychrotolerant, *pp* psychrophilic, *ps* piezosensitive

revealed to be a major process in both frozen and unfrozen samples (Mountfort et al. 2003). Facing a limited supply of organic nutrients in the absence of photosynthesis, a microbial assemblage beneath the Taylor glacier was found to cycle sulfur with Fe(III) as terminal electron acceptor (Mikucki et al. 2009). In deep lake sediments, a community of psychrophilic methanogens was analyzed, with maximal rates of methane production occurring at 6°C (Nozhevnikova et al. 2003). However, permafrost sediments and other cold environments could also be a sink for methane, since methanotrophic (methane-oxidating) bacteria have been found in Siberian permafrost sediments (Khmelenina et al. 2002), in anoxic cold seep sediments (Orphan et al. 2002), and elsewhere (for a review, see Trotsenko and Khmelenina (2005)).

Removal of soil contamination is another important issue, especially at low temperature. Certain microorganisms are able to degrade phenol and hydrocarbons including petroleum, the most widespread contaminants in the environment, and other organic wastes in cold environments (Eriksson et al. 2003; Soares et al. 2003; Margesin 2007; Delille and Coulon 2008). Bacteria

Table 18.2
Selected articles 2002–2011 reviewing bacterial life at low temperatures

Scope	Title	References
Biodiversity and environments	Biology of extremophilic and extremotolerant methanotrophs	Trotsenko and Khmelenina (2002)
	Extremophiles and the search for extraterrestrial life	Cavicchioli (2002)
	Biological invasions in the Antarctic: extent, impacts and implications	Frenot et al. (2005)
	Cold-adapted archaea	Cavicchioli (2006)
	Microbial growth in the polar oceans-role of temperature and potential impact of climate change	Kirchman et al. (2009)
	Microbial genesis, life and death in glacial ice	Price (2009)
	Diversity and ecology of psychrophilic microorganisms	Margesin and Miteva (2011)
Cold sensors and regulators	Mechanisms of bacterial adaptation to low temperature	Chattopadhyay (2006)
	Thermosensors in eubacteria: role and evolution	Schumann (2007)
	Microbial thermosensors	Klinkert and Narberhaus (2009)
	How do bacteria sense and respond to low temperature?	Shivaji and Prakash (2010)
	Diversity in transcripts and translational pattern of stress proteins in marine extremophiles	Ambily Nath and Loka Bharathi (2011)
Adaptations of the membrane	Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress	Sakamoto and Murata (2002)
	Psychrophilic microorganisms: challenges for life	D'Amico et al. (2006)
	Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures	Chan and Wiedmann (2009)
	How do bacteria sense and respond to low temperature?	Shivaji and Prakash (2010)
Adaptation of proteins and ribosomes to low temperature	Psychrophilic enzymes: revisiting the thermodynamic parameters of activation may explain local flexibility	Lonhienne et al. (2000)
	Molecular basis of cold adaptation	D'Amico et al. (2002)
	Psychrophilic enzymes: hot topics in cold adaptation	Feller and Gerday (2003)
	Some like it cold: biocatalysis at low temperatures	Georlette et al. (2004)
	Cold-adapted enzymes	Siddiqui and Cavicchioli (2006)
	Cold-adapted enzymes from marine Antarctic microorganisms	Marx et al. (2007)
	Life at low temperatures: is disorder the driving force?	Feller (2007)
Metabolism and growth	The phosphotransferase system of *Lactobacillus casei*: regulation of carbon metabolism and connection to cold shock response	Monedero et al. (2007)
	Microbial genesis, life and death in glacial ice	Price (2009)
	Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism	Kennedy et al. (2010)
The cold shock response	Control of transcription termination in bacteria by RNA-binding proteins that modulate RNA structures	Stülke (2002)
	Bacterial cold shock responses	Weber and Marahiel (2003)
	Transcriptional and post-transcriptional control of cold-shock genes	Gualerzi et al. (2003)
	Cold shock response and adaptation at near-freezing temperature in microorganisms	Inouye and Phadtare (2004)
	Recent developments in bacteria cold-shock response	Phadtare (2004)
	Molecular components of physiological stress responses in *Escherichia coli*	Wick and Egli (2004)

Table 18.2 (continued)

Scope	Title	References
	Cold shock proteins aid coupling of transcription and translation in bacteria	El-Sharoud and Graumann (2007)
	Structure and function of bacterial cold shock proteins	Horn et al. (2007)
	RNA remodeling and gene regulation by cold shock proteins	Phadtare and Severinov (2010)
Long-term adaptation of psychrotolerant bacteria	Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures	Chan and Wiedmann (2009)
Biotechnological applications	Low-temperature extremophiles and their applications	Cavicchioli et al. (2002)
	Cold-inducible promoters for heterologous protein expression	Baneyx and Mujacic (2003)
	Bioprospecting for microbial products that affect ice crystal formation and growth	Christner (2010)
Food	Psychrotrophs in dairy products: their effects and their control	Champagne et al. (1994)
	Bacterial membranes: the effects of chill storage and food processing	Russell (2002)
	Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria	Galvez et al. (2008)
	Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk	De Jonghe et al. (2010)
	Persistence of *Listeria monocytogenes* in food industry equipment and premises	Carpentier and Cerf (2011)
Ecology of human pathogens in cold habitats	Transcriptional regulation in *Yersinia*: an update	Marceau (2005)
	Environmental predators as models for bacterial pathogenesis	Hilbi et al. (2007)
	Bacterial strategies to overcome insect defences	Vallet-Gely et al. (2008)

with this capability are already present in pristine soils but increase in number as a result of contamination (Margesin et al. 2003). Similarly, psychrotolerant bacteria from the genera *Shewanella* and *Arthrobacter* have been isolated from oil-reservoir water and are therefore considered to be of potential use for bioremediation (Kato et al. 2001).

The poles of the earth, comprising environments such as sea ice, deep lakes, and glaciers, are a research target as they represent unique bacterial habitats (Thomas and Dieckmann 2002). Scientists seek to investigate Lake Vostok in 2011, despite irreversibly spoiling by drilling (*Figs. 18.1*, *18.2*). The discovery of numerous lakes beneath the Antarctic ice shield and the streams of subglacial water between them, however, brings into question the reason for touching this putative ecosystem even more (Frenot et al. 2005). Indeed, other Antarctic habitats have been examined, and microbes have only recently been isolated from the sediment environment beneath the ice shield (Lanoil et al. 2009). In the permanently frozen Lake Fryxell, a diverse range of phototrophic purple bacteria and sulfate-reducing prokaryotes was found (Karr et al. 2003; Sattley and Madigan 2010). Halophilic bacteria and proteorhodopsin-bearing bacteria are active in the ice-sealed Lake Vida and Lake Ross, respectively (Mondino et al. 2009; Koh et al. 2010) (*Fig. 18.3*). These findings were surprising because organisms in Antarctic habitats commonly not only face continuous low temperatures but also poor light conditions. However, bacteria that are associated with particles or surfaces are respiring even in Arctic wintertime sea-ice cores (Junge et al. 2004). Here, they are encased in caverns filled with liquid, hyperoxide brines characterized by high concentrations of dissolved organic, nutrient limitations, carbon dioxide depletion, and high ammonia concentrations (Thomas and Dieckmann 2002).

Cold Sensors and Regulators

Temperature is one of the most important parameters monitored by free-living microbes, as their physiology is required to readjust when exposed to sudden temperature changes (Klinkert and Narberhaus 2009). The physicochemical mechanisms that underlie temperature sensing are conformational changes of either DNA, RNA, proteins, or membranes (for reviews, see Shivaji and Prakash 2010; Schumann 2007; Eriksson et al. 2002; Phadtare and Severinov 2010). These mechanisms might act simultaneously to sense low temperature and overlap as in the case of H-NS.

Sensing Through Alteration in DNA Conformation

In bacteria, the expression of genes depends on DNA conformation, and the degree of DNA superhelicity is known to vary in response to temperature changes (Eriksson et al. 2002). In the

Table 18.3
Selected "omic" studies on psychrotolerant and psychrophilic bacteria

Genome sequences	Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment	Moran et al. (2004)
	Molecular adaptations to psychrophily: the impact of "omic" technologies	Casanueva et al. (2010)
Transcriptome analysis	Genome-wide transcriptional profiling of the *Bacillus subtilis* cold-shock response	Kaan et al. (2002)
	Genome-wide transcriptional analysis of the cold shock response in *Bacillus subtilis*	Beckering et al. (2002)
	Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature	Liu et al. (2002)
	Changes in *Escherichia coli* transcriptome during acclimatization at low temperature	Polissi et al. (2003)
	Genome-wide transcriptional analysis of the cold shock response in wild-type and cold-sensitive, quadruple-*csp*-deletion strains of *Escherichia coli*	Phadtare (2004)
	Functional genomics of stress response in *Pseudomonas putida* KT2440	Reva et al. (2006)
	Global transcriptome analysis of the cold shock response of *Shewanella oneidensis* MR-1 and mutational analysis of its classical cold shock proteins	Gao et al. (2006)
	Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log- and stationary-phase cells	Chan et al. (2007)
	Psychrobacter arcticus 273–4 uses resource efficiency and molecular motion adaptations for subzero temperature growth	Bergholz et al. (2009)
Proteome analysis	A proteomic determination of cold adaptation in the Antarctic archaeon, *Methanococcoides burtonii*	Goodchild et al. (2004)
	Proteomic analysis of *Psychrobacter cryohalolentis* K5 during growth at subzero temperatures	Bakermans et al. (2007)
	Proteomic studies of an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, for global identification of cold-inducible proteins	Kawamoto et al. (2007)
	Cold adaptation in the marine bacterium, *Sphingopyxis alaskensis*, assessed using quantitative proteomics	Ting et al. (2010)
	Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*	Cacace et al. (2010)
Combined approach	Adaptation of *Bacillus subtilis* to growth at low temperature: a combined transcriptomic and proteomic appraisal	Budde et al. (2006)
Metagenomics	Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome	Simon et al. (2009)
	Comparative metagenomic analysis of a microbial community residing at a depth of 4,000 m at station ALOHA in the North Pacific subtropical gyre	Konstantinidis et al. (2009)
	Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism	Kennedy et al. (2010)

cyanobacterium *Synechocystis*, DNA supercoiling is involved in the expression of cold-induced genes including desaturases (Los 2004; Prakash et al. 2009). Supercoiling in *Escherichia coli* is mainly regulated by topoisomerases I and II (Tse-Dinh et al. 1997; Hurme and Rhen 1998), but proteins such as H-NS play a role in fine-tuning the DNA conformation. H-NS binds to curved regions of DNA and is responsible for the cold repression of bacterial genes, possibly by preventing open promoter complex formation necessary for transcription (Williams and Rimsky 1997). An example is the *Shigella* virulence regulator VirF whose expression is suppressed at low temperature by H-NS (Tobe et al. 1993). Similarly, the catabolite repression protein (CRP)-dependent promoters of the histone-like proteins HUα and HUβ are differentially affected by low temperature. The proteins itself in turn affect DNA structure and thus regulate transcription of several genes under certain conditions (Giangrossi et al. 2002).

An interesting aspect is the proposed involvement of GATC methylation by the DNA methyltransferase Dam in the cold shock response. According to this hypothesis, Dam is limited in fast growing cells inside a host, resulting in a hemi-methylated DNA that is more stable and displays a higher melting point (Riva et al. 2004). After shedding of *E. coli* from warm blooded animals into the environment, the cells experience a cold shock and the transcription of genes containing a GATC cluster ceases due to the high stability of hemi-methylated DNA. This effect might explain the decrease in transcription of certain down regulated genes.

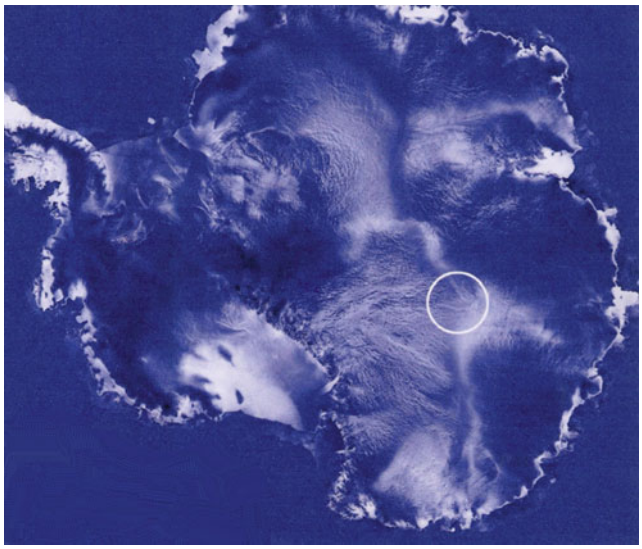


Fig. 18.1
Lake Vostok (*circled*) has lain undisturbed below the ice sheets of Antarctic for many years (From Gavaghan (2002))

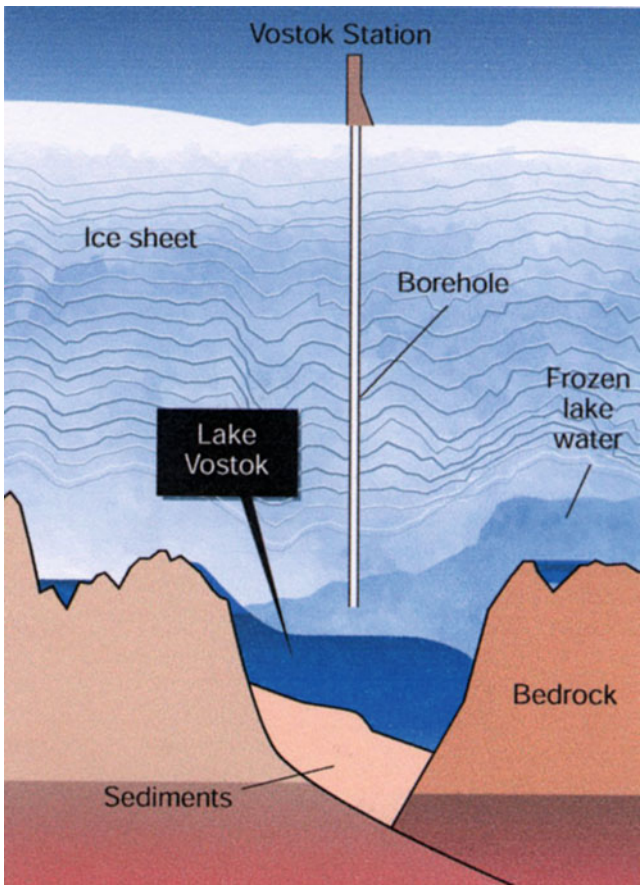


Fig. 18.2
Schematic view of the borehole to Lake Vostok. It extends beneath Vostok station into areas of frozen lake water but does not reach the lake (From Gavaghan (2002))

Sensing Through Alteration in RNA Conformation and Stability

Translational control by RNA thermometers plays an important role in bacterial regulation (Chowdhury et al. 2003; Narberhaus et al. 2006). Canonical examples for temperature-dependent RNA secondary structures affecting translation are the transcripts of the virulence regulators LrcF in *Yersinia pestis* and PrfA in *Listeria monocytogenes*. In both cases, the secondary structure of the mRNA inhibits translation at 25°C. At body temperature however, melting of stem-loop structures makes the ribosomal binding site accessible (Hoe and Goguen 1993; Johansson et al. 2002). A similar mechanism, however, is not known for translation at low temperature, although mRNA restructuring and stabilization is a known response to a downshift in temperature. The 159 nucleotides long 5′ UTR of the *E. coli cspA* mRNA acts as a cold sensor that controls translation of CspA by alternating RNA structures (Yamanaka et al. 1999b; Giuliodori et al. 2010). The mRNA of CspA appears as a thermodynamically unstable folding intermediate at high temperature and is rapidly degraded. The transcript is stabilized at low temperature and more efficiently translated, thus enabling cold shock induction.

Translation of σ^{32} is another example for cold sensing via mRNA. At lower temperature, the mRNA of this sigma factor is folded and can therefore not be translated. At higher temperature, it becomes accessible to the ribosome upon unfolding. Translated σ^{32} then activates the heat shock response (Morita et al. 1999).

Another mechanism was described for σ^{S}, a stationary phase sigma factor. The transcription of *rpoS* encoding σ^{S} depends on *dsrA*, a small regulatory RNA that probably stabilizes the *rpoS* mRNA. The half-life of *dsrA* is prolonged at 25°C compared to its half-life at 37°C (Repoila and Gottesman 2003). In an *E. coli* microarray analysis, the RpoS- and DsrA-dependent induction of biofilm and cold shock genes at low temperature was revealed (White-Ziegler et al. 2008).

The Ribosome as Temperature Sensor

The translational machinery may also act as a sensor for both heat and cold shock (*Fig. 18.4*). After heat shock, the ribosomal A-site is empty, whereas it is blocked following cold shock due to a stop in initiation and translation of misfolded mRNA. High and low temperatures lead to an increase or decrease, respectively, of the concentration of the stringent response regulators guanosine 5′-triphosphate-3′-diphosphate and guanosine 5′-diphosphate-3′-diphosphate (collectively abbreviated [p]ppGpp). Thus, a [p]ppGpp decrease might provoke a cold shock response (VanBogelen and Neidhardt 1990). For example, a nutritional upshift is coupled with a decrease in (p)ppGpp and leads to induction of CspA (Weber and Marahiel 2003; Wick and Egli 2004). Additionally, a mutant lacking RelA ([p]ppGpp synthetase) and SpoT ([p]ppGpp hydrolase) is unable to produce (p)ppGpp and has

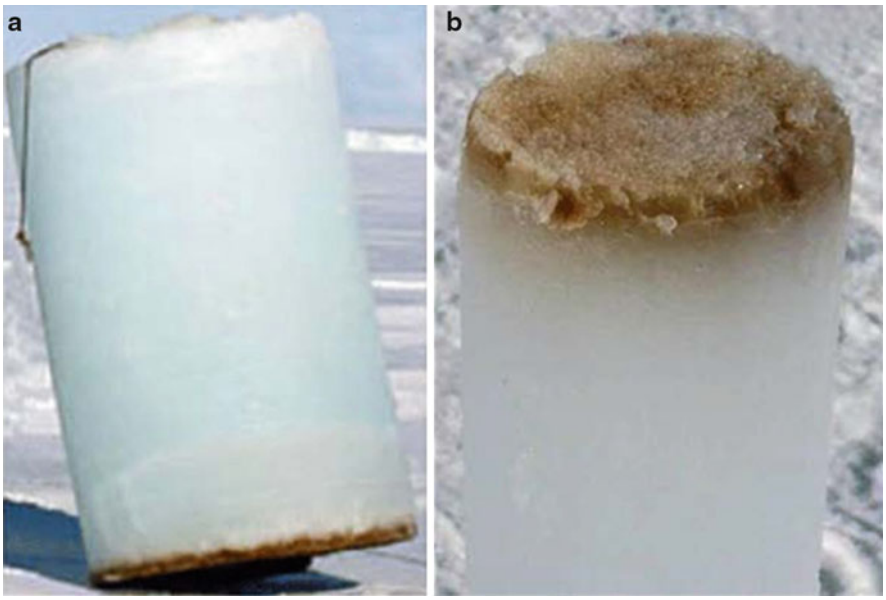


Fig. 18.3
(**a**) A 1-m-diameter by 2.3-m-thick cylinder drilled from Antarctic sea ice at Terra Nova Bay. (**b**) Bottom section of a 13-cm-diameter ice core sample. The *brown* coloration on the *bottom* of the ice at the ice-water interface is due to the high concentration of algal and bacterial cells forming a layer that can be up to 10-cm thick (From Koh et al. (2010))

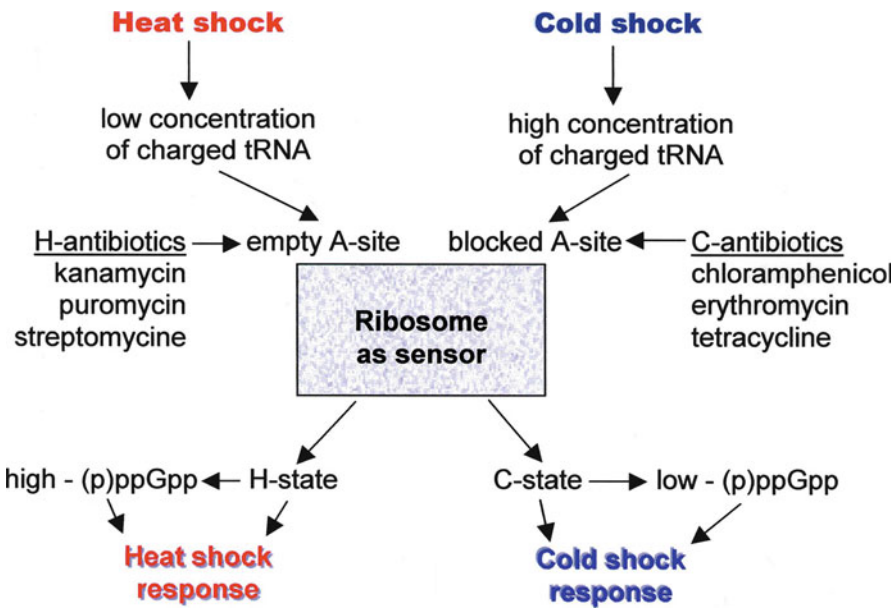


Fig. 18.4
Model of the ribosome as a temperature sensor in bacteria. After heat shock, translation proceeds faster than charged tRNA can be supplied, which may result in an empty A-site that is also affected by H-antibiotics. This could signal the ribosomal induction of the heat shock response (H-state) and increase of the guanosine 5′-triphosphate-3′-diphosphate and guanosine 5′-diphosphate-3′-diphosphate [(p)ppGpp] concentration. In contrast, cold shock leads to a reduced translational capacity of the cell and thus blocking the A-site (as is also achieved by C-antibiotics) due to a high concentration of charged tRNA. As a consequence, the cold shock response is induced and the levels of (p)ppGpp are lowered (Legend modified and figure redrawn from Graumann et al. (1996))

a higher induction of cold shock proteins after a cold shock. This mutant phenotype seems to be preadapted to low temperature (Jones et al. 1992a). A mutant of *L. monocytogenes* carrying a knockout of *pgpH* encoding a phosphohydrolase showed an accumulated level of (p)ppGpp and was cold sensitive (Liu et al. 2006).

Sensing Through Alteration in Protein Conformation

Changes in protein conformation are more pronounced following temperature increase (Eriksson et al. 2002). An intriguing example of low-temperature sensing by protein interaction is the aspartate chemotaxis of *E. coli* in which the transmembrane chemoreceptors Tap that mediate chemotaxis to dipeptides act as a cold sensor (Nara et al. 1991). During cold adaptation, receptor methylation (catalyzed by the methyltransferase CheR) and demethylation (catalyzed by the methylesterase CheB) regulate the histidine kinase activity of the sensors. Thermosensing is probably achieved due to the specific temperature dependency of the methylation-demethylation equilibrium and occurs via methylation of a single glutamyl residue in the presence of aspartate (Nara et al. 1996; Nishiyama et al. 1999).

The activity of H-NS on DNA structure itself depends to some extent on the conformation of this protein. H-NS function is associated with oligomerization by means of a coiled-coil structure. This flexible structure stiffens at lower temperatures thus improving oligomerization and subsequent DNA binding of H-NS (Smyth et al. 2000; Dame et al. 2006).

The Cytoplasmic Membrane as Cellular Thermometer

Another mechanism of cold-temperature sensing involves the physical state of the membrane (Sakamoto and Murata 2002). In *Bacillus subtilis*, the membrane-bound two-component signal transduction system DesK/DesR regulates the cold induction of the *des* gene coding for a Δ5-lipid desaturase (Mansilla and de Mendoza 2005). Unsaturated fatty acids (UFAs), which are the product of Des, act as negative signaling molecules for *des* transcription (Aguilar et al. 2001). Apparently, the physical state of the cytoplasmic membrane regulates the two-component system (TKS): a temperature downshift, which results in a more rigid membrane, provokes the phosphorylation of a dimeric response regulator DesR by the sensor kinase DesK. The phosporylated DesR then binds to the promoter of *des* and induces synthesis of UFAs. After restoring the fluid state of the membrane, DesK dephosphorylates DesR and inactivates it (Cybulski et al. 2004). Recent studies revealed that under aerobic conditions, cold adaptation of the membrane is mediated by the composition of branched-chain fatty acids rather than an increase of desaturated lipids and that the multimembrane-spanning domain of DesK is sensitive to membrane thickness (Cybulski et al. 2010; Beranova et al. 2010). The regulatory loop of DesK, DesR, Des, and UFAs is shown in *Fig. 18.5*.

In the cyanobacterium *Synechocystis*, two histidine kinases and a response regulator have been identified which regulate several genes at cold temperature (Suzuki et al. 2000). Interestingly, the membrane-bound histidine kinase Hik33 not only senses cold but also osmotic and salt stress, and the induced gene sets partially overlap (*Fig. 18.6*). When the membranes were artificially rigidified by gene engineering, certain cold-inducible genes were expressed at higher levels (Inaba et al. 2003). Another thermoresponsive TKS is involved in upregulating phytopathogenic factors in *Pseudomonas syringae*. This system consists of the membrane-bound histidine protein kinase CorS, which mediates thermosensing, and two transcriptional regulators, CorR and CorP, which induce the phytotoxin coronatine (Smirnova et al. 2002; Braun et al. 2008).

Adaptations of the Membrane

Membrane adaptation to different growth temperatures has long been a target of research (Russell 1997; Sakamoto and Murata 2002). A survey of the increasing "-omic" literature shows that membrane composition including (unsaturated) fatty acid and polysaccharide biosynthesis is often affected by low temperature (Kaan et al. 2002; Gao et al. 2006; Chan et al. 2007; Riley et al. 2008; Allen et al. 2009).

Strategies of Fatty Acid Alteration

The lipid composition of the cytoplasmic membrane is of great importance for cellular processes such as nutrient uptake, electron flow in respiration or photosynthesis, and ATP synthesis. If bacteria are subjected to rapid chilling or freezing, a variety of damages can occur, like the release of lipopolysaccharides or alteration of membrane permeability (Kempler and Ray 1978; Boziaris and Adams 2001; Riva et al. 2004). The lipid composition of the membrane, in combination with the temperature, controls the phase transition from the fluid phase to the semi-crystalline or solid phase (Jones et al. 2002). To grow at low temperature, cells need cytoplasmic membranes that retain sufficient fluidity to maintain a physical state supportive of the multiple functions of the membrane, a concept that has been termed "homeoviscous adaptation" (Sinensky 1974). The temperature-dependent alterations in fatty acyl chain composition are thus mainly aimed at maintaining the proton permeability of the cytoplasmic membrane at a rather constant level (Albers et al. 2000). For instance, low proton permeability in the cold must be counteracted by an appropriate adaptation of the membrane lipids in *Psychrobacter immobilis* (*Fig. 18.7*). Several fatty acid changes such as unsaturation and chain shortening are known to increase or decrease membrane fluidity in bacteria (*Table 18.4*). Palmitoleate, for example, is not present in *E. coli* grown at 30°C but constitutes 11% of the fatty acid content in cells grown at 12°C. The *lpxP* gene encoding a palmitoleoyl transferase, which acts late in the lipid A synthesis (*Fig. 18.8*), was found to be 30-fold cold induced 2 h after

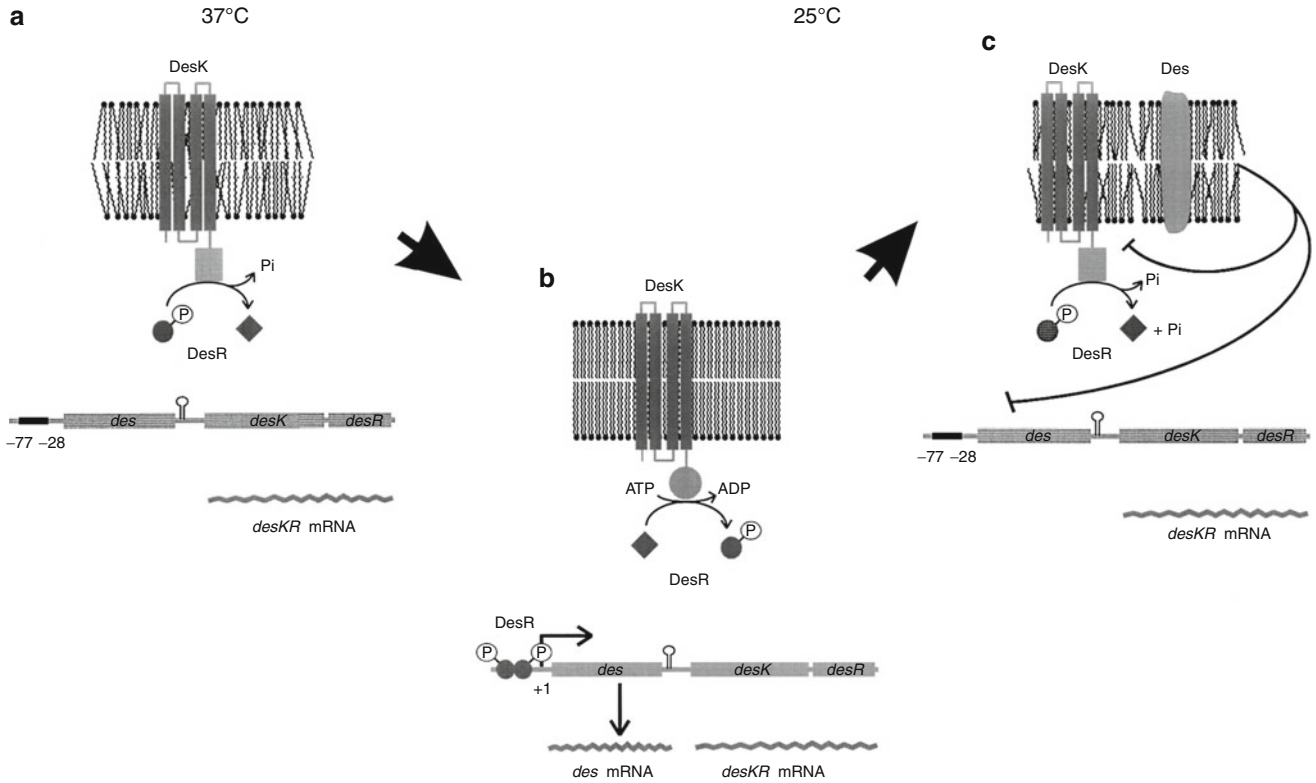


Fig. 18.5

Model of *des* transcriptional control by two-component temperature signal transduction proteins. It is proposed that DesK assumes different signaling states in response to a temperature-induced change in membrane fluidity. This is accomplished by regulating the ratio of kinase to phosphatase activity such that a phosphatase-dominant state is present at 37°C, when membrane lipids are disordered (a), whereas a kinase-dominant state predominates upon an increase in the proportion of ordered membrane lipids after a temperature downshift to 25°C (b). DesK-mediated phosphorylation of DesR results in transcriptional activation of *des* (b) leading to synthesis of Des, which desaturates the acyl chains of membrane phospholipids (c). These newly synthesized UFAs inhibit *des* transcription either by favoring DesK dephosphorylation of DesR-P or by causing dissociation of DesR-P from its binding site (c) (Adapted from Aguilar et al. (2001) and Cybulski et al. (2004))

a temperature drop. Thereafter, the activity gradually declines but does not disappear (Carty et al. 1999). As a possible advantage, the palmitoleate content of the outer membrane provides a more effective barrier to harmful chemicals at low temperature (Vorachek-Warren et al. 2002).

Response of Desaturases to Low Temperature

Both anaerobic and aerobic mechanisms are responsible for the synthesis of UFAs in bacteria. The anaerobic pathway, elucidated in detail for *E. coli*, produces *cis*-UFA by a specific 2,3-dehydrase acting at the C_{10} level (Cronan and Rock 1996). A second mechanism is the introduction of double bonds into the fatty acids by iron-containing desaturases (Mansilla and de Mendoza 2005). Their reaction is characterized by oxygen-dependent desaturation of full-length fatty acyl chains. Desaturases have not been found in archaea, but are present and cold induced in psychrophilic bacteria such as the marine Antarctic and Arctic bacteria *Pseudoalteromonas haloplanktis, Psychrobacter arcticus*, and *Psychromonas ingrahamii*, helping to cope with increased solubility of oxygen at low temperature (Medigue et al. 2005; Bergholz et al. 2009; Riley et al. 2008).

The molecular basis of the response of fatty acid adaptation to cold shock was studied in more detail in unicellular cyanobacteria (Sakamoto et al. 1997; Los and Murata 1999). In *Synechocystis*, four desaturase genes (*desA–desD*) were reported, three of which (*desA*, *B*, and *D*) are cold inducible (*Fig. 18.9*). The up regulation appeared to be due to an increased stability of *des* mRNA at low temperature. Inactivation of both *desA* and *desD* in *Synechocystis* led to a cold-sensitive phenotype that prevented this mutant from propagating at low temperature (Tasaka et al. 1996).

In contrast to cyanobacteria, *Bacillus subtilis* has only one desaturase, Δ5-Des (Aguilar et al. 1998; Weber et al. 2001). Cold shock induction of *des* occurs within 30 min and is almost exclusively controlled at the level of transcription. Unlike the situation in cyanobacteria, the stability of mRNA is not increased. Its transient induction after cold shock would imply that desaturation does not occur through de novo synthesis of

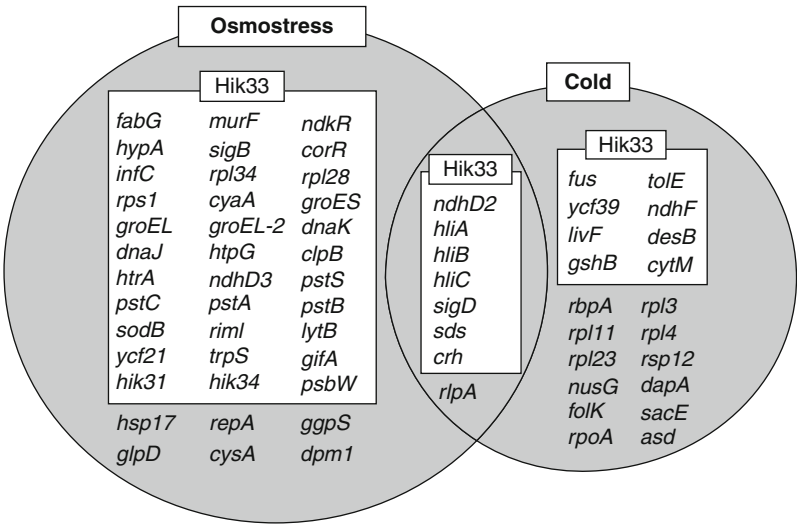


Fig. 18.6

Osmostress-inducible and cold-inducible genes that were regulated by the sensor histidine kinase Hik33 in wild-type *Synechocystis* cells. *Large* and *small circles* enclose genes whose expression was induced by osmotic stress and cold stress, respectively. *Rectangles* enclose genes whose expression was regulated to a greater or lesser extent by Hik33 in cells under hyperosmotic stress and under cold stress. Genes outside *rectangles* appeared to be insensitive to the mutation in Hik33 in terms of their responses to the respective stresses. The rectangle in the overlapping region of the two *circles* marks genes whose Hik33-regulated expression was observed under both kinds of stress (From Mikami et al. (2002))

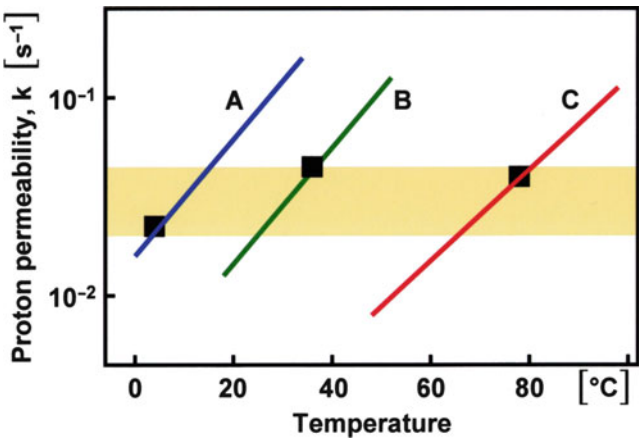


Fig. 18.7

Graphic representation of the proton permeability of the psychrophilic bacterium *Psychrobacter immobilis* (*line A*), five mesophilic species represented by *line B* (*B. subtilis*, *E. coli*, *Methanosarcina barkeri*, *Halobacterium salinarum*, and *Halorubrum vacuolatum*), and the hyperthermophilic *Sulfolobus acidocaldarius*, *line C*. The black squares represent measured proton permeabilities and the colored area indicates the rather narrow range within which proton permeability is maintained and growth is possible. Note that some thermophilic and hyperthermophilic bacteria have higher proton permeability (From (Albers et al. (2000))

Table 18.4

Fatty acid changes influencing membrane fluidity in bacteria

Increase of fluidity		Decrease of fluidity
Unsaturation	↔	Saturation
Cis double bond	↔	*Trans* double bond
Chain shortening	↔	Chain lengthening
Methyl branching	↔	Straight chain
Cis-unsaturation	↔	Straight chain

From Gounot and Russell (1999)

fatty acids. Surprisingly, a *des* null mutant of *B. subtilis* showed a cold-sensitive phenotype in the absence of isoleucine, probably due to the role of isoleucine as a precursor for *anteiso*-branched fatty acids (Klein et al. 1999) (*Fig. 18.10*).

Membranes in Psychrotolerants

The psychrotolerant *Listeria monocytogenes* is a foodborne pathogen that can grow well at refrigeration temperature. Due to its medical importance, the membrane composition of *L. monocytogenes*, which lacks a desaturase, has been studied in more detail. When grown in continuous culture at 10°C in contrast to 30°C, this bacterium contains a lower proportion

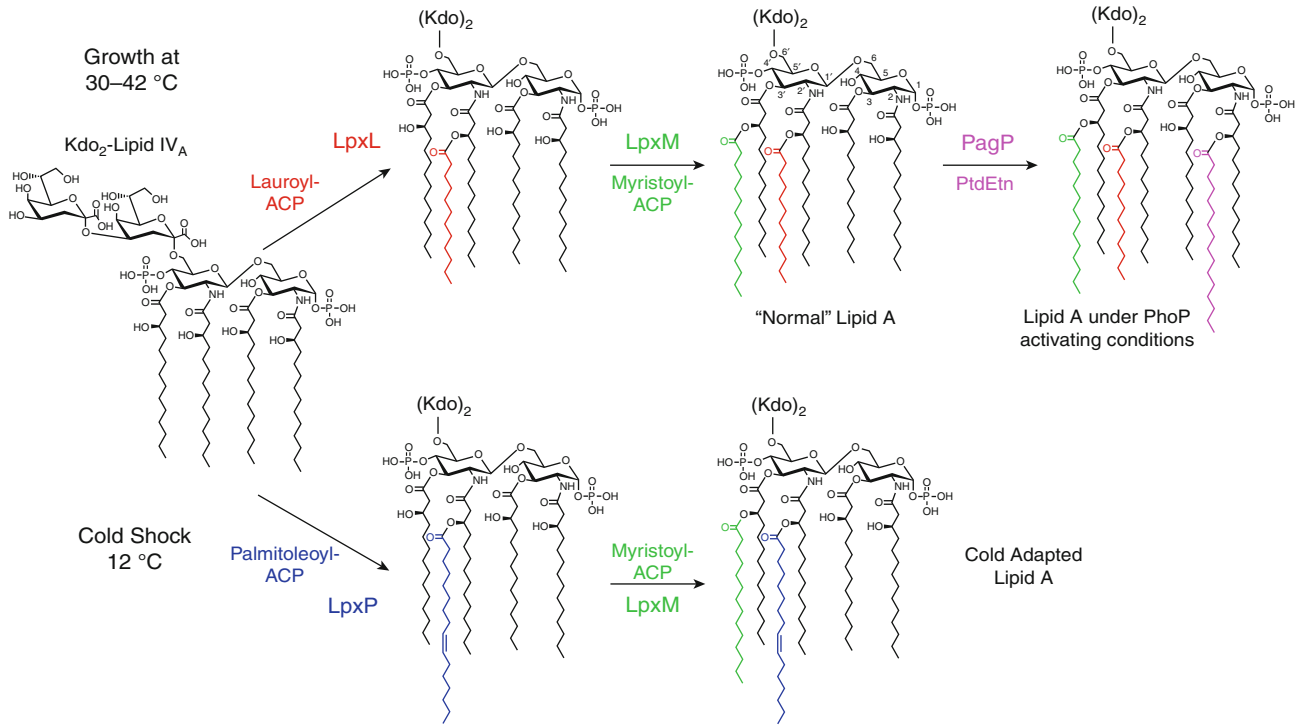


Fig. 18.8

Biosynthesis of Kdo_2-lipid A during cold shock in *E. coli*. In cells grown at 30°C or above, the key precursor Kdo_2-lipid IVA is utilized solely by the lauroyltransferase LpxL. However, in cold-shocked cells an additional acyltransferase, LpxP, is induced, which is proposed to incorporate palmitoleate at the same site normally reserved for laurate. In wild type cells, the action of LpxL and LpxP is followed rapidly by the myristoyltransferase, LpxM, generating hexa-acylated lipid A. About two-thirds of the hexa-acylated lipid A isolated from cells grown overnight at 12°C contains palmitoleate, and the remainder contains laurate. When the PhoP/PhoQ system is activated, a portion of the lipid A molecules contains a palmitate residue at position 2, which is incorporated by the outer membrane enzyme PagP using glycerophospholipids as palmitate donors (Original legend with references see Vorachek-Warren et al. (2002))

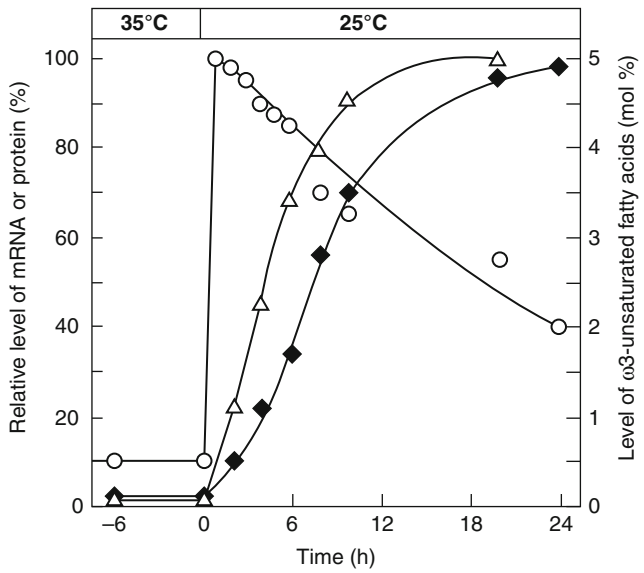


Fig. 18.9

Changes of levels of *desB* mRNA (*open circles*), the encoded 3-desaturase (*open triangles*) and ω-3-UFAs (*closed diamonds*) in *Synechocystis* after a temperature downshift from 35°C to 25°C (From Los and Murata (1999))

of *anteiso*-$C_{17:0}$ and a higher proportion of *anteiso*-$C_{15:0}$ and short chain fatty acids (Jones et al. 1997). Similarly, *L. monocytogenes* responding to cold displayed an increased amount of *anteiso*-$C_{15:0}$ in all lipid classes (Mastronicolis et al. 1998; 2006). The fatty acid profiles of the *L. monocytogenes* wild type and a cold-sensitive mutant deficient in branched-chain fatty acid synthesis suggested that the fatty acid 12-methyltetradecanoic acid (*anteiso*-$C_{15:0}$) plays a critical role in low-temperature growth of *L. monocytogenes*, presumably by maintaining membrane fluidity (Edgcomb et al. 2000). Interestingly, even between strains of the same species, differences in the membrane adaptation to low temperature were found (Badaoui Najjar et al. 2007; Arguedas-Villa et al. 2010). The *L. monocytogenes* strains Scott A and CNL 895897 differ in their pattern of branched fatty acids in response to low temperatures. In addition to odd-numbered branched fatty acids found in both strains, the CNL strain uses substantial amounts of even-numbered branched fatty acids (Chihib et al. 2003). Similarly, it was reported that one *Sphingomonas* strain uses UFAs, whereas another strain shifts from even-chain to odd-chain fatty acids (Männistö and Puhakka 2001).

The production of increased proportions of membrane UFAs correlates with bacterial growth at low temperature or

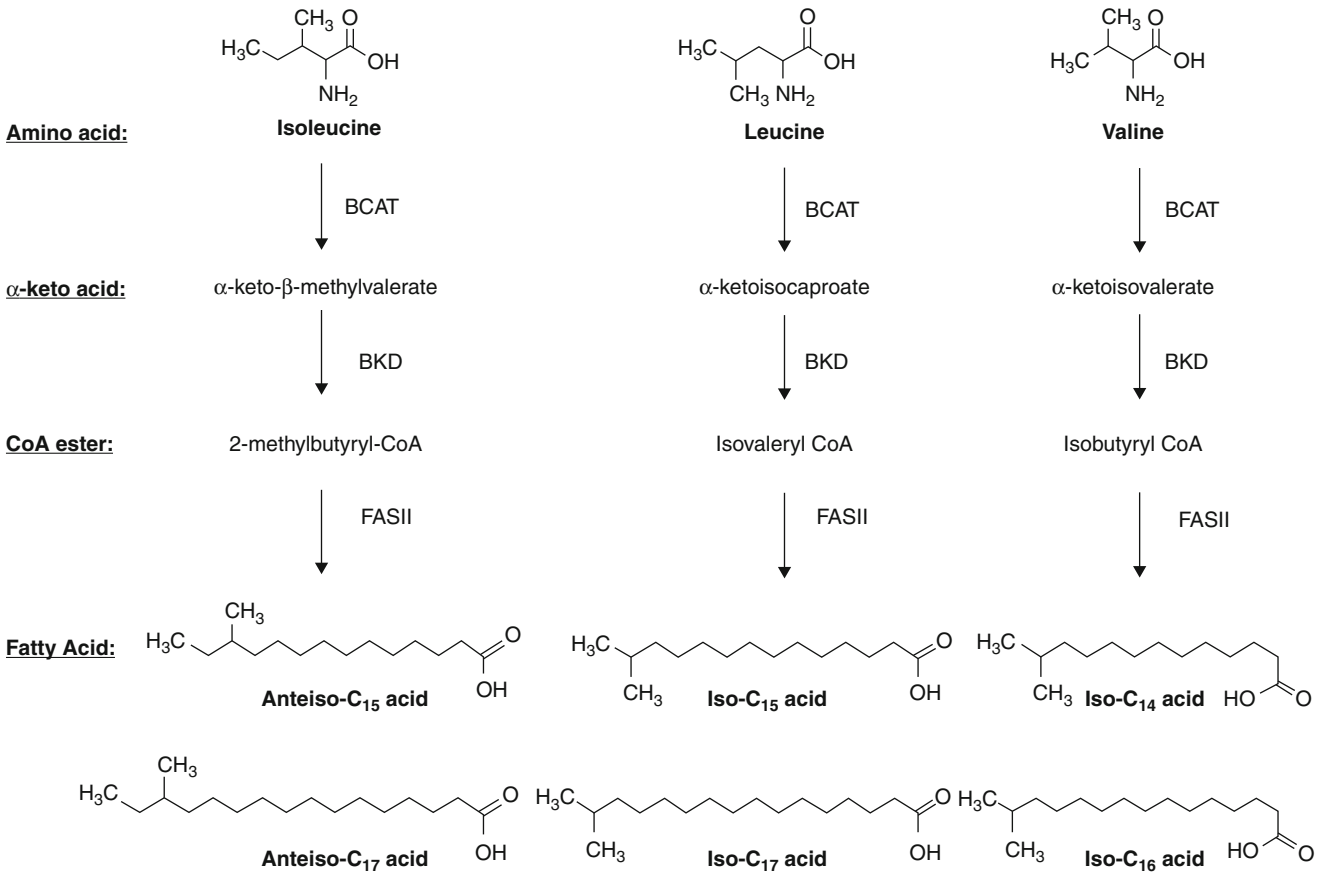


Fig. 18.10

Pathways of branched-chain fatty acid biosynthesis in *B. subtilis* and its dependence on external supply of valine, leucine, and isoleucine. The conversion of amino acids to α-keto acids is catalyzed by branched-chain amino acid transaminase (BCAT) and the synthesis of acyl-CoA esters by branched-chain α-keto dehydrogenases (BKD). Fatty acid synthase (FASII) uses acyl-CoA substrates to yield the branched-chain fatty acids (From Chan and Wiedmann (2009))

high pressure (Allen and Bartlett 2002). Allen et al. (1999) investigated the fatty acids produced by the deep-sea bacterium *Photobacterium profundum*, grown at various temperatures and pressures, and isolated oleic acid-auxotrophic mutants. One of these mutants, strain EA3, was deficient in the production of monounsaturated fatty acids and was sensitive to both low-temperature and high-pressure in the absence of exogenous 18:1 fatty acid. The authors conclude that monounsaturated, but not polyunsaturated fatty acids, are required for growth of *P. profundum* both at high pressure and low temperature. In contrast, in the psychrotolerant bacterium *Psychrobacter* sp. isolated from Siberian permafrost, unsaturated C_{18} fatty acids and C_{16} methyl esters are predominant both at 4°C and 5% NaCl (Ponder et al. 2005).

However, the fatty acid content does not always change dramatically in response to temperature. Könneke and Widdel (2003) examined a range of sulfate-reducing bacteria in their response of the fatty acid content (unsaturated vs. saturated). The highest levels of *cis*-UFAs was measured in the psychrophilic species, but a substantial response in terms of increasing amounts of UFAs at low temperature was only found in the genus *Desulfobacter* (Fig. 18.11). All other genera responded with only slight changes. A similar result was formerly reported for psychrotolerant *Pseudomonas* species (Bhakoo and Herbert 1980).

Differences Between Closely Related Mesophilic and Psychrotolerant Strains

Randomly selected strains of a bacterial collection of marine sea-ice bacteria from Antarctic were analyzed to obtain a profile of the membrane fatty acids. Results showed that short-chain saturated and UFAs were more common in the psychrotolerants when compared to psychrophiles. In contrast, branched-chain fatty acids were more abundant in the psychrophiles (Rotert et al. 1993).

Such observations raise the question of whether differences in the capability of membrane adaptation to low temperature between closely related psychrotolerant and mesophilic strains (i.e., belonging to the same species) are responsible for the thermal type. Some species comprise psychrotolerant as well as mesophilic strains. One example is *Rhizobium leguminosarum*, which is known as a mesophilic species growing poorly at

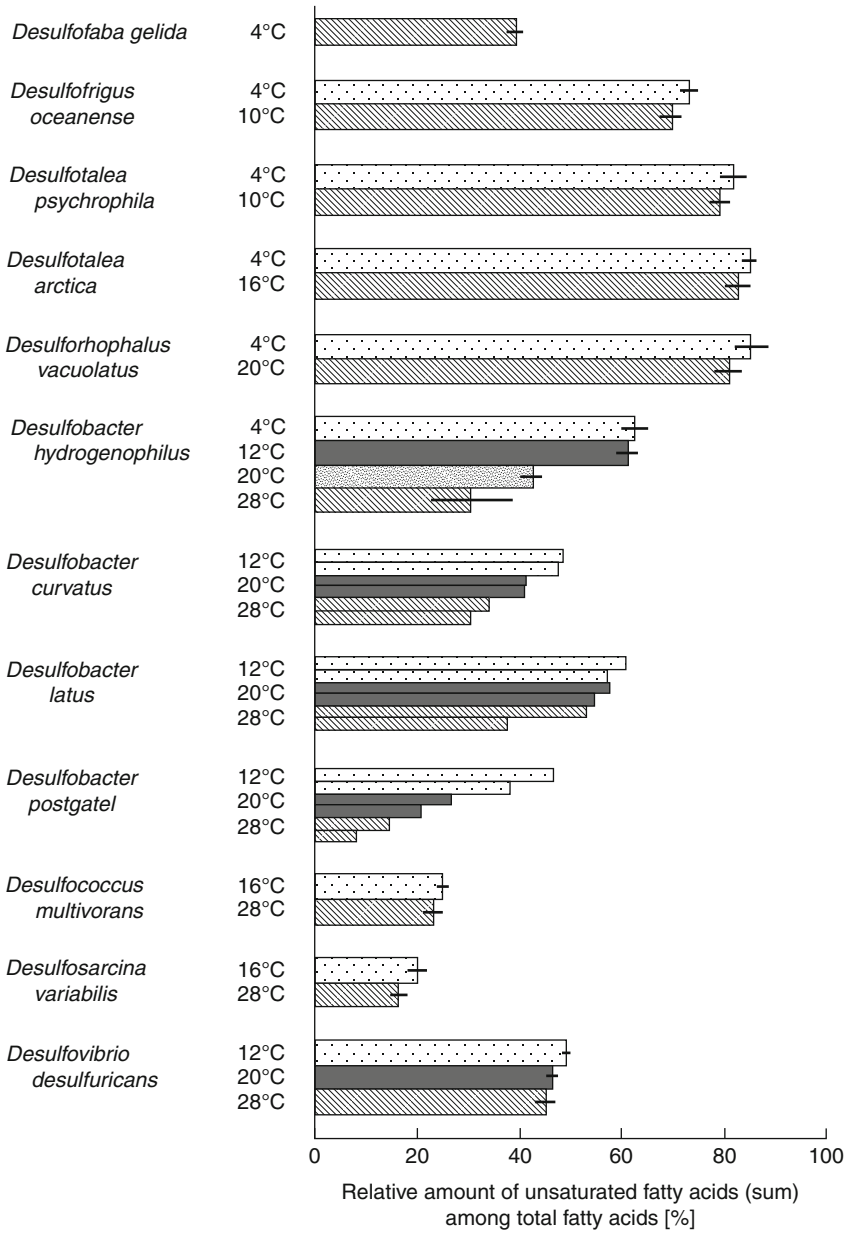


Fig. 18.11

Proportions of UFAs among total fatty acids in psychrophilic and mesophilic species of sulfate-reducing bacteria grown at different temperatures. Analyses were carried out while cells were still growing and had reached three-quarters of the maximum (final) optical density. Note that a substantial increase in UFAs is only visible in *Desulfobacter* species. In all other species examined, virtually no increase in UFAs can be found (Adapted from Könneke and Widdel (2003))

temperatures below 10°C (Graham 1992). However, psychrotolerant strains have been isolated from the Arctic legumes *Astralagus* and *Oxytropis*, and nitrogenase activity in Arctic nodules was detectable down to 0°C. The minimal and maximal growth temperature of isolates was 0°C and 27–30°C, respectively (for a review on Arctic rhizobia, see Prévost et al. (1999)). Psychrotolerant and mesophilic *R. leguminosarum* strains have also been isolated from the legume species *Lathyus japonicus* and *L. pratensis* (Drouin et al. 2000). These authors have determined the fatty acid profiles after growth at 25°C, at 5°C, and after cold shock from 25°C to 5°C. Interestingly, the degree of psychrotolerance of the strains did not correlate with their fatty acid composition.

There is a vast body of literature concerning mesophilic and psychrotolerant isolates of the *B. cereus*, a soil bacterium that poisons food (Granum and Lund 1997; Ehling-Schulz et al. 2011). The difference in growth rate of the mesophilic and psychrotolerant *B. cereus* strains is shown in ❯ *Fig. 18.12*. We derived a branched-chain fatty acid profile of a mesophilic *B. cereus* strain and a very closely related psychrotolerant *B. weihenstephanensis* strain (❯ *Table 18.5*). *Iso-* and *anteiso*-branched fatty acids are predominant,

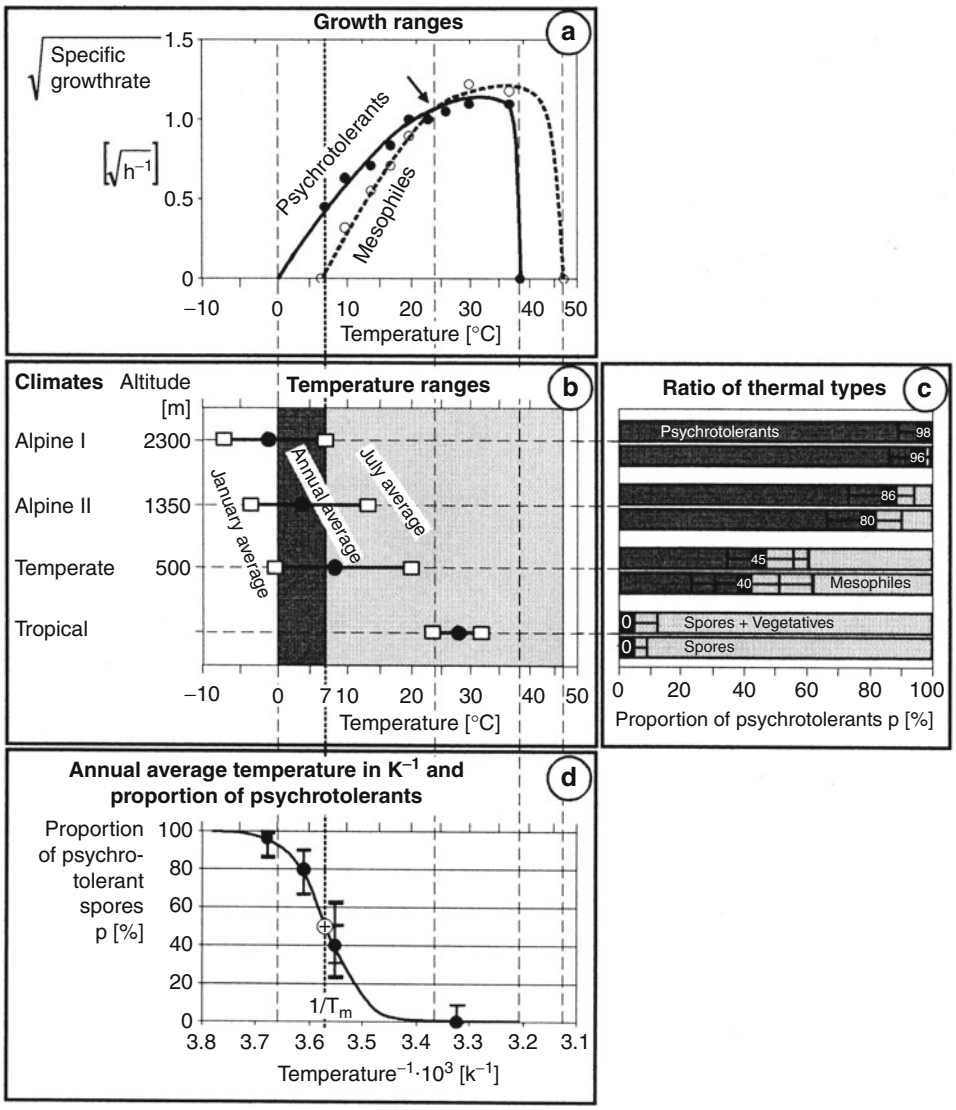


Fig. 18.12

Difference in growth rate of a mesophilic *B. cereus* and a psychrotolerant *B. weihenstephanensis* strain and influence of different climates on the ratio of mesophilic and psychrotolerant *cspA* genotypes. For a comprehensive view, the diagrams have common axes. (b) and (c) share the climate axis, and (a), (b), and (d) share the temperature axes. Temperatures are displayed as both °C and K^{-1}. (a) Comparison between the growth ranges of psychrotolerant and mesophilic strains. (b) Temperature ranges of a tropical climate, and a climatic sequence, consisting of one temperate climate at 500-m altitude and two temperate high-mountain climates at 1,350-m and 2,300-m altitude designated as alpine II and alpine I. January and July average temperatures (□) and annual average temperatures (●) are indicated. The white area indicates temperatures below 0°C, where no substantial growth occurs; the darker area marks the range between 0°C and 7°C, where only psychrotolerant strains grow well, and the brighter area highlights the growth range of the mesophilic strains. (c) The ratios (in %) of psychrotolerant (*dark bar*) to mesophilic (*bright bar*) *cspA* genotypes are displayed for each climate. The *upper* bar of each pair gives this ratio for the total population, consisting of spores and vegetative cells; the *lower* bar shows the ratio for spores only. The 95% confidence intervals of the individual assays are indicated by *error* bars. The *bold outer* error bars of the temperate sample indicate the estimated intraclimatic mean variation. (d) Proportion of psychrotolerant spores over the annual average temperature. This relation can be described by a tangens hyperbolicus function, with its point of inflection (⊕) shifted to 7°C (From von Stetten et al. (1999))

a feature that is a characteristic observed in all species of *Bacillus* studied so far (Kämpfer 1994). In both strains, *iso*-branched fatty acids increased about 6–7% at 12°C in comparison to 25°C due to a higher amount of *iso*-13:0 and *iso*-16:0. A further increase of *iso*-16:0 in the psychrotolerant strain at 7°C raised the fraction of the branched isoform to nearly 50% of all fatty acids. Upon lowering the temperature, straight-chain fatty acid and monounsaturated fatty acid levels decreased in response to changes in C_{16} fatty acid levels. These data confirm the hypothesis that bacilli adapt to

Table 18.5
Major fatty acids of a mesophilic *B. cereus* WSBC 10030 and a psychrotolerant *B. weihenstephanensis* WSBC 10226 grown at different temperatures in percent[a]

RT (min)[b, c]	Fatty acid	Mesophilic strain grown at		Psychrotolerant strain grown at		
		12°C	25°C	7°C	12°C	25°C
12.95	*i*-12:0	0.9	1.0	1.6	2.1	1.4
16.37	*i*-13:0	13.4	8.5	14.7	15.3	8.9
19.82	*i*-14:0	4.9	5.6	3.8	4.1	3.4
23.32	*i*-15:0	16.6	18.1	7.5	10.1	13.3
26.48	*i*-16:0	7.0	2.0	18.0	6.8	2.6
29.86	*i*-17:0	2.4	3.1	0.8	2.5	5.1
Branched, iso		*45.2*	*38.3*	*46.4*	*40.9*	*34.7*
16.64	*a*-13:0	3.9	3.7	6.2	8.8	5.3
23.60	*a*-15:0	7.2	10.2	6.5	8.9	9.0
26.63	*a*-16:0	1.6	4.1	0.0	1.6	3.6
Branched, anteiso		*13.7*	*20.2*	*13.2*	*20.7*	*20.9*
14.19	12:0	0.4	0.8	1.9	1.8	1.4
21.12	14:0	3.7	5.6	4.4	4.3	4.9
27.88	16:0	7.8	10.4	2.9	5.5	8.1
Straight, even		*11.9*	*16.8*	*9.2*	*11.6*	*14.4*
27.38	16:1	7.5	10.3	4.3	8.4	11.6
33.35	18:1	1.4	1.5	1.8	1.0	1.7
Unsaturated, even		*8.9*	*11.8*	*6.1*	*9.4*	*13.3*
25.71	u.i.	1.4	1.2	1.9	1.0	1.4
27.00	u.i.[b]	8.7	4.9	10.1	5.7	3.3
29.00	u.i.[b]	3.7	2.2	7.7	5.3	4.9
29.27	u.i.[b]	0.7	0.7	1.1	1.2	1.4
Unidentified		*14.5*	*9.0*	*20.8*	*13.2*	*11.0*

WSBC Weihenstephan bacillus collection, Chair of Microbial Ecology, Freising, *RT* retention time, *i*- iso-, *a*- anteiso, *u.i.* unidentified
[a]T. Kaplan and S. Scherer, unpublished results
[b]Small peaks, representing less than 1% of total fatty acids are not listed
[c]See Byun et al. (2003)

decreasing environmental temperature by replacing the saturated straight-chain acids with the lower melting branched-chain acids, or by changing to fatty acid branching instead of fatty acid unsaturation (Suutari and Laakso 1992). Kaneda (1991) reported that mainly 12- and 13-methyltetradecanoic acids (equivalent of *a*-15:0 and *i*-15:0) controlled the fluidity of membranes with branched-chain fatty acids. Indeed, these fatty acids constituted the major fraction in our study at 25°C but decreased at 12°C. We did not observe a correlation of the minimum growth temperature with the fatty acid composition. The nearly identical fatty acid pattern of the mesophilic and psychrotolerant *B. cereus* indicates that differences in lipid-dependent membrane architecture may not be responsible for the substantially different growth rates of these strains at 12°C.

Carotenoids and Polysaccharides

Synthesis of a particular type of carotenoids also contributes to cold adaptation of some species (Chattopadhyay 2006; Shivaji and Prakash 2010). In the two psychrophilic bacteria *Sphingobacterium antarcticum* and *Micrococcus roseus*, a higher amount of polar carotenoids in comparison with their less polar counterparts was identified. In vitro studies with synthetic membranes of phosphatidylcholine demonstrated that the major pigments zeaxanthin, β-cryptoxanthin, and β-carotene were bound to these membranes, thus compensating the fluidizing effect of the UFAs (Jagannadham et al. 2000). Interestingly, Gram-positive bacteria collected from the Antarctic region showed a predominance of pigmented isolates. In *Arthrobacter agilis*, this pigmentation is due to a C50-carotenoid whose

synthesis is induced at low temperature. Such carotenoids are assumed to stabilize the membrane, since such C50-carotenoids are only reported from other extremophiles and archaea capable of coping with salt, cold, and radiation stress (Fong et al. 2001). This speculation fits to the observation that some carotenoids are only present in the thylakoid membranes of *Cylindrospermopsis raciborskii* at low temperature, possibly protecting the cyanobacterium from reactive radicals (Varkonyi et al. 2002).

In addition, exopolysaccharides (EPS) were recently suggested to play a specific role in the maintenance of protein activity as they not only mediate cell adhesion but also trap the extracellular enzymes as well as their substrates and products (Marx et al. 2007); alternatively, EPS were suggested to lower the freezing point, thus keeping the surrounding water available (Riley et al. 2008; Ayala-del-Rio et al. 2010).

Compatible Solutes

Cold shock studies revealed that low-molecular-weight compounds play an important role in response to cold stress and thus in adaptation to low temperature. Examples for these so-called compatible solutes are glycine betaine, carnitine, and trehalose. However, how those highly soluble polyhydroxylated compounds protect the cells against low temperature remains unclear. Four mechanisms have been suggested (Kandror et al. 2002): (1) compatible solutes act as "chemical chaperones" against low temperature denaturation or aggregation, (2) cold stress causes oxidative stress, and substances such as trehalose act as free radical scavengers, (3) compatible solutes protect the membrane, or (4) compatible solutes are induced to anticipate a possible temperature drop below freezing.

L. monocytogenes uses at least three compatible solute transporters. The porters I (BetL), a Na^+ symporter, and II (Gbu), which acts as an ATP-dependent transporter, are responsible for glycine betaine uptake. Its cold-activated uptake has been shown to be most rapid between 7°C and 12°C (Mendum and Smith 2002; Angelidis and Smith 2003). Carnitine accumulates after osmotic or low temperature stress via the transporter OpuC that is induced after chilling and contributes to cryoprotection (Wemekamp-Kamphuis et al. 2004a; Angelidis et al. 2002a). This reminds of similar observations made in *B. subtilis* by Brigulla et al. (2003). However, if Gbu is blocked, the increased carnitine uptake cannot completely restore the cryoprotective effect (Angelidis et al. 2002b). If all three osmolyte transporters are deleted, *L. monocytogenes* is severely impaired in growth at low temperature, but growth is not completely abolished (Wemekamp-Kamphuis et al. 2004a. The activation of the betaine transporter BetP of *Corynebacterium glutamicum* depends on osmolarity, temperature, and lipid composition (Ozcan et al. 2007). In *E. coli*, the trehalose synthesis genes *otsAB* and the cryptic promoter (P1) of the *proU* transporter, which is important for mediating cytoplasmic accumulation of compatible solutes, are induced during low-temperature growth (Schiefner et al. 2004). Thus, the cellular trehalose content increases up to eightfold after cold shock (Kandror et al. 2002; Rajkumari and Gowrishankar 2001). If compatible solutes are added to the medium, both *L. monocytogenes* and *E. coli* survive better at low temperature (Shahjee et al. 2002; Dykes and Moorhead 2001). Genes involved in transport and syntheses of compatible solutes are also up regulated in psychrophilic bacteria (Ayala-del-Rio et al. 2010).

In addition, the presence of antifreeze proteins has been reported for some strains (Gilbert et al. 2005). Low-temperature-induced ice-nucleating agents (INAs) comprising outer membrane proteins, lipids, phospholipids, and carbohydrates have been reported to serve as templates for ice crystallization and to prevent desiccation (Lundheim 2002; Ponder et al. 2005; Christner 2010).

Another concept is based on chaotropic substances such as fructose, which are known to disorder cellular macromolecules (Hallsworth et al. 2003). These solutes were postulated to determine microbial survival at extreme temperatures and pressure (Chin et al. 2010).

Adaptation of Proteins and Ribosomes to Low Temperature

Many cold enzymes mainly isolated from Antarctic and Arctic microorganisms have been purified and characterized in detail at both the biochemical and structural level. The reader is referred to the reviews indicated in *Table 18.2* and to *Table 18.6*, which lists enzymes from psychrotolerant organisms. The application of cold enzymes offers considerable potential for the detergent and food industries, the production of fine chemicals, and bioremediation processes (Russell 1998; Gerday et al. 2000) (section "Biotechnological Applications").

The Thermodynamic Challenge

Chemical reactions are characterized by a strong dependency of the reaction velocity on the reaction temperature. The effect of temperature on a chemical reaction is basically described by the Svante Arrhenius equation

$$k = Ae^{-Ea/RT}$$

where k is the rate constant, A the pre-exponential factor that is reaction specific, E_a the activation energy, R the gas constant (8.31 kJ mol^{-1}), and T the temperature in kelvins. Thus, a temperature decrease induces an exponential decrease of k. As a rule, a temperature drop by 10°C reduces the reaction rate by a factor of 1.5–4, the so-called Q_{10} value. Notably, the greater the activation energy E_a, the stronger the temperature dependency of the reaction rate. Reactions with low activation

Table 18.6
Selected examples of cold adapted enzymes from psychrophilic/psychrotolerant organisms

Enzyme	Source	References
Adenylate kinase	*Marinibacillus marinus*	Davlieva and Shamoo (2009)
Alkaline phosphatase	*Shewanella* sp.	Murakawa et al. (2002)
Alkaline protease	*Exiguobacterium* sp. SKPB5	Kasana and Yadav (2007)
Aminopeptidase	*Colwellia psychrerythraea*	Huston et al. (2004)
α-Amylase	*Pseudoalteromonas haloplanktis*	Claverie et al. (2003)
Alkaliphilic esterase	Antarctic desert soil metagenome	Heath et al. (2009)
Catalase	*Bacillus* sp. N2a	Wang et al. (2008)
Cellulase	*Pseudoalteromonas haloplanktis*	Violot et al. (2005)
Chitinase	*Moritella marina*	Stefanidi and Vorgias (2008)
Chitobiase	*Arthrobacter* sp. TAD1	Lonhienne et al. (2001)
Dihydrofolate reductase	*Moritella profunda*	Xu et al. (2003b)
Esterases	*Pseudoalteromonas haloplanktis*	Aurilia et al. (2008)
β-Galactosidase	*Planococcus* sp. L4	Hu et al. (2007)
Glutamate dehydrogenase	*Psychrobacter* sp. TAD1	Camardella et al. (2002)
Hydrolytic enzymes	Divers	Groudieva et al. (2004)
Isocitrate lyase	*Colwellia psychrerythraea*	Sato et al. (2008)
β-Lactamase	*Pseudomonas fluorescens*	Michaux et al. (2008)
Lipase	*Acinetobacter* sp. CR9	Kasana et al. (2008)
Lipase	Deep-sea sediment metagenome	Jeon et al. (2009)
Malate dehydrogenase	*Moritella* sp. strain 5710	Saito and Nakayama (2004)
Metalloprotease	*Flavobacterium psychrophilum*	Secades et al. (2003)
NAD^+-dependent dehydrogenases	*Shewanella* PA-43	Irwin et al. (2001)
Ornithine carbamoyltransferase	*Moritella abyssi*	Xu et al. (2003a)
Pectate lyase	*Mrakia frigida*	Margesin et al. (2005)
Peptidyl-prolyl *cis-trans* isomerase	*Shewanella* sp. SIB1	Suzuki et al. (2004)
Phosphoglycerate kinase	*Pseudomonas* sp. TACII18	Bentahir et al. (2000)
Replication protein Rep	*Psychrobacter* sp. TA144	Duilio et al. (2001)
Serine hydrolase	*Moritella* sp. 2-5-10-1	Yang et al. (2008)
Subtilisin-like serine protease	*Vibrio* sp. PA44	Arnorsdottir et al. (2002)
L-Threonine dehydrogenase	*Cytophaga* sp. KUC-1	Kazuoka et al. (2003)
Tyrosine phosphatase	*Shewanella* sp.	Tsuruta et al. (2004)
Xylanase	*Pseudoalteromonas haloplanktis*	Van Petegem et al. (2003)

energies will only slightly depend on the reaction temperature. As examples, the temperature-dependent activities of a β-galactosidase and an α-amylase isolated from a psychrophilic, mesophilic, and thermophilic bacterium are shown in *Fig. 18.13*.

The influence of the reaction temperature on the reaction rate is more complicated when enzyme-catalyzed reactions are considered (for reviews, see *Table 18.2*). In this case, substrate and enzyme concentration as well as the enzyme-substrate interaction play an important role. At non-saturating substrate concentration, the reaction velocity also depends on the K_m, the substrate concentration required to produce 50% of the maximal activity.

The Trilogy of Activity-Stability-Flexibility: Structural Adaptations

The relative effect of temperature on the activity of psychrophilic and mesophilic enzymes is mainly characterized by enzyme activity, stability, and flexibility (Georlette et al. 2004): (1) Cold-adapted enzymes exhibit a high specificity and are catalytically efficient at low and moderate temperatures; their specific activity is higher than that of their mesophilic counterparts at 0–30°C, but lower when compared to that of the mesophilic enzymes at their own environmental temperature. (2) The thermostability of cold adapted enzymes is reduced significantly as reflected by the shift of their maximal activity

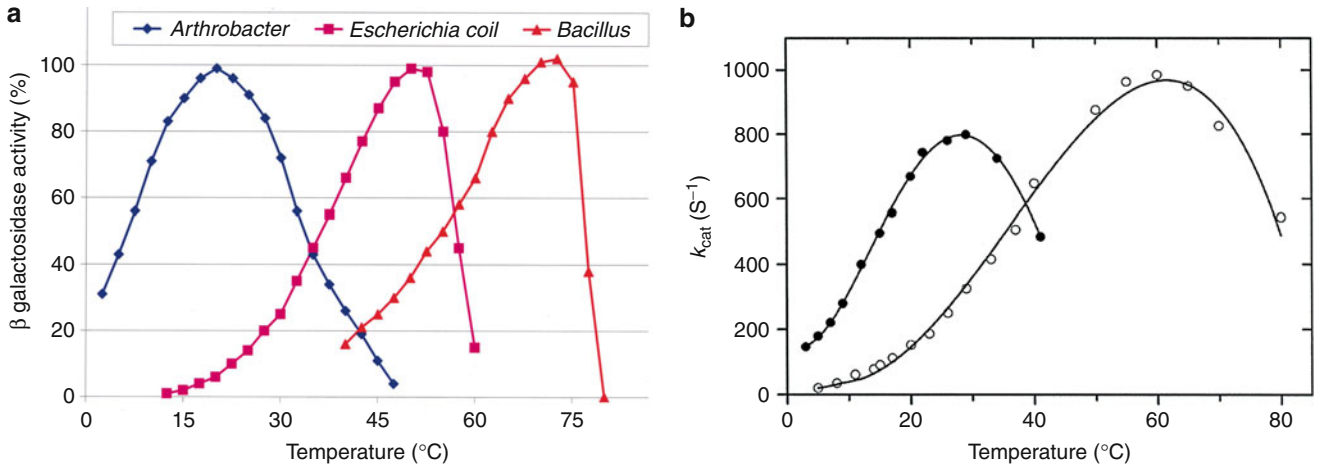


Fig. 18.13

Graphic comparison of the thermodependence of enzymes. (a) β-Galactosidase from the psychrophile *Arthrobacter* D2, the mesophile *E. coli*, and a thermophilic *Bacillus* (Adapted from Brenchley (1996)). (b) α-Amylase from a psychrophilic (●) and a mesophilic (○) organism D'Amico et al. (2002)

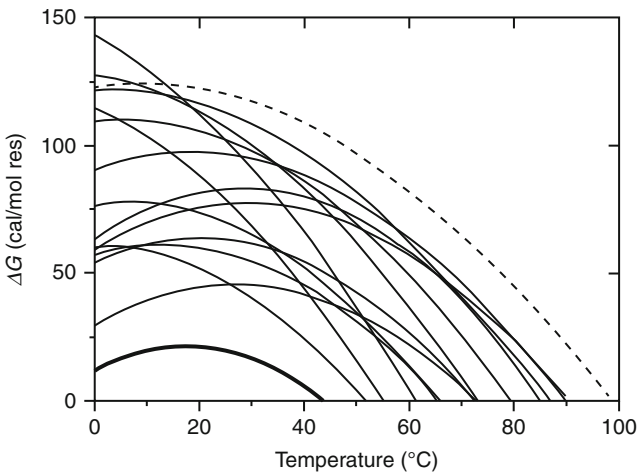


Fig. 18.14

Free enthalpy (Gibbs free energy (per mole of residue) of unfolding or conformational stability. Curves for a psychrophilic α-amylase (*heavy line*), several mesophilic proteins (*continuous lines*), and a thermophilic protein (*dashed line*) are indicated. Extrapolation of the curve below 0°C predicts cold unfolding at approximately −10°C (From Georlette et al. (2004))

toward low temperature and their inactivation by a slight increase of temperature. (3) Psychrophilic enzymes exhibit an increased flexibility of either a selected area (the enzymatically active site), the catalytic domain, or the overall protein structure (D'Amico et al. 2002). The concept of "localized increase in flexibility" (Feller and Gerday 2003) is confirmed by the observation that the thermal inactivation of psychrophilic enzymes often precedes structure unfolding (Collins et al. 2002).

The relationship between enzyme stability, flexibility, and activity has to be properly adjusted to the low temperature experienced by psychrotolerant or psychrophilic bacteria. Function of proteins requires an adequate balance between often opposing features such as structural rigidity and catalytic flexibility (Georlette et al. 2004). The cold adaptation of enzymes is mainly achieved through a reduction in the activation energy so that their activity becomes less temperature-dependent (D'Amico et al. 2003). More importantly, psychrophilic enzymes proceed with a low enthalpy change (Feller and Gerday 2003) due to a weakening or reduction of intramolecular interactions (*Fig. 18.14*). This in turn leads to an increase of local thermostability, for example, at the active site. Flexible active sites, however, are expected to bind their substrates weakly, and it was therefore suggested that many psychrophilic enzymes increase their reaction rate at the expense of K_m (Feller and Gerday 2003). Secreted enzymes required for nutrient scavenging are possible exceptions (Siddiqui and Cavicchioli 2006). K_m is influenced by the nature of the interaction of the enzyme with the substrate. An electrostatic interaction will be weakened by an increase in temperature, while hydrophobic interactions tend to be stabilized. Thus, the reaction velocity of enzymes will be differentially influenced by temperature, due to the relative contribution of electrostatic versus hydrophobic forces. In summary, several adaptations to low temperature are found in different enzymes from psychrophilic bacteria (*Table 18.7*). Notably, in no case have all of these adaptations been realized in one protein; each protein displays a couple of such changes which is sufficient to render the enzyme cold active.

Another aspect of psychrophilic proteins is that they tend to dissociate easier into nonfunctional monomers because of a weakening of hydrophobic bonds (Jahns and Kaltwasser 1993; Ramstein et al. 2003). An example is the H-NS-like protein from the psychrophilic *Psychrobacter*. The α-helical domain of this protein displays weaker intermolecular interactions, which may account for the low thermal stability at 37°C

Table 18.7
Adaptation of cold active enzymes in comparison to their mesophilic counterparts

More polar and less hydrophobic residues
Additional glycine residues and low arginine/lysine ratio
Fewer hydrogen bonds, aromatic interactions, and ion pairs
Lack of or fewer salt bridges
Additional or extended surface loop(s) with increased polar residues or decreased proline content (improves solvent interactions) or both
Modified α-helix dipole interactions
Reduced hydrophobic interactions between subunits
Weaker calcium binding

From Russell (2000) and Arnorsdottir et al. (2002)

(Tendeng et al. 2003). In *E. coli*, H-NS has two isoforms, HUα and the cold-inducible isoform HU*β*. HU*β* homodimers show weaker intermolecular interactions (Ramstein et al. 2003).

Extrinsic factors for cold adaptation of enzymes are the role of exopolysaccharides (section "Adaptations of the Membrane") and the cold-induced peptidyl-prolyl *cis-trans* isomerases with activity to trap and refold denatured proteins. This was observed in *Sphingopyxis alaskensis*, *Thermococcus* sp. and *Methanococcoides burtonii* (Ting et al. 2010; Ideno et al. 2001; Goodchild et al. 2004).

Differences Between Thermotypes of Archaea as Example

A comparative genomics approach between the two cold-adapted *Methanogenium frigidum* and *Methanococcoides burtonii* and other mesophile or (hyper-)thermophile archaea revealed trends in amino acid and tRNA composition and structural features of proteins, which are, to some extent, applicable to eubacteria (Saunders et al. 2003). A component analysis of cold-adapted enzymes revealed a higher content of noncharged polar amino acids, particularly glutamine and threonine, and a lower content of hydrophobic amino acids, especially leucine. This was confirmed by the investigation of proteins from psychrophilic or psychrotolerant bacteria that are also characterized by a higher content of noncharged polar amino acids (Feller and Gerday 2003). Consistent with increased protein flexibility, proline, arginine, and lysine residues were found to be reduced in *Psychrobacter arcticus* (Ayala-del-Rio et al. 2010). The charged residue arginine is generally thought to enhance protein thermostability by facilitating a greater number of electrostatic interactions (Siddiqui and Cavicchioli 2006), and a higher number of glycine residues provides localized chain mobility. As exemplified for archaeal proteins, the surfaces of cold-adapted proteins show a tendency to a higher proportion of hydrophobic residues at the expense of charged residues, resulting in a destabilization of the surface (Saunders et al. 2003).

Cold Adaptation of the Ribosomal Apparatus

Protein synthesis of mesophilic bacteria at low temperature requires a proper function of tRNA, and many posttranscriptional modifications of tRNA are known in cold-adapted microorganisms. The study of three psychrophilic bacteria from the genera *Moritella* and *Vibrio* revealed that, among other posttranscriptional modifications, these organisms contained 40–70% more dihydrouridine than mesophilic bacteria (Dalluge et al. 1997). Nuclear magnetic resonance studies showed that dihydrouridine leads to a higher local flexibility of RNA molecules (Dalluge et al. 1996). Apparently, the role of the elevated content of this modified nucleoside is to increase local conformational flexibility of tRNA under low temperature conditions where thermal motions and intermolecular interactions of biomolecules are compromised.

Cold-adapted microorganisms may therefore have structurally different ribosomes when compared to mesophilic bacteria. The comparison of mesophilic and psychrotolerant isolates from the *B. cereus* group showed a systematic difference in the structure of 16S rRNA (Lechner et al. 1998; von Stetten et al. 1998). Both signatures systematically contain A or T in psychrotolerant strains and G or C in mesophilic strains. It may therefore be speculated that the flexibility of the ribosome at low temperature is increased in some parts of the molecule in the psychrotolerant isolates. However, the occurrence of specific sequence motifs in psychrotolerant strains is not necessarily due to a positive selection pressure associated with this ribosome's function, but could be a consequence of neutral drift processes. Therefore, further analysis of the genomic DNA from a wide range of isolates was undertaken (B. Prüß and S. Scherer, unpublished data). This analysis showed that *B. cereus* group strains harbor between six and ten copies of 16S rDNA. Moreover, a number of these environmental strains have both rDNA operons with psychrotolerant signatures and rDNA operons with mesophilic signatures. The ability of these isolates to grow at low temperatures correlates with the prevalence of rDNA operons having psychrotolerant signatures, indicating specific nucleotides within the 16S rRNA play a role in psychrotolerance (*Fig. 18.15*). In vivo measurement of protein synthesis in a psychrotolerant *B. weihenstephanensis* and a mesophilic *B. cereus* clearly showed that ^{35}S-methionine incorporation at low temperature occurs faster by a factor of 4 (T. Kaplan and S. Scherer, unpublished data).

Metabolism and Growth

Metabolic Activity

If kept at temperatures below the minimal growth temperature, cells may die over time due to oxidative damage, radiation, or exhaustion of nutrients (Price 2009). Estuarine and marine *Vibrio* species seem to disappear from their habitat under low temperature conditions (e.g., below 15°C) but reappear with increasing temperatures. Such organisms enter a so-called viable

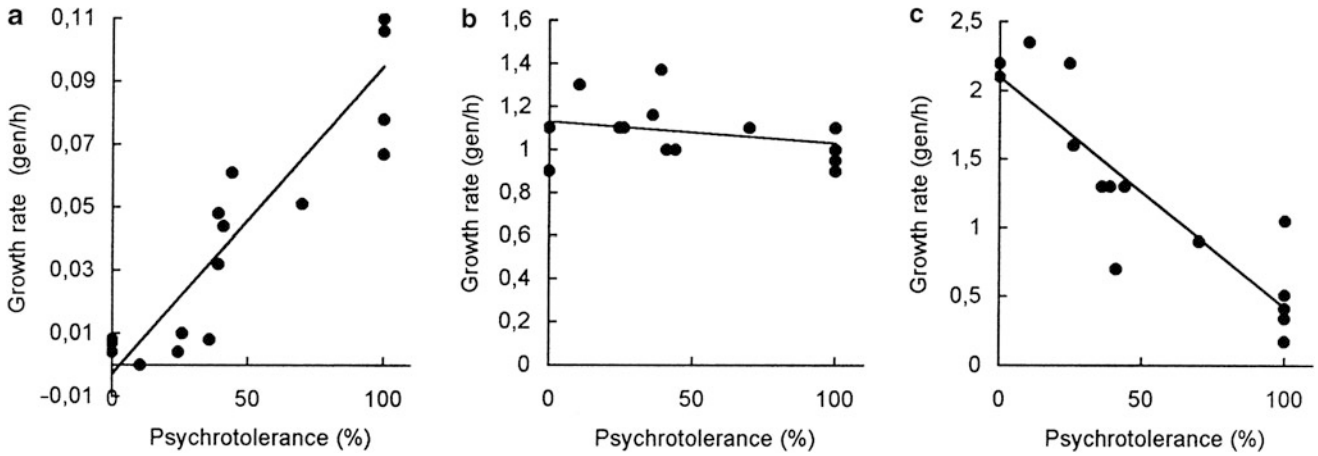


Fig. 18.15

Comparison of growth rates and psychrotolerance indices using *Bacillus cereus* strains. Cultures were grown at 10°C (a), 28°C (b), and 42°C (c). The growth rates are plotted against the percentage of psychrotolerant signatures. A psychrotolerance index of 50% means that one half of the operons of this strain carry the psychrotolerant signature and the other half the mesophilic signature (From Prüß et al. (1999))

but not culturable (VBNC) state. During the VBNC state, the formerly rod-shaped cells become coccoid and their metabolism is barely maintained. This dormant state might allow microbes to become resistant to temperatures below the permissive threshold and to survive with a minimal metabolic rate (Smith and Oliver 2006; Ponder et al. 2008). The lowest temperatures at which metabolically active bacterial communities exist have been reported to be −12°C to −17°C (Carpenter et al. 2000), and this was confirmed by detecting the incorporation of labeled thymidine and leucine into macromolecules at −15°C (Christner 2002).

Spore-forming, psychrotolerant bacteria isolated from Siberian permafrost did not grow or metabolize at temperatures below the freezing point. Gram-positive isolates from the same sample metabolized but did not grow, and one Gram-negative isolate grew at −10°C with a doubling time of 39 days, with a requirement of more energy that is used to maintain cell physiology below 4°C (Bakermans et al. 2003). Price and Sowers (2004) therefore categorized the rate of microbial metabolism to correspond to exponential growth with unlimited nutrients, to the maintenance of functions at nutrient levels too low for growth, and to survival of bacteria captured in ice, rock, or sediments. In the survival state, energy is used to repair macromolecular damages by cold, oxidants, or natural radioactivity (Amato et al. 2010).

The amount, quality, and availability of nutrients in the deep sea or soil can be unpredictable, requiring bacterial adaptations including sensing, uptake, and utilization of a broad spectrum of carbon, nitrogen, phosphorous, and energy sources (Kennedy et al. 2010; Amato and Christner 2009). The fact that low-temperature adaptation also requires nutrient sensing is reflected by many studies on psychrophilic bacteria reporting cold-induced expression of flagellar and chemotaxis genes (*Table 18.3*), as well as of regulators such as the cyclic-diGMP signaling second messenger involved in motility (Junge et al. 2003; Duchaud et al. 2007; Riley et al. 2008; Kawamoto et al. 2007).

Nutrient Uptake

At low temperature transport systems are required to cope with lower rates of diffusion and transport across the membrane and for the transport of compatible solutes (Nedwell 1999). In *Psychromonas ingrahamii*, 11 sets of tripartite ATP-independent periplasmic transporters (TRAP) were identified, exceeding in number those of *E. coli*, *S. oneidensis*, and *V. cholerae* (Riley et al. 2008). TRAPs are specialized in the transport of C_4-dicarboxylic organic acids; inactivation of a cold-induced substrate-binding TRAP subunit of *Psychrobacter arcticus*, DctT, resulted in a growth rate decrease in the presence of glutamate, butyrate, fumarate, and acetate (Bakermans et al. 2009). Acetate is assumed to be present under the water-logged conditions during tundra summer and to serve as a basis for energy metabolism (Ayala-del-Rio et al. 2010). In *Silicibacter pomeroyi* and *P. ingrahamii*, several ABC-type transporters for peptides and (branched-chain) amino acids were identified, but only few for sugars (Moran et al. 2004; Riley et al. 2008), suggesting that proteins are an important carbon source for these bacteria.

On the basis of the different nitrate and ammonium uptake responses to temperature, dependency on ammonium as an inorganic nitrogen source was suggested to increase at low temperatures (Ray et al. 1999). Indeed, four transporters for ammonium and one for urea are present in *S. pomeroyi*, while genes for the assimilation of nitrate and nitrite are missing (Moran et al. 2004). The $^{14}CH_3NH_3^+$ uptake activity of a psychrophilic marine bacterium *Vibrio* sp. was found to be

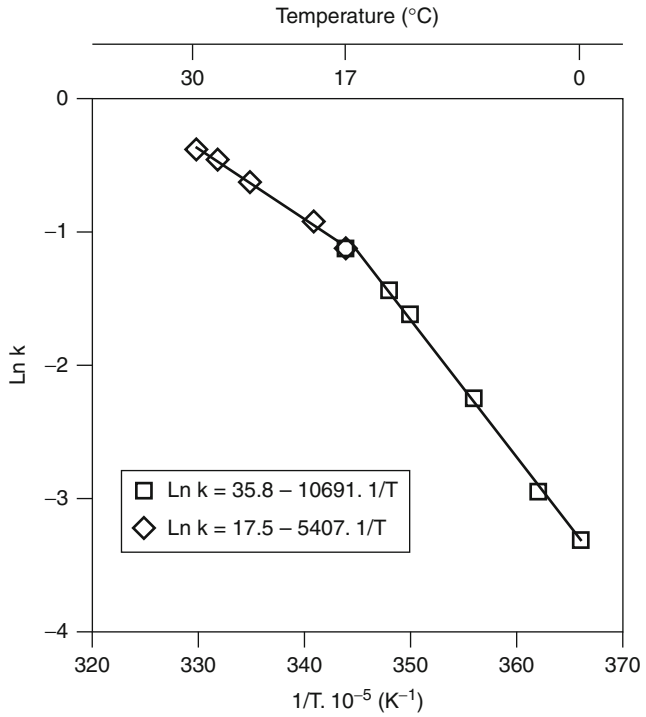


Fig. 18.16
Biphasic Arrhenius plot of the growth rate of *Pseudomonas fluorescens* (From Guillou and Guespin-Michel (1996))

higher at low temperatures, and the apparent K_m value for the uptake of $^{14}CH_3NH_3^+$ did not change significantly over the temperature range 0–25°C (Chou et al. 1999). Assessment of the temperature dependency of this system demonstrated its unusual psychrophilic properties (Fig. 18.16). Another metabolic adaptation has recently been reported for *Shewanella* spp. that recruited Na^+ –dependent nutrient transporters to use the high Na^+ content as an energy source (Zhao et al. 2010). Iron-uptake systems such as transporters for ferric iron, a TonB-dependent receptor for iron and a bacterioferritin iron storage protein in marine bacteria are often found to be up regulated in cold-adapted bacteria (Bakermans et al. 2007; Ting et al. 2010), possibly pointing to the requirement of iron-dependent enzymes such as desaturases.

Central Metabolism

Cold stress modulates the carbon flow of a given organism, either by increasing cold sensitive key enzymes necessary for certain metabolic pathways or by switching to alternative pathways or cold-adapted isoenzymes. For example, cold stress induces a switch from respiratory metabolism to anaerobic lactate formation in psychrotolerant *Rhizobium* strains (Sardesai and Babu 2000), and free fatty acids are utilized for an efficient energy production as an adaptation of *Sphingopyxis alaskensis* to the cold (Ting et al. 2010). Temperature-specific carbon source utilization was reported for psychroactive bacteria isolated from Siberian permafrost (Ponder et al. 2005). The result of this resource efficiency response is rapidly generated energy to overcome the stress (Bergholz et al. 2009).

Two proteins from the glyoxylate cycle, malate dehydrogenase and isocitrate lyase, are up regulated in *Psychrobacter cryohalolentis* at low temperature, indicating an increased need of carbohydrates or intermediates (Bakermans et al. 2007). To cope with cold, psychrophilic *Colwellia maris* expresses a thermolabile isocitrate lyase that is able to utilize its substrate at lower temperatures because of a lower temperature optimum. The same organism has two isocitrate dehydrogenase isoenzymes: one with mesophilic and the other with psychrophilic characteristics (Ochiai et al. 1979). Accordingly, both the tricarboxylic acid and glyoxylate cycles are important for growth in cold (Watanabe et al. 2002).

The inability of many psychrophiles to use glucose as a carbon source is reflected by a lack of PEP-dependent phosphotransferase systems (PTS) in the Antarctic bacteria *Pseudoalteromonas haloplanktis* and *P. arcticus* (Medigue et al. 2005; Ayala-del-Rio et al. 2010; Duchaud et al. 2007). *P. haloplanktis* possesses a pyruvate kinase with homology to the cold-adapted PykA of *E. coli*, and gluconate is its preferred carbon source providing pyruvate via the Entner-Doudoroff pathway. *P. ingrahamii* also has a restricted capability of utilizing carbohydrates but metabolizes macromolecules for nutrition and energy. It encodes 48 peptidases, again demonstrating the role of amino acids as central carbon sources (Riley et al. 2008). Similar findings were reported for the fish pathogen *Flavobacterium psychrophilum* (section "Ecology of Human Pathogens in Cold Habitats") that does not use carbohydrates, but degrades lipids to fatty acids and is rich in peptidases, too (Duchaud et al. 2007). Interestingly, this is in contrast to the archaeon *Methanococcoides burtonii* that lacks peptide transporters, corresponding with its inability to grow on peptides; nitrate reductase and nitrate transporters were also not identified in this organism (Allen et al. 2009).

Genes for ammonium and urea assimilation are present in many psychrophilic bacteria including crenarchaea (Moran et al. 2004; Konstantinidis et al. 2009). The ammonia incorporation pathway is induced in *P. arcticus* (Bergholz et al. 2009), while arginine and *N*-acetyl-glucosamine, ubiquitous substrates in marine environments, are used by *P. haloplanktis* as nitrogen and carbon source (Medigue et al. 2005).

Transcriptomic analysis revealed an up regulation of genes for the biosynthesis of proline, methionine, histidine, and tryptophan in *P. arcticus*, while those for the synthesis of branched-chain amino acids (BCAA), arginine, and lysine were down regulated at low temperature (Bergholz et al. 2009). In *Sphingopyxis alaskensis*, the genes for histidine and proline biosynthesis were found to be induced under similar conditions (Ting et al. 2010). The adaptation mechanisms of this marine bacterium are summarized in Fig. 18.17. Possible reasons for a specific requirement of a limited number of certain

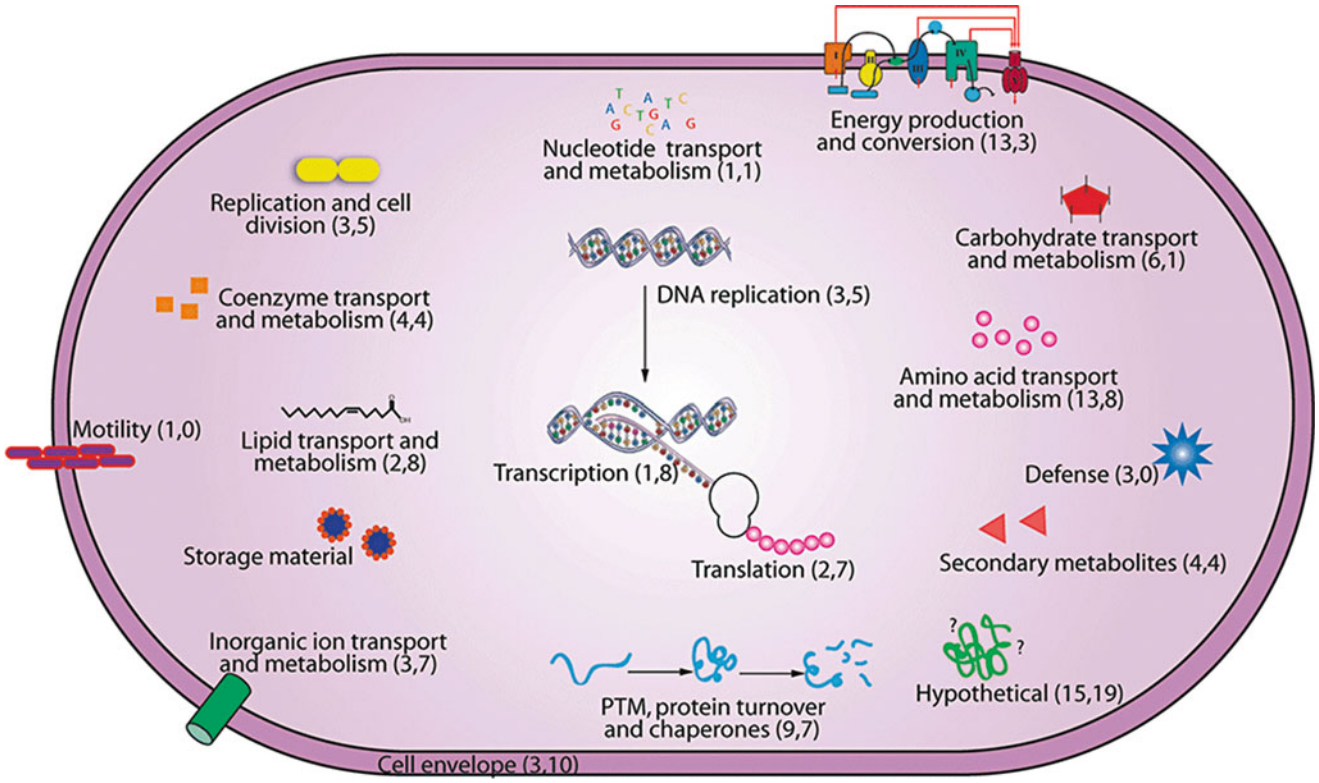


Fig. 18.17

Summary of the cellular processes important for adaptation of *S. alaskensis* at low temperature. The number in parentheses represent the number of proteins with significant increases in abundance at 10°C versus 30°C, followed by the number of proteins with significant increases in abundance at 30°C versus 10°C (From Ting et al. (2010))

amino acids may be (1) flexibility of proteins (section "Adaptation of Proteins and Ribosomes to Low Temperature"), (2) regulation of the concentration of intracellular solutes (Feller 2003), or (3) thermal compositional biases of proteins (Allen et al. 2009).

Growth Rates

As a result of their metabolic adaptations, psychrophiles have a higher growth rate at low temperature and a lower minimal growth temperature in comparison to mesophiles. Among other factors, the minimal growth temperature is determined by the freezing temperature of the cytosol. Most cells remain unfrozen at −10°C to −15°C because of the physical properties of the aqueous solvent systems inside and outside the cells (see Russell 1990). The maximal specific growth rate of a psychrotolerant *P. fluorescens* with respect to temperature was studied, yielding an Arrhenius plot with a drastic change in slope at 17°C. Over the cold domain (0–17°C), the temperature characteristic was twofold higher than over the suboptimal temperature domain (17–30°C; Guillou and Guespin-Michel 1996). The authors suggested that protein degradation is inhibited below 17°C. This influence of low temperature on protein turnover has also been reported for a psychrotolerant *Arthrobacter globiformis* (Potier et al. 1990) and could be an explanation for the higher temperature characteristic of the Arrhenius plot in the low-temperature range. A biphasic behavior of the growth rate Arrhenius plot was also reported for a *Pseudomonas putida* strain (Chablain et al. 1997).

Table 18.8 lists selected doubling times at low temperatures. Monitoring incorporation of ^{14}C-labeled acetate into lipids, the doubling times of a bacterial population from Siberian permafrost was shown to range from 1 day at 5°C to 160 days at −20°C (Rivkina et al. 2000) (*Fig. 18.18*). For an individual bacterium, subzero metabolic activity was first reported for *P. ingrahamii* with a generation time of 240 h at −12°C (Breezee et al. 2004). Four plots of growth rate versus temperature (*Fig. 18.19*) demonstrate very different growth rates for psychrotolerant and psychrophilic strains. The terms psychrophilic and psychrotolerant are defined here by the growth ranges and do not reflect growth rates. For instance, a psychrotolerant *Pseudomonas* has a doubling time of 3 h at 10°C, while a psychrophilic *Desulfotalea* species grows with a doubling time of 27 h at the same temperature. Beside other factors, the growth rate depends on the substrate used in the experiment. Some bacteria such as *Methanogenium* or *Desulfofrigus* are notoriously slow-growing organisms, irrespective of the growth temperature.

Table 18.8
Selected doubling times of cold-adapted bacteria

Species	TT	Temperature (°C)	dt	References
Psychromonas ingrahamii	Pp	−12	10 days	Breezee et al. (2004)
Psychrobacter sp. Str.1	Pt	−10	39 days	Bakermans et al. (2003)
Frigoribacterium aff. *faeni*	Pt	−10	294 days	Bakermans et al. (2003)
Rhodococcus sp.	Pt	−10	370 days	Bakermans et al. (2003)
Bacillus psychrophilus	Pp	−5	7 h	Morita (1975)
Bacillus sp.	Pp	−2	48 h	Inniss (1975)
Pseudomonas fluorescens	Pt	0	28 h	Guillou and Guespin-Michel (1996)
Methanogenium frigidum	Pp	0	42 days	Franzmann et al. (1997)
Yersinia enterocolitica	Pt	0	27 h	Neuhaus (2000)
Carnobacterium funditum	Pt	1	19 h	Franzmann et al. (1991)
Vibrio marinus	Pp	3	4 h	Morita and Albright (1965)
Leuconostoc mesenteroides	Pt	4	24 h	Hamasaki et al. (2003)
Leuconostoc citreum	Pt	4	52 h	Hamasaki et al. (2003)
Bacillus sp.	Nd	5	8 h	Brenchley (1996)
Psychromonas antarcticus	Pp	5	36 h	Mountfort et al. (1998)
Rhodoferax antarcticus	Pp	5	60 h	Madigan et al. (2000)
Pseudomonas sp.	Pt	10	3 h	Morita (1975)
Clostridium gasigenes	Pp	10	9 h	Broda et al. (2000b)
Bacillus weihenstephanensis	Pt	10	11 h	B. Prüß and S. Scherer (unpublished data)
Desulfotalea psychrophila	Pp	10	27 h	Knoblauch et al. (1999)
Desulfofrigus fragile	Pp	10	169 h	Knoblauch et al. (1999)
Bacillus cereus	Mp	10	90 h	B. Prüß and S. Scherer (unpublished data)
Methanogenium frigidum	Pp	15	5 days	Franzmann et al. (1997)
Yersinia enterocolitica	Pt	15	4 h	Neuhaus (2000)

TT thermal type, *Pp* psychrophilic, *Pt* psychrotolerant, *Mp* mesophilic, *Nd* not determined, *dt* doubling time in hours (h) or days (d)

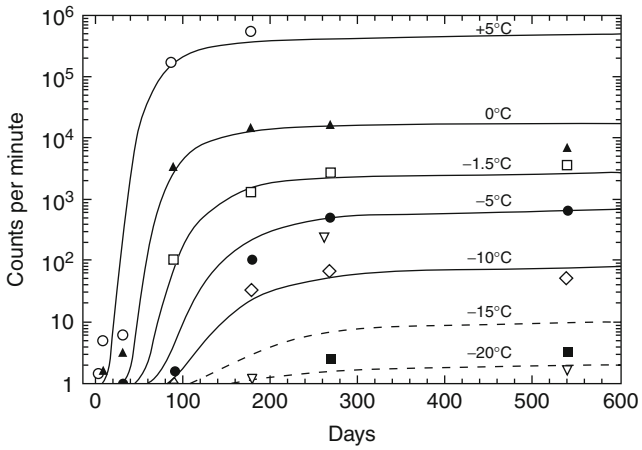


Fig. 18.18
Incorporation of ^{14}C-labeled acetate by the native bacterial population in Siberian permafrost over a 550-day period (From Rivkina et al. (2000))

The Cold Shock Response

As described above, life at low temperature requires a set of long-term adaptations involving membrane composition, protein activity, and metabolism. If microorganisms are exposed to a sudden drop in temperature, they respond to this stress with a specific pattern of transient gene expression termed cold shock response, which precedes long-term adaptation (Weber and Marahiel 2003; *Fig. 18.20*). It is worth of note that the literature on this response is based nearly exclusively on observations made in cultures growing exponentially in a liquid broth. Comparison of cold-shocked gel-entrapped *E. coli* cells to free-floating cells revealed significant differences in the protein response (Perrot et al. 2001). What one defines as a specific cold shock stimulon therefore depends on the experimental procedures. To our knowledge, virtually no report deals with the cold shock response of stationary phase cells, despite the fact that many bacteria spend most of their lifetime in the stationary phase (Kjelleberg 1993). Other stresses can mimic a cold shock

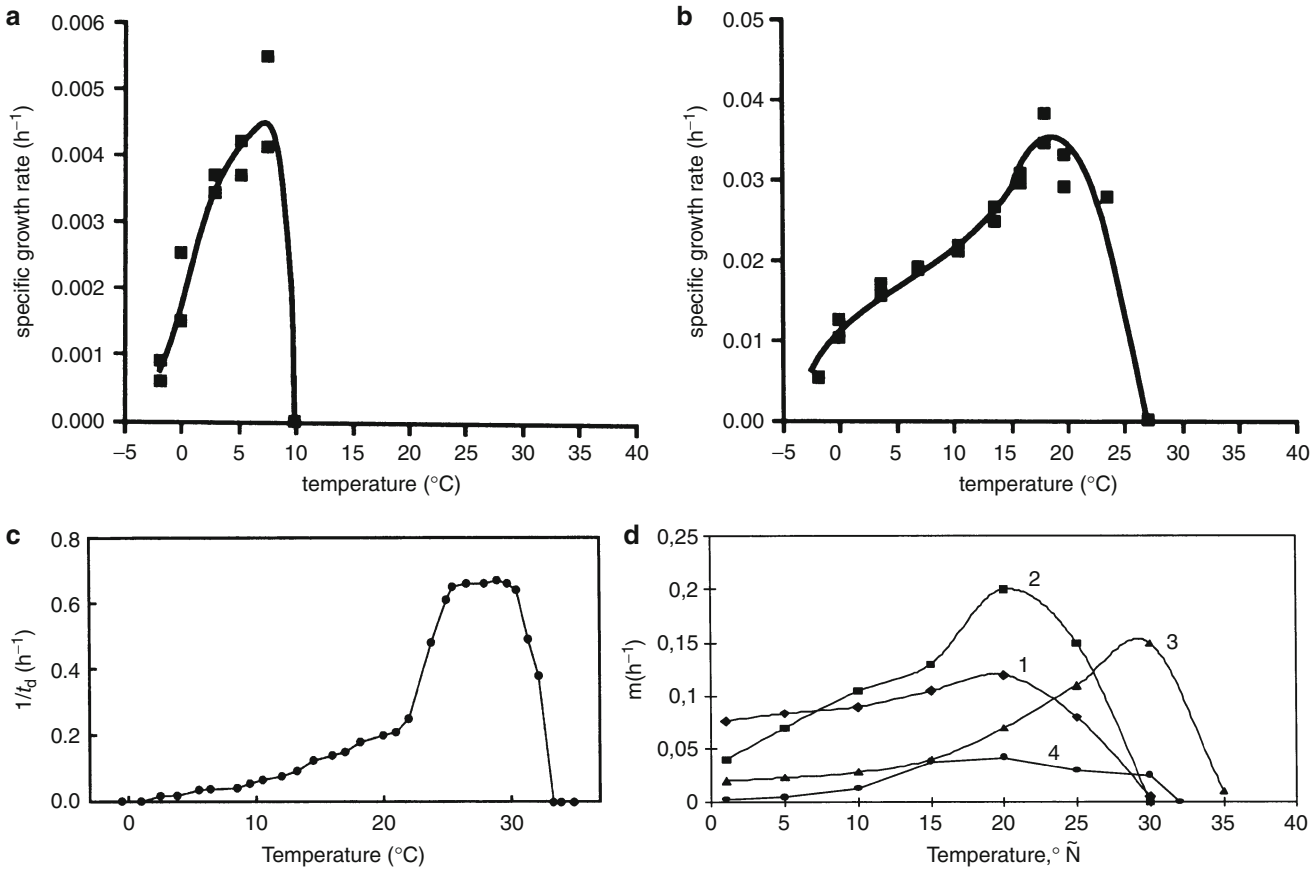


Fig. 18.19

Thermodependence of growth rates in psychrophilic and psychrotolerant species of bacteria. (a) *Desulfofaba* sp. is an extreme psychrophile, (b) *Desulfofrigus* sp. is a moderate psychrophile, and (c) *Clostridium algidixylanolyticum* is a typical psychrotolerant. (d) Different species of *Acetobacterium* are compared. (1) *A. bakii* (psychrotolerant, from pond sediments), (2) *A. paludosum* (psychrotolerant, from fen), (3) *A. fimetarium* (mesophile, from manure), and (4) *A. tundrae* (psychrophile, from tundra) (From Broda et al. (2000), Knoblauch and Jørgensen (1999), and Nozhevnikova et al. (2001b))

response or at least induce the cold shock stimulon to some extent (Wick and Egli 2004). The hypothesis that every event stopping or stalling the ribosomes leads to an induction of the cold shock response was supported by the finding that many cold-inducible genes are also expressed under non-cold-shock conditions (Walker et al. 2004). This is most obvious for the cold shock response itself (Gualerzi et al. 2003), the use of certain antibiotics affecting the translational speed (VanBogelen and Neidhardt 1990), or the provision of nutrients (Brandi et al. 1999).

Shifting a culture of *E. coli* from 37°C to 8°C or below resulted in polysomal run-off and accumulation of free ribosomes (Xia et al. 2002). The most severe problem following sudden temperature decline is therefore the initiation and translation of bulk mRNA in the cold, resulting in a new equilibrium of the cellular components with an adapted pattern of protein concentration (Broeze et al. 1978; Jones and Inouye 1994; Horn et al. 2007).

Major Cold Shock Proteins: CspA–CspI

Cold shock proteins (CSPs) are a subset of cold-inducible proteins (CIPs). They are small and mostly acidic proteins that have been identified in most free-living bacteria including the hyperthermophilic phyla Aquificales and Thermotogota, but neither in archaea nor in the human pathogens *Helicobacter pylori*, *Campylobacter jejuni*, or *Mycoplasma genitalium* (Francis and Stewart 1997; Wouters et al. 2000b). CSPs display homology to CspA, a 7.4-kDa cytoplasmic protein first discovered in *E. coli* (Jones et al. 1987) (reviewed in Ermolenko and Makhatadze (2002)). Since CspA and its homologs show the highest induction level after a downshift from 37°C to 10°C, these proteins are often termed "major cold shock proteins" (MCSPs). Besides CspA, *E. coli* contains a family of eight highly similar CSPs, termed CspB–CspI. The three-dimensional structure of the CspA-homolog CspB of *B. subtilis* is shown in *Fig. 18.21*. All these different CspA homologs are believed

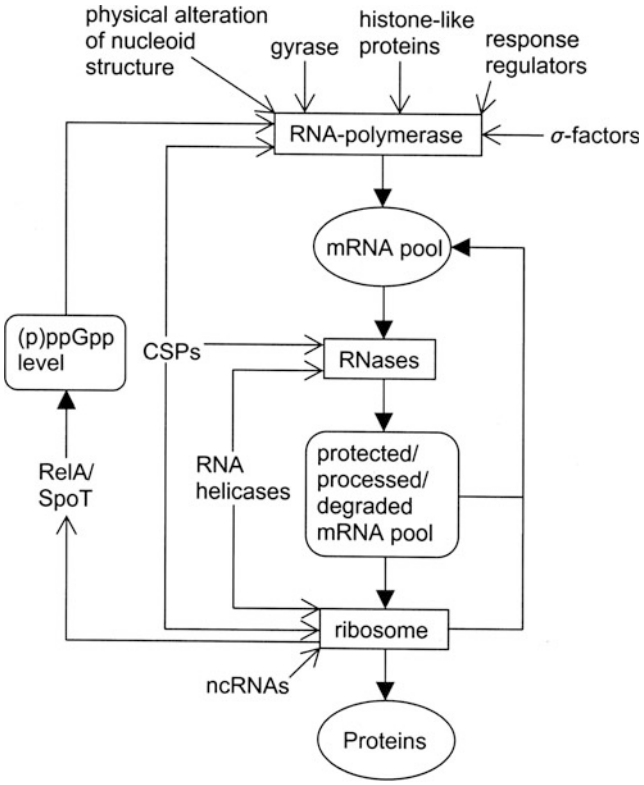


Fig. 18.20
Regulation of bacterial cold shock responses as a multiple filter model. The *arrows* indicate the flow of genetic information from DNA (*via* RNA polymerase, *top*) to protein (*bottom*) or the production of effector molecules. The T-*arrows* have to be read as "modulates activity of." The filter systems are *boxed; circles* represent their substrates or products (From Weber and Marahiel (2003))

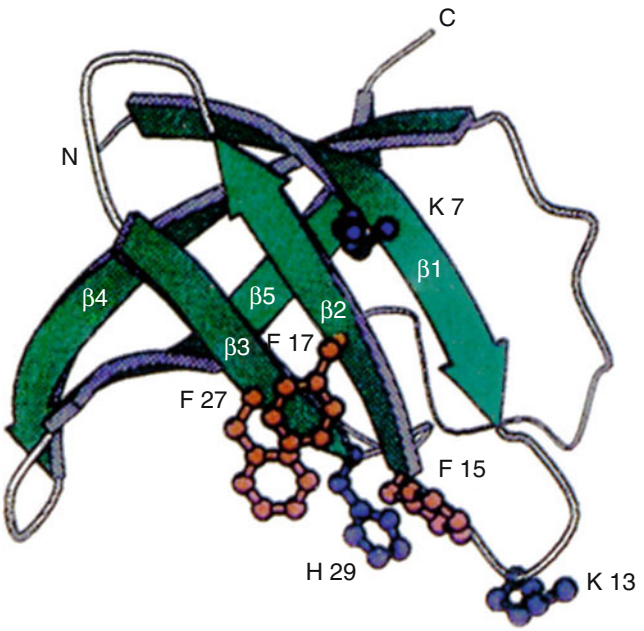


Fig. 18.21
Three-dimensional structure of CspB from *B. subtilis* features five antiparallel *β*-sheets that form a barrel (From Graumann and Marahiel (1996))

to be stress adaptation proteins for different tasks, although the cold-inducible CSPs can replace each other to some extent (Graumann et al. 1997; Yamanaka et al. 1998). A quadruple deletion mutant missing CspA, CspB, CspE, and CspG was cold sensitive and formed filamentous cells at 15°C. This phenotype was suppressed by overexpression of each member of the cold shock protein family except CspD, which caused lethality when present in a higher copy number (Xia et al. 2001b; Phadtare and Inouye 2004). Synthesis of CspA is increased 30-fold under certain conditions, and the MCPs of *E. coli* (CspA, B, G, and I) account for up to 13% of the total protein synthesis (Goldstein et al. 1990; Lee et al. 1994).

Low temperature increases the tendency of mRNAs to misfold with respect to efficient translation. *E. coli* CspA and *B. subtilis* CspB are considered to act as mRNA chaperons opening the secondary structures of mRNAs that otherwise block the initiation of translation (Jiang et al. 1997; Hunger et al. 2006); see *Fig. 18.22*. This post-transcriptional mechanism was extended to a model in which CSPs couple transcription and translation.

Other Cold-Inducible Proteins

CspA induces and is part of the cold stimulon that directly or indirectly regulates 30 proteins such as H-NS or GyrA (Madan Babu and Teichmann 2003; Martínez-Antonio and Collado-Vides 2003). GyrA is part of topoisomerase II (Maxwell and Howells 1999). The promoters of those secondary CSPs contain one or more of the so-called Y-box motifs CCAAT. Recognized by CspA, this Y-box motif subsequently activates transcription of the genes encoding H-NS, GyrA, and possibly other proteins (Qoronfleh et al. 1992; Jones et al. 1992b). The enhanced level of GyrA, together with that of H-NS and Hu*β*, increases the negative supercoiling of plasmids and chromosomal DNA (Giangrossi et al. 2002). The DNA twisting itself regulates the induction of *recA*, which encodes another CSP of *E. coli* (Wang and Syvanen 1992). However, the increase in negative supercoiling was found to be transient after cold shock (*Fig. 18.23*). This shows that the open complex formation of transcription turned cold insensitive after the adaptation of the entire system to low temperature (Krispin and Allmansberger 1995; Lopez-Garcia and Forterre 1999). In addition, most promoters of the "–10/–35 type" are not active below 15°C in vitro, and this may in turn prevent protein synthesis for vital proteins below a certain threshold temperature (Minakhin and Severinov 2003).

Antitermination, which is mediated by CspA and other cold shock–induced CSPs, was proposed to induce the genes of secondary cold-induced proteins (such as NusA, InfB, RbfA, and Pnp) located in the region of the *metY-rpsO* operon. These CSPs probably prevent secondary structure formation in the

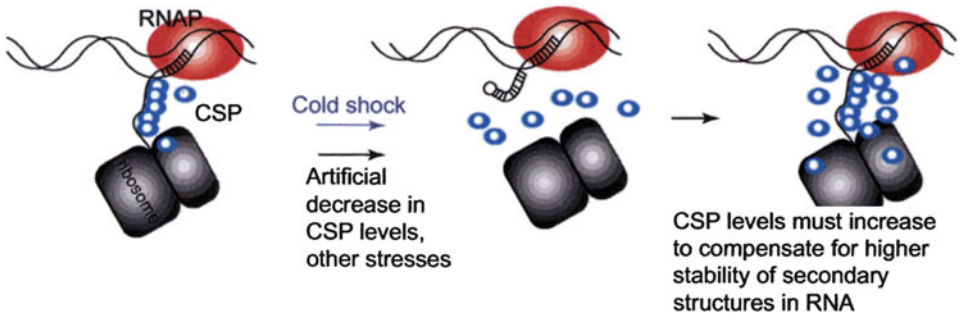


Fig. 18.22
Model for the function of cold shock proteins (CSPs) as RNA chaperones that couple transcription and translation of mRNA. During growth at 37°C, CSPs bind to mRNA as it protrudes from the RNA-polymerase complex (RNAP) and stabilizes the RNA in a linear form. The ribosome then displaces CSPs, which have only low affinity for RNA, and initiates translation. Accordingly, an artificial decrease in the CSP concentration would lead to the formation of secondary structure in RNA and prevent translation. After cold shock or other stresses such as carbon starvation, an increase in the CSP concentration is needed to counterbalance the increased stability of RNA secondary structure (Redrawn from Graumann and Marahiel (1998))

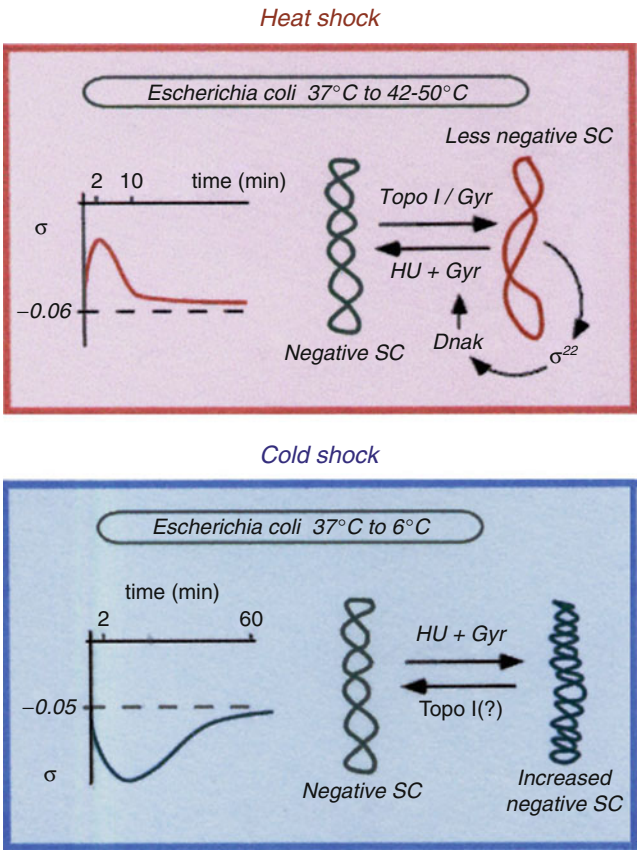


Fig. 18.23
Effects of heat shock and cold shock on plasmid DNA. The *left* side of each panel shows the variation in the plasmid-specific linking difference ($\sigma = \Delta Lk/Lk_o$), dependent on the time of exposure to the shock temperature. *SC* supercoiling, *Topo* topoisomerase, *Gyr* gyrase, and HU, a small and basic heat-stable DNA-binding protein (From Lopez-Garcia and Forterre (1999))

nascent RNA, which causes antitermination in ρ-independent terminator regions. The read-through produces a higher transcript level, which in turn increases the translation of such proteins (Bae et al. 2000; Zangrossi et al. 2000). *nusA* is an essential gene, and NusA governs transcriptional elongation, pausing, termination, and antitermination. The core RNA polymerase associates with the sigma factor A (SigA) to form the holoenzyme that is capable of promoter recognition. As the polymerase complex enters the transcriptional elongation phase, NusA replaces SigA in the complex (Gopal et al. 2001). RbfA associates with the 30S subunit of the ribosome, enabling 16S rRNA maturation and interaction with mRNA (Xia et al. 2003).

Lists of cold stress–induced proteins in *B. subtilis* and *E. coli* have been published (Graumann and Marahiel 1999; Gualerzi et al. 2003). The list of cold shock–induced genes has been extended recently by a genome-wide transcriptional analysis of cold-shocked *E. coli* cells. New factors found by this study are responsible for the transport or metabolism of diverse sugars and molecular chaperones (*mopA*, *mopB*, *htpG*, and *ppiA* (Phadtare and Inouye 2004)).

Regulation of MCSPs

Transcriptional and posttranscriptional regulation of cold shock genes, including the MCSPs, was reviewed in detail by (Gualerzi et al. 2003), and an overview of some aspects is given in *Fig. 18.24*. Most cold-inducible MCSPs have an unusually long mRNA leader region of 156–256 bp upstream of the translational start. The major transcription start +1 of *cspA* is located 159 bp upstream from the translational starting point. The promoter seems to be σ-70 dependent, since the −35 region (TTGCAT) and the −10 region (CTTAAT) are found to be similar to a σ-70 consensus sequence (TTGACA for the −35 and TATAAT for the −10) (Qoronfleh et al. 1992; Tanabe et al. 1992). The 5′ end of the *cspA* mRNA contains a regulatory

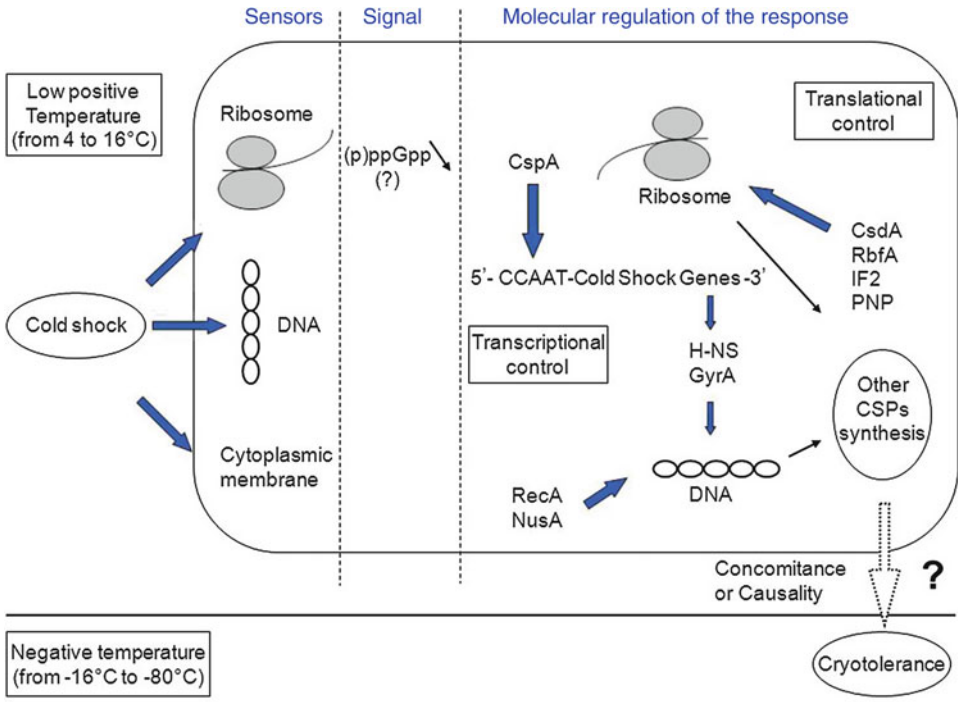


Fig. 18.24
Cold shock responses (From Panoff et al. (1998))

sequence (cold box), which stabilizes the mRNA at low temperature and enables cold shock induction (Xia et al. 2002). The consensus cold box sequence (5′ UGACGUACAGA) is found in *cspA*, *cspB*, and *csdA* (Jiang et al. 1996a).

However, if the 5′ end of *cspA* is overproduced, the expression of cold shock genes is no longer transient, and the synthesis of bulk proteins is impaired (Jiang et al. 1996b; Xia et al. 2002). Also, the cessation of regrowth after cold shock is prolonged. This fits well with the observation that *cspA* mRNA in excess is poisonous to the cell (Etchegaray and Inouye 1999; Xia et al. 2001a). This robust translatability of *cspA* mRNA depends on initiation, and the ribosome appears to be preadapted to translate *cspA* mRNA. Since overproduction of CspA together with the overproduction of the 5′-end restores the normal cold shock response, CspA itself probably interacts with the cold box (Jiang et al. 1996a). Furthermore, Giuliodori et al. (2004) could demonstrate the preferential translation of cold shock mRNAs after cold shock in vitro. The ribosomes are adapted to display translational selectivity for MCSP mRNAs. The *trans*-acting factors involved in ribosomal adaptation are (1) CspA itself, which increases translatability of mRNA in the cold, and (2) the cold shock–induced stoichiometric imbalance between the initiation factors IF1, IF2, and IF3, and the ribosomes. Possible *cis*-acting elements discussed by the same authors are the secondary or tertiary structures of the unusual long 5′ leader sequences of MCSP mRNAs.

In addition to CspA-mediated autoregulation, CspE revealed as a repressor for *cspA*. CspE is abundantly produced at 37°C, and *cspA* is derepressed in a *cspE* mutant (Fang et al. 1998). In vitro, CspE and CspA cause transcriptional pausing just behind the cold box of *cspA*, and CspA production is inhibited by addition of CspE to the translating ribosomes (Bae et al. 1999; Phadtare and Inouye 1999).

Cold Shock and mRNA Degradation

Adaptation to low temperature after cold shock includes the establishment of a new equilibrium of the transcriptome. This is obtained not only by transcriptional changes but also by modified activity of the mRNA degradosome (Prud'homme-Genereux et al. 2004). Given that the machinery for mRNA degradation becomes inefficient upon sudden temperature drop, the induction of a new decay pathway is required to prevent unnecessary mRNA accumulation upon cold adaptation (Gualerzi et al. 2003). RNases possibly involved here include the polynucleotide phosphorylase (PNPase) and RNase H, which are cold shock induced, and RNase II or RNase E, which are not (Clarke and Dowds 1994; Cairrão et al. 2003). Bacteria without PNPase are cold sensitive (Luttinger et al. 1996; Goverde et al. 1998). The cold-temperature induction of PNPase in *E. coli* occurs by reversal of its autoregulation. At 37°C, ribonuclease III cleaves the leader of the *pnp* mRNA, whereupon PNPase represses its own translation via an unknown mechanism. This latter step is inhibited after cold shock (Beran and Simons 2001; Mathy et al. 2001). It is interesting to mention here that

cold-inducible RNases (PNPase and RNase H) were found to be important for full virulence of *Shigella*, enteroinvasive *E. coli*, and *S. enterica* (Cairrão et al. 2003; Ygberg et al. 2006).

The induction of CspA expression is mainly due to an increase in mRNA stability. The half-life of the *cspA* mRNA is 12 s at 37°C but between 15 and 30 min at 15°C in *E. coli* (Jiang et al. 1993; Fang et al. 1997). If the coding region of *cspA* is fused to the constitutive *lpp* promoter, it is still cold inducible. This observation is explained by a strong vulnerability of the transcript to RNase E degradation at 37°C. As in *E. coli*, the transcripts of *cspB* and *cspC* in *B. subtilis* are also dramatically stabilized having a half-life of 1 min at 37°C and more than 30 min at 15°C (Kaan et al. 1999). A similar observation was made in *Rhodobacter capsulatus* with a *cspA* transcript half-life of around 4 min at 32°C and 47 min at 10°C (Jäger et al. 2004).

Down regulation of MCSP mRNA is an important step, at least in enterobacteria, before growth can resume. This phenomenon is mainly due to the exceptionally strong ability of MCSP mRNAs to initiate at the ribosome. Therefore, MCSP mRNA outcompetes bulk mRNA and thus prevents growth (Neuhaus et al. 2000; Xia et al. 2001a). In the above-mentioned PNPase-deficient strains, the decay of *cspA* mRNA is delayed, subsequently preventing regrowth (Neuhaus et al. 2000). In *Yersinia enterocolitica*, the *cspA* tandem mRNA is cleaved at multiple specific cut sites, with an AGUAAA consensus (termed "cold shock cut box") to downregulate the MCSP mRNA. After these initial cleaving steps, the fragments are removed rapidly and growth can resume (Neuhaus et al. 2003). Cleavage of the *cspA* transcript within the coding sequence and subsequent rapid removal of the fragments was also found in *Rhodobacter capsulatus* (a member of the α-proteobacteria), but no consensus cut sequence could be detected (Jäger et al. 2004). CspE was found to interfere with both the PNPase and RNase E of the degradosome machinery, inhibiting internal cleavage and removal of the poly(A) tails from mRNAs, thus stabilizing particular mRNAs (Feng et al. 2001).

Protein Synthesis and the Cold Shock Response

The discovery by (Broeze et al. 1978) that the initiation of mRNA transcription is impaired at low temperature indicated that the ribosome is a target of cold shock (Hurme and Rhen 1998; Perrot et al. 2000). Acting as an RNA chaperone, CspA facilitates initiation and elongation of translation after cold shock (Jiang et al. 1997). A ribosomal protein S21 homolog, which is encoded by *rpsU*, is cold induced in *Sinorhizobium meliloti* (O'Connell and Thomashow 2000) as well as in the cyanobacterium *Anabaena variabilis* (Sato 1994). This protein may facilitate the binding of mRNA to the ribosome. Interestingly, *rpsU* is located downstream of *cspA* in *S. meliloti*. It may thus support the ribosome to function at low temperatures in the same way as other CSPs such as RbfA (Jones et al. 1996). Possibly, small CSPs (e.g., CspA), which appear to be synthesized continuously in some organisms (Graumann et al. 1997; Yamanaka et al. 1999a), may help transform the ribosomes into a cold-insensitive state. Colocalization of CspB and ribosomal protein L1 was demonstrated for *B. subtilis* (Mascarenhas et al. 2001).

The level of inactive ribosomes determines the extent of the cold shock response. Once a balanced translational capacity is achieved, the cold shock response is repressed. At least four proteins (RbfA, IF2, CsdA/DeaD, and pY/Yfia) have been proposed as mediators of the ribosome's transformation into a cold-insensitive state (Jones et al. 1996). The ribosomal binding factor A (RbfA) was found to be a suppressor of a cold-sensitive mutation in the 16 S rRNA. Cells lacking RbfA exhibit a cold-sensitive phenotype because the 16 S RNA is not processed properly (Dammel and Noller 1995; Bylund et al. 1998). CsdA of *E. coli* and CshA/CshB of *B. subtilis* belongs to the DEAD-box helicases and possesses RNA unwinding activity, thus relieving RNA secondary structures (Hunger et al. 2006). A CsdA mutant is impaired in growth at low temperatures and has the cold-sensitive phenotype of elongated cells (Jones et al. 1996), and can be complemented by another helicase, RhlE, CspA, and the cold-inducible RNaseR (Awano et al. 2010). IF2 is needed for initiation of mRNA translation at the ribosome (Moreno et al. 2000). Ribosomes of cold-shocked *E. coli* were shown to be associated with a protein called "PY" or "Yfia" (Rak et al. 2002). However, this protein apparently disappears when the growth arrest is resolved (Kalinin et al. 2002). PY blocks the P as well as the A site of the ribosome, inhibiting translation initiation during cold shock but not under normal growth conditions. By blocking the translation of all but CSPs, the cell diverts all translation factors to the synthesis of CSPs, thus ensuring survival in the cold (Vila-Sanjurjo et al. 2004). This finding might explain the initiation inhibition after cold shock, firstly observed by Broeze et al. (1978).

O'Connell et al. (2000) found that the transcription of all three *rrn* operons of *Sinorhizobium meliloti* is induced by cold shock. Since the number of ribosomes is usually positively correlated with growth rate, one would expect ribosome synthesis inhibition when growth at low temperature is down regulated. However, the cell may upregulate ribosome synthesis because protein synthesis is severely inhibited at low temperature but is needed for survival.

Cross-Protection

The cold shock response is, as shown above, not a single event nor a clearly defined reaction. A few reports on cross-protection against other stresses upon cold shock include the induction of barotolerance in *Lactobacillus sanfranciscensis* after cold stress (Scheyhing et al. 2004) and of NaCl tolerance in *Shewanella putrefaciens* (Leblanc et al. 2003). A similar phenotype was observed with a *L. monocytogenes* mutant of lmo1078 encoding a putative UDP-glucose pyrophosphorylase (Chassaing and Auvray 2007). A cold shock response has a positive impact on *Vibrio parahaemolyticus* survival after crystal violet challenge (Lin et al. 2004) and on *L. monocytogenes* resistance toward pH, high pressure, and freezing stress (Wemekamp-Kamphuis et al. 2004b). Another example is the CSP-family proteins of

L. monocytogenes that contribute to oxidative stress adaptation; their deletion significantly impaired the capability of this foodborne pathogen to invade epithelial cells (Loepfe et al. 2010). Pfennig and Flower (2001) showed that BipA, a member of the ribosome binding GTPase superfamily that regulates pathogenicity of enterohemorrhagic *E. coli* (EHEC), contributes to cold adaptation in *E. coli* MC4100. This was confirmed by a similar observation in *P. putida* (Reva et al. 2006).

Long-Term Adaptation of Psychrotolerant Bacteria

In contrast to the cold shock response of psychrotolerant bacteria, our knowledge about their long-term response to cold and their adaptation to the modified growth conditions is yet limited. Beside common patterns such as alterations of the membrane composition (Kaan et al. 2002; Riley et al. 2008; Chan et al. 2007), some interesting differences between the cold-adaptation of psychrophilic and psychrotolerant bacteria were found (section ❯ "Metabolism and Growth").

To identify *L. monocytogenes* genes that are induced during growth at low temperature as compared to 37°C, transcriptional analyses based on selective capture of transcribed sequences (SCOTS) and microarrays were performed (Liu et al. 2002; Chan et al. 2007). According to the overall results of the SCOTS analysis, adaptation of this pathogen to 10°C includes the up regulation of genes involved in amino acid biosynthesis (*hisJ*, *trpG*, and *aroA*), general stress response (*groEL*, *clpP*, *clpB*, *flp*, and *trxB*), cell surface alterations (*fbp*, *psr*, and *flaA*), modification of the degradative metabolism (*eutB*, *celD*, and *mleA*), and regulatory adaptive response (*rpoN*, *lhkA*, *yycJ*, *bglG*, *adaB*, and *psr*). The whole-genome microarray approach not only confirmed the down regulation of listerial virulence factors and the up regulation of the carnitine and glycine betaine transporter OpuC and GbuC at low temperature but additionally revealed the induction of several PTS and CodY-repressed genes involved in biosynthesis of BCAA. The latter group comprises isoleucine, an important precursor for the synthesis of anteiso-branched-chain fatty acids.

Peptides are a major source of amino acids at low temperature as indicated by the finding that the oligopeptide transporter Opp is essential for listerial growth at refrigerator temperatures (Borezee et al. 2000). The overall results of a proteomics approach suggest an enhanced demand of *Listeria* for energy to adapt to low temperature (Cacace et al. 2010). The induction of glycolytic activity, mediated by the catabolite control protein A (CcpA), is a common pattern of the low-temperature response of *L. monocytogenes*, *Lactobacillus lactis* and *P. fluorescens* (Cacace et al. 2010; Wouters et al. 2000a; Lynch and Franklin 1978). A link between carbon catabolite repression and cold shock response via PTS was also demonstrated in *Lactobacillus casei* (Monedero et al. 2007). In *B. subtilis* and *L. monocytogenes*, the genes encoding Pta and AckA, as well as those responsible for biosynthesis of BCAAs, were found to be up regulated upon a decline in temperature (Shivers et al. 2006; Cacace et al. 2010). These factors are controlled by CcpA, which is up regulated in the cold. Pta and AckA are involved in the pyruvate metabolism and control the intracellular level of acetyl phosphate.

In *B. subtilis*, the transcription factor SigB that is important for continuous growth and sporulation at low temperature is induced by cold shock (Budde et al. 2006). Growth of a corresponding mutant is severely impaired at 15°C, but the mutant is rescued by the addition of glycine betaine (Brigulla et al. 2003; Mendez et al. 2004). Genes for arginine and tryptophan biosynthesis, as well as the urease operon, are up regulated by low temperature. Conflicting data have been reported for genes involved in BCAA biosynthesis, probably due to an only temporary induction after cold shock (Budde et al. 2006; Beckering et al. 2002). In contrast to *L. monocytogenes*, major anabolic and catabolic routes including glycolysis are repressed, likely reflecting the slower growth rate at low temperature (Budde et al. 2006).

In *E. coli*, a higher transcriptional level 5 h after a cold shock to 15°C was observed for genes involved in motility, iron transport, and pyruvate metabolism, while glycolysis and amino acid biosynthesis appear to be down regulated (Phadtare and Inouye 2004). The utilization of histidine as carbon and nitrogen source is another catabolic pathway that is up regulated by psychrotolerant bacteria such as *Y. enterocolitica* and *P. syringae* during growth at low temperature (Bresolin et al. 2006b; Janiyani and Ray 2002). In *P. putida*, genes involved in fatty acid metabolism and LPS biosynthesis are essential at 4°C, as well as the uptake of phosphate, possibly regulated by PhoB-PhoR, a key regulator that controls membrane phospholipid composition (Reva et al. 2006). The TKS CbrA/CbrB that controls the utilization of carbon and nitrogen sources is also required for *P. putida* growth in the cold. With respect to human and animal pathogens among the psychrotolerant bacteria, implications for their yet underinvestigated ecology could be derived from a recent adaptation study (Bresolin et al. 2006a) (section ❯ "Ecology of Human Pathogens in Cold Habitats").

Biotechnological Applications

Although there is considerable biotechnological potential of the factors involved in cold-adaptation of bacteria (❯ *Table 18.9*), few have been commercialized (Cavicchioli et al. 2002). Possible advantages of cold-active enzymes and factors are a shortening of process times, the saving of energy costs, the prevention of volatile compound loss, the performance of reactions involving thermosensitive compounds, and a reduced risk of contamination (Margesin et al. 2007), whereas weak thermostability might be a severe drawback. Of emerging interest are psychrophiles that contribute to the production of biofuels (Barnard et al. 2010; Amaretti et al. 2010). The archaea also revealed as a novel biotechnological resource and their possible applications include the generation of renewable energy and synthetic chemicals (Cavicchioli 2011). In the following, we will highlight some aspects in environmental biotechnology, expression systems, and pharmaceutical applications.

Table 18.9
Applications of cold-active cells, enzymes, and factors in biotechnology (Modified from (Margesin et al. 2007))

Field of application	Advantage	Involved enzymes and factors
Environment	In situ/on-site bioremediation of organic contaminants	Mono- and dioxygenases, transferases, hydrolases
	Low-energy wastewater treatment	Enzymes involved in anaerobic degradation
	Low-temperature methane production	Enzymes involved in anaerobic degradation (cellulases, hemicellulases), methanogenic archaea
	Low-temperature composting	Enzymes involved in litter degradation
	Production of artificial snow, manufacture of frozen food, substitution for silver iodide in cloud seeding, construction of transgenic crops	Bacterial INAs
Agriculture	Increased legume production in cold regions	Arctic rhizobia
	Biocontrol of plant diseases	Antibiotics, insecticidal toxins
	Frost protection of plants	INA negative bacterial strains (''ice-minus'')
Molecular biology	Expression systems	
	Selective enzyme inhibition	Proteases
	Efficient protoplast formation	Cellulases, xylanases
	Ligation, PCR, transcription, mutagenesis	Ligases, uracil DNA glycosylases, alkaline phosphatase, DNA/RNA polymerases
Pharmaceuticals	Debridement of necrotic tissue, digestion promotion, chemonucleolytic agents	Multienzyme systems
	Temperature-sensitive vaccines	Biotransformation of essential psychrophilic genes
	Cleaning of contact lens	Subtilisin
Textiles	Improved quality after desizing, biopolishing, and stone-washing of fabrics	Cellulases, amylases
	Washing at low temperature: additives in detergents	Proteases, lipases, α-amylases, cellulases, oxygenases
Food industry	Reduced incubation time for lactose hydrolysis in milk and dairy products	ß-galactosidase
	Improved juice clarification, increased juice yield, wine industry, cheese ripening	Pectinases, cellulases, pectate lyase
	Breadmaking	Amylases, xylanases
	Efficient and gentle removal of fish skin, meat tenderization	Proteases, carbohydrases
	Cold pasteurization, food preservation	Catalases, lysozymes, glucose oxidases
	Dietary supplements for humans, livestock, and fish	Polyunsaturated fatty acids
Organic synthesis	Synthesis of volatile and heat-sensitive compounds (flavors, fragrances)	Lipases, esterases, proteases
	Synthesis of acrylamide	Nitrile hydratase
	Asymmetric chemical synthesis	3-isopropylmalate dehydrogenase
	Organic phase biocatalysis (increased solvent choice, product yield, and biocatalysis stability)	Enzymes operating at low water conditions
Biosensors	Selective, sensitive, and rapid online monitoring of low-temperature processes; quality control	Lactate-dehydrogenases

Biotechnology to Decontaminate Water or Soil

An important step in wastewater treatment is the removal of water pollutants by microorganisms. Even in a moderate climate, wastewater temperature may drop to 10°C or 15°C in winter, eventually inhibiting growth of the microbial flora. Different technical solutions have been proposed to treat wastewater successfully at 13°C (for a review,

see Lettinga et al. (2001)), and cold-adapted bacteria feasible for wastewater remediation in the cold were successfully isolated from an Arctic freshwater pond and seawater sediment, respectively (Gratia et al. 2009; Xing et al. 2010). Another problem is the huge amount of solids entering sewage treatment facilities. Anaerobic digestion might decrease their amount, but results in fouling and biogas emission including the formation of methane (Nozhevnikova et al. 2001a). Conversely, psychrotolerant nitrifying bacteria may pose a threat to drinking water quality in cold climates (Lipponen et al. 2002).

Of great concern is soil contamination with petroleum hydrocarbons, chlorinated solvents, and pesticides by human activity. Cold regions might increasingly be affected as vast petroleum reserves have been identified in the Arctic and the Antarctic. The long-term investigation of oil tanker accidents showed that nitrogen availability, the oil loading, and the progress of natural biodegradation, but not temperature, restricted the effectiveness of petroleum bioremediation by cold-adapted, indigenous microbes (Bragg et al. 1994; Kasai et al. 2001). Those capable to mineralize petroleum hydrocarbons are searched for in Antarctic and alpine soils (Delille and Coulon 2008; Margesin 2007).

Acid mine drainages posing environmental problems have also been examined for bacterial activities. A metagenomic analysis of an acidophilic biofilm elucidated microbial survival strategies under extreme conditions (Tyson et al. 2004). Cold-adapted *Acidothiobacillus ferrooxidans* strains mediate the bioleaching of metal sulfides, thus allowing the extraction of metals from their ores at environmental temperature (Margesin et al. 2007).

Expression Systems

Cold-adapted organisms, artificially expressed proteins, or promoters activated at low temperatures might have certain advantages for the production of thermolabile, toxic, or proteolytically sensitive factors, or for proper folding, increased solubility, or enhanced stability of proteins (Gonzalez et al. 2003; Tutino et al. 2001; Mujacic et al. 1999; Takeuchi et al. 2003). A TKS from *Pseudoalteromonas* was recently adapted as an effective cold-inducible expression system to produce soluble and catalytically active β-galactosidase and α-glucosidase (Papa et al. 2007). For more information about the usage of cold-inducible promoters in *E. coli*, the reader is referred to the review by Baneyx (1999) or the methodological papers by Baneyx and Mujacic (2003), Qing et al. (2004), and Duilio et al. (2004).

Pharmaceuticals

Possible applications of cold-adapted bacteria and their products in (veterinary) medicine are compatible solutes in skin care products, drug excipients, treatments for respiratory diseases, peptide antibiotics, and anticancer therapeutics (Irwin 2010). Ether-linked lipids from archaea have been proposed for application in the production of liposomes for drug and vaccine delivery (Patel and Sprott 1999). Nano and colleagues replaced genes such as *ligA*, which encodes an NAD-dependent DNA ligase, of mammalian pathogens with their counterparts from psychrophilic bacteria. The recombinant strains died at temperature above 35°C, potentially giving rise to the creation of temperature-sensitive vaccines (Duplantis et al. 2010).

Food

Cold-adapted bacteria may be responsible for severe food spoilage, poisoning, or pathogenic contamination and are an important economic factor for the food industry (Russell 2002). Spoilage by psychrotolerant organisms includes toxin expression, organoleptic changes (structure, texture, and color), gas formation, production of biogenic amines, and transmission of pathogenic bacteria. In contrast, psychrotolerants intrinsically protect food products during storage. The most common psychrotolerant pathogens found in food are *L. monocytogenes*, *Y. enterocolitica*, non-proteolytic *Clostridium botulinum*, certain *B. cereus*-group strains, and *Aeromonas hydrophila*.

Food preservation by refrigeration and freezing usually inhibits or reduces the activity of foodborne microorganisms. However, bacteria differ in their capability to survive during freezing, with Gram-negative strains being less resistant than Gram-positive bacteria or vegetative clostridial strains, whereas endospores and food-poisoning toxins are apparently not affected by low temperatures (Jay 2000).

The predominant genera involved in food spoilage by psychrotolerant bacteria include *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Hafnia*, and *Serratia* (Brightwell et al. 2007; Dogan and Boor 2003; De Jonghe et al. 2010). In recent years, several new studies have been published on the occurrence of psychrotolerant bacteria in food matrices. The smoky odor in chocolate milk stored at 4–9°C is due to guaicol produced by the spoiling organism *Rahnella aquatilis*, an opportunistic pathogen causing surgical wound infection (Maraki et al. 1994). This was the first identification of an organism responsible for this type of spoilage (Jensen et al. 2001). Psychrotolerant lactic acid bacteria (LAB) have been identified as causative agents for spoilage in cooked meat products (Hamasaki et al. 2003). Psychrophilic *C. estertheticum* and *C. gasigenes* cause spoilage in vacuum-packed chilled meats, resulting in severe pack distension, a phenomenon which has been termed "blown pack spoilage." The source of psychrophilic clostridia in spoiling vacuum-packed chilled meat products is most likely soil particles and fecal material introduced at the abattoir (Boerema et al. 2003).

The control of *L. monocytogenes* is of major concern for the food industry as most cases of human listeriosis are caused by consumption of refrigerated ready-to-eat foods contaminated with high levels of *L. monocytogenes* (Chan and Wiedmann 2009), and due to its capability to grow well at 4°C. Moreover, it efficiently invades epithelial cells after a chilling period of up to 4 weeks (Larsen et al. 2010). Neither cold nor carbon dioxide

induces a viable but nonculturable state in *L. monocytogenes* (Li et al. 2003). However, reduction in the number of this organism is achieved by using essential oils and freezing (Cressy et al. 2003), or by a combination of high pressure (200 MPa), subzero temperatures (−18°C), and low pH (4.5) (Ritz et al. 2008). Combination of organic acids such as lactate and diacetate synergistically inhibit growth of this pathogen at refrigerator temperatures (Stasiewicz et al. 2010). Cross-protection as described above has significant implications for control measures. Sodium salts also prevent listerial growth (Taormina 2010), but exposure to low temperature may inadvertently cross-protect *Listeria* cells against NaCl stress upon CSP induction (Schmid et al. 2009). This reminds of a similar finding described for the spoilage bacterium *S. putrefaciens* (section ❯ "The Cold Shock Response"). Interestingly, growth of *L. monocytogenes* at 37°C had a higher impact on its freeze-thaw tolerance than growth at 4°C (Azizoglu et al. 2009).

The physiology of a pathogen growing in the food matrix is often poorly understood. An elegant in situ method for monitoring the psychrotolerant pathogen *Y. enterocolitica* in cheese samples was reported by Maoz et al. (2002). A full-length *luxCDABE* operon was introduced in the genome of this organism, which carried a constitutive promoter. The emitted light, corresponding to colony forming unit (cfu) counts, was monitored with a sensitive, charge-coupled device (CCD) camera. This system does not need the addition of any further substance like antibiotics (to maintain a plasmid) or substrate for the light-producing LuxAB enzymes. The influence of bioprotective cultures and other means to control the pathogen can be monitored in situ without laborious cfu plate countings. This technique was already used to monitor the behavior of *B. cereus* and *S. enterica* in food (Dommel et al. 2010; Srikumar and Fuchs 2011).

Ecology of Human Pathogens in Cold Habitats

The role and containment of psychrotolerant pathogenic bacteria in food have been mentioned in the section above. We highlight here some aspects of the multifaceted ecology of several psychrotolerant and mesophilic human pathogens.

Virulence Factors

Pathogenicity toward mammalian hosts is a recent adaptation in geological terms. It requires exceptional regulatory mechanisms that repress or induce virulence genes where appropriate. Therefore, the expression of virulence factors is often controlled by temperature-dependent mechanisms that allow the pathogens to determine whether they are inside or outside a host (DiRita et al. 2000; Eriksson et al. 2002). Sophisticated systems to control virulence factor expression in a temperature-dependent manner have evolved in the foodborne pathogen *Y. enterocolitica* (Marceau 2005). Examples are the *lrcF* mRNA-thermometer (section ❯ "Cold Sensors and Regulators") and the two virulence modulators YmoA and RovA that are accessible to proteolytical degradation at 37°C (Herbst et al. 2009). Among the virulence determinants of yersiniae that are strongly expressed at environmental but not body temperature are a heat-stable enterotoxin, lipopolysaccharides, the primary internalization factor invasin, and iron-scavenging systems (Straley and Perry 1995). One of the major proteins induced in *L. monocytogenes* is a ferritin-like protein (Dussurget et al. 2005; Hebraud and Guzzo 2000). A similar finding has been reported from *Streptococcus thermophilus* that expresses an iron-binding protein upon cold shock (Nicodeme et al. 2004). The virulence-associated factor *hms* of *Y. pestis* is also known to be expressed at low temperature (Lillard et al. 1999). These examples hint to a yet neglected field of research: the association of human pathogens with invertebrates.

Invertebrates as Hosts

A well-known example of a pathogen-invertebrate association as a prerequisite for human infection is *Yersinia pestis* transmission by fleas. *Y. pestis* cycles between fleas and a rodent host and is thus exposed to a broad temperature spectrum. Within the flea midgut, the pathogen rapidly multiplies, a step that requires the activity of an *ymt*-encoded phospholipase D. Subsequent colonization of the proventriculus involves *hms*-dependent biofilm formation, resulting in a blockage of food uptake into the midgut. Repeated feeding attempts result in the regurgitation of bacteria into the feeding lesion of the new host (Vallet-Gely et al. 2008). Much more widespread are bacteria-invertebrate association that do not affect mammals, but offer an excellent opportunity for microbes to gain access to otherwise rare nutrients. Examples are *Legionella* that interacts with *Acanthamoeba castellanii* and *Dictyostelium discoideum*, and *Pseudomonas* that exploits *Caenorhabditis elegans*. These nonmammal hosts are bacterial predators and naturally phagocytose and feed on bacterial pathogens (Hilbi et al. 2007). In addition, pathogens such as *L. monocytogenes* were found to kill *Drosophila melanogaster* upon infection (Mansfield et al. 2003). These invertebrates are proven models to study bacterial pathogenesis as they allow the identification of virulence factors directed against humans (Sifri et al. 2005).

Few bacteria, such as *B. thuringiensis* and *Photorhabdus luminescens*, are able to kill insects. Such an activity was also revealed for the enteropathogen *Y. enterocolitica* that expresses insecticidal genes at 10°C and kills *Manduca sexta*, the tobacco hornworm, and *Galleria mellonella*, the greater wax moth (Bresolin et al. 2006a; Fuchs et al. 2008). It is assumed that a large number of factors directed against invertebrate hosts and their immune response remain to be uncovered (Heermann and Fuchs 2008). Consequently, bacteria-invertebrate interactions are assumed to have shaped the evolution of human pathogens (Waterfield et al. 2004). Low-temperature expression analyses might shed further light on the ecology of pathogenic bacteria.

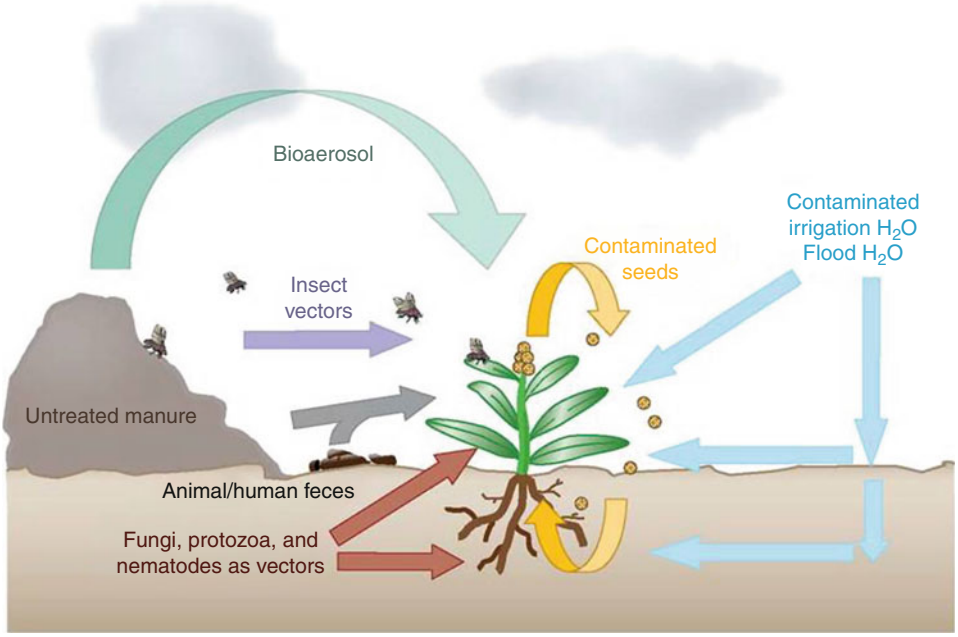


Fig. 18.25
Schematic illustration of factors that can contribute to the contamination of fruit and vegetables with human enteric pathogens in the field (From Brandl (2006))

Fish Pathogens

Few cold-adapted bacteria are pathogenic toward fish; economically relevant examples are *Mycobacterium* spp. causing mycobacteriosis, *Moritella viscosa* infecting salmon and cod, and *Yersinia ruckeri*, the etiological agent of enteric redmouth disease in salmons and trouts. The genes responsible for ruckerbactin production, an iron acquisition siderophore, show increased expression upon temperature decrease (Fernandez et al. 2004). This finding links to *Aliivibrio* (formerly *Vibrio*) *salmonicida* that produces significant amounts of iron siderophores and further iron uptake systems suggested as important virulence factors only at low temperatures (Colquhoun and Sorum 2001).

Proteomic approaches demonstrated the importance of motility, which is induced in many bacteria during growth in the cold, for a successful infection by this etiological agent of cold-water vibriosis in farmed Atlantic salmon (Karlsen et al. 2008). While classical virulence factors of Vibrionaceae, such as the cholera toxin CT, the thermostable hemolysin TDH, and the metalloprotease VVP, are absent in the *A. salmonicida* genome, novel virulence factors were predicted (Hjerde et al. 2008). Their expression and function in low-temperature environments remain to be elucidated. Another psychrophilic fish pathogen, *Flavobacterium psychrophilum*, is not only known to produce a metalloprotease, a heat-labile hemolysin and siderophores (Secades et al. 2003; Hogfors-Ronnholm and Wiklund 2010; LaFrentz et al. 2009), but also harbors genes for gliding motility proteins, adhesins, and toxins. Its psychrophilic character is underlined by the presence of desaturases, a carotenoid biosynthesis pathway, CSPs, and enzymes inactivating reactive oxygen species (ROS). Proteins with antioxidant activity help psychrophiles to withstand not only the ROS production of their hosts, but also to cope with the increase of ROS due to a higher gas solubility at low temperatures (D'Amico et al. 2006).

Association with Plants

Ecosystems of human pathogens include the association with plants (Tyler and Triplett 2008; *Fig. 18.25*), hinting to a low-temperature growth modus to play a role also for mesophilic bacteria. Infected fruits and produce are nowadays considered to be major vehicles of foodborne pathogens, including *Salmonella*, EHEC, and *Listeria* (Brandl 2006). While virulence factors in plant-associated bacteria are known to be thermoregulated (Smirnova et al. 2001), respective knowledge is scarce for non-psychrotolerant pathogens. It has been shown by Schikora et al. (2008) that *Salmonella* is not only able to infect *Arabidopsis thaliana* but can also cause an illness phenotype of the plant. The EHEC pathovar of *E. coli* is able to multiply in plant material (Brandl 2006), and one of the largest outbreaks was caused by contaminated radish sprouts in Japan (Michino et al. 1999). Interestingly, EHEC were found to be better adapted to cold stress than *E. coli* K12 strains, partially due to the RpoS-mediated expression of proteins involved in adaptation to cold shock and proteins that play a role in central metabolic pathways (Vidovic et al. 2011). Growth of EHEC in biofilms is influenced by plant-secreted chemicals and also affects its central metabolism (Lee et al. 2010). On the other hand, *L. monocytogenes* thrives in cooled food of plant origin

(Walter et al. 2009). Interestingly, the antibacterial activity of carvacrol, an oregano and thyme ingredient, is reduced at lower temperatures against *L. monocytogenes* (Veldhuizen et al. 2007). The above-mentioned pathogens have to compete with the native plant surface flora, which is also influenced by temperature (Rasche et al. 2006). Competitors from soil, *Enterobacter asburiae* and a distinct group of psychrotolerant *Pseudomonas* species, are able to protect plants from colonization by pathogens (Cooley et al. 2003; Johansson and Wright 2003).

References

Aguilar PS, Cronan JE Jr, de Mendoza D (1998) A *Bacillus subtilis* gene induced by cold shock encodes a membrane phospholipid desaturase. J Bacteriol 180(8):2194–2200

Aguilar PS, Hernandez-Arriaga AM, Cybulski LE, Erazo AC, de Mendoza D (2001) Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. EMBO J 20(7):1681–1691

Alam SI, Dixit A, Reddy GS, Dube S, Palit M, Shivaji S, Singh L (2006) *Clostridium schirmacherense* sp. nov., an obligately anaerobic, proteolytic, psychrophilic bacterium isolated from lake sediment of Schirmacher Oasis, Antarctica. Int J Syst Evol Microbiol 56:715–720

Albers SV, van de Vossenberg JL, Driessen AJ, Konings WN (2000) Adaptations of the archaeal cell membrane to heat stress. Front Biosci 5:D813–D820

Allen EE, Bartlett DH (2002) Structure and regulation of the ω-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium *Photobacterium profundum* strain SS9. Microbiology 148:1903–1913

Allen EE, Facciotti D, Bartlett DH (1999) Monounsaturated but not polyunsaturated fatty acids are required for growth of the deep-sea bacterium *Photobacterium profundum* SS9 at high pressure and low temperature. Appl Environ Microbiol 65(4):1710–1720

Allen MA, Lauro FM, Williams TJ, Burg D, Siddiqui KS, De Francisci D, Chong KW, Pilak O, Chew HH, De Maere MZ, Ting L, Katrib M, Ng C, Sowers KR, Galperin MY, Anderson IJ, Ivanova N, Dalin E, Martinez M, Lapidus A, Hauser L, Land M, Thomas T, Cavicchioli R (2009) The genome sequence of the psychrophilic archaeon, *Methanococcoides burtonii*: the role of genome evolution in cold adaptation. ISME J 3(9):1012–1035

Amaretti A, Raimondi S, Sala M, Roncaglia L, De Lucia M, Leonardi A, Rossi M (2010) Single cell oils of the cold-adapted oleaginous yeast *Rhodotorula glacialis* DBVPG 4785. Microb Cell Fact 9:73

Amato P, Christner BC (2009) Energy metabolism response to low-temperature and frozen conditions in *Psychrobacter cryohalolentis*. Appl Environ Microbiol 75(3):711–718

Amato P, Doyle SM, Battista JR, Christner BC (2010) Implications of subzero metabolic activity on long-term microbial survival in terrestrial and extraterrestrial permafrost. Astrobiology 10(8):789–798

Ambily Nath IV, Loka Bharathi PA (2011) Diversity in transcripts and translational pattern of stress proteins in marine extremophiles. Extremophiles 15(2):129–153

Angelidis AS, Smith GM (2003) Role of the glycine betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. Appl Environ Microbiol 69(12):7492–7498

Angelidis AS, Smith LT, Hoffman LM, Smith GM (2002a) Identification of *opuC* as a chill-activated and osmotically activated carnitine transporter in *Listeria monocytogenes*. Appl Environ Microbiol 68(6):2644–2650

Angelidis AS, Smith LT, Smith GM (2002b) Elevated carnitine accumulation by *Listeria monocytogenes* impaired in glycine betaine transport is insufficient to restore wild-type cryotolerance in milk whey. Int J Food Microbiol 75(1–2):1–9

Arguedas-Villa C, Stephan R, Tasara T (2010) Evaluation of cold growth and related gene transcription responses associated with *Listeria monocytogenes* strains of different origins. Food Microbiol 27(5):653–660

Arnorsdottir J, Smaradottir RB, Magnusson OT, Gudmundur SH, Kristjansson MM (2002) Characterization of a cloned subtilisin-like serine proteinase from a psychrotrophic *Vibrio* species. Eur J Biochem 269(22):5536–5546

Auman AJ, Breezee JL, Gosink JJ, Kämpfer P, Staley JT (2006) *Psychromonas ingrahamii* sp. nov., a novel gas vacuolate, psychrophilic bacterium isolated from Arctic polar sea ice. Int J Syst Evol Microbiol 56:1001–1007

Auman AJ, Breezee JL, Gosink JJ, Schumann P, Barnes CR, Kämpfer P, Staley JT (2010) *Psychromonas boydii* sp. nov., a gas-vacuolate, psychrophilic bacterium isolated from an Arctic sea-ice core. Int J Syst Evol Microbiol 60:84–92

Aurilia V, Parracino A, D'Auria S (2008) Microbial carbohydrate esterases in cold adapted environments. Gene 410(2):234–240

Awano N, Rajagopal V, Arbing M, Patel S, Hunt J, Inouye M, Phadtare S (2010) *Escherichia coli* RNase R has dual activities, helicase and RNase. J Bacteriol 192(5):1344–1352

Ayala-del-Rio HL, Chain PS, Grzymski JJ, Ponder MA, Ivanova N, Bergholz PW, Di Bartolo G, Hauser L, Land M, Bakermans C, Rodrigues D, Klappenbach J, Zarka D, Larimer F, Richardson P, Murray A, Thomashow M, Tiedje JM (2010) The genome sequence of *Psychrobacter arcticus* 273–4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. Appl Environ Microbiol 76(7):2304–2312

Azizoglu RO, Osborne J, Wilson S, Kathariou S (2009) Role of growth temperature in freeze-thaw tolerance of *Listeria* spp. Appl Environ Microbiol 75(16):5315–5320

Badaoui Najjar M, Chikindas M, Montville TJ (2007) Changes in *Listeria monocytogenes* membrane fluidity in response to temperature stress. Appl Environ Microbiol 73(20):6429–6435

Bae W, Phadtare S, Severinov K, Inouye M (1999) Characterization of *Escherichia coli cspE*, whose product negatively regulates transcription of *cspA*, the gene for the major cold shock protein. Mol Microbiol 31(5):1429–1441

Bae W, Xia B, Inouye M, Severinov K (2000) *Escherichia coli* CspA-family RNA chaperones are transcription antiterminators. Proc Natl Acad Sci USA 97(14):7784–7789

Bakermans C, Tsapin AI, Souza-Egipsy V, Gilichinsky DA, Nealson KH (2003) Reproduction and metabolism at – 10 degrees C of bacteria isolated from Siberian permafrost. Environ Microbiol 5(4):321–326

Bakermans C, Tollaksen SL, Giometti CS, Wilkerson C, Tiedje JM, Thomashow MF (2007) Proteomic analysis of *Psychrobacter cryohalolentis* K5 during growth at subzero temperatures. Extremophiles 11(2):343–354

Bakermans C, Sloup RE, Zarka DG, Tiedje JM, Thomashow MF (2009) Development and use of genetic system to identify genes required for efficient low-temperature growth of *Psychrobacter arcticus* 273–4. Extremophiles 13(1):21–30

Baneyx F (1999) Recombinant protein expression in *Escherichia coli*. Curr Opin Biotechnol 10(5):411–421

Baneyx F, Mujacic M (2003) Cold-inducible promoters for heterologous protein expression. Methods Mol Biol 205:1–18

Barnard D, Casanueva A, Tuffin M, Cowan D (2010) Extremophiles in biofuel synthesis. Environ Technol 31(8–9):871–888

Beckering CL, Steil L, Weber MH, Völker U, Marahiel MA (2002) Genomewide transcriptional analysis of the cold shock response in *Bacillus subtilis*. J Bacteriol 184(22):6395–6402

Bentahir M, Feller G, Aittaleb M, Lamotte-Brasseur J, Himri T, Chessa JP, Gerday C (2000) Structural, kinetic, and calorimetric characterization of the cold-active phosphoglycerate kinase from the antarctic *Pseudomonas* sp. TACII18. J Biol Chem 275(15):11147–11153

Beran RK, Simons RW (2001) Cold-temperature induction of *Escherichia coli* polynucleotide phosphorylase occurs by reversal of its autoregulation. Mol Microbiol 39(1):112–125

Beranova J, Mansilla MC, de Mendoza D, Elhottova D, Konopasek I (2010) Differences in cold adaptation of *Bacillus subtilis* under anaerobic and aerobic conditions. J Bacteriol 192(16):4164–4171

Bergholz PW, Bakermans C, Tiedje JM (2009) *Psychrobacter arcticus* 273–4 uses resource efficiency and molecular motion adaptations for subzero temperature growth. J Bacteriol 191(7):2340–2352

Bhakoo M, Herbert RA (1980) Fatty acid and phospholipid composition of five psychrotrophic *Pseudomonas* spp. grown at different temperatures. Arch Microbiol 126(1):51–55

Bidle KD, Manganelli M, Azam F (2002) Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. Science 298(5600):1980–1984

Boerema JA, Broda DM, Bell RG (2003) Abattoir sources of psychrophilic clostridia causing blown pack spoilage of vacuum-packed chilled meats determined by culture-based and molecular detection procedures. Lett Appl Microbiol 36(6):406–411

Borezee E, Pellegrini E, Berche P (2000) OppA of *Listeria monocytogenes*, an oligopeptide-binding protein required for bacterial growth at low temperature and involved in intracellular survival. Infect Immun 68(12):7069–7077

Boziaris IS, Adams MR (2001) Temperature shock, injury and transient sensitivity to nisin in gram negatives. J Appl Microbiol 91(4):715–724

Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the *Exxon Valdez* oil spill. Nature 368:413–418

Brakstad OG, Lodeng AG (2005) Microbial diversity during biodegradation of crude oil in seawater from the North Sea. Microb Ecol 49(1):94–103

Brandi A, Spurio R, Gualerzi CO, Pon CL (1999) Massive presence of the *Escherichia coli* 'major cold-shock protein' CspA under non-stress conditions. EMBO J 18(6):1653–1659

Brandl MT (2006) Fitness of human enteric pathogens on plants and implications for food safety. Annu Rev Phytopathol 44:367–392

Braun Y, Smirnova AV, Schenk A, Weingart H, Burau C, Muskhelishvili G, Ullrich MS (2008) Component and protein domain exchange analysis of a thermoresponsive, two-component regulatory system of *Pseudomonas syringae*. Microbiology 154:2700–2708

Breezee J, Cady N, Staley JT (2004) Subfreezing growth of the sea ice bacterium "*Psychromonas ingrahamii*". Microb Ecol 47(3):300–304

Brenchley JE (1996) Psychrophilic microorganisms and their cold-active enzymes. J Ind Microbiol 17:432–437

Bresolin G, Morgan JA, Ilgen D, Scherer S, Fuchs TM (2006a) Low temperature-induced insecticidal activity of *Yersinia enterocolitica*. Mol Microbiol 59(2):503–512

Bresolin G, Neuhaus K, Scherer S, Fuchs TM (2006b) Transcriptional analysis of long-term adaptation of *Yersinia enterocolitica* to low-temperature growth. J Bacteriol 188(8):2945–2958

Brightwell G, Clemens R, Urlich S, Boerema J (2007) Possible involvement of psychrotolerant Enterobacteriaceae in blown pack spoilage of vacuum-packaged raw meats. Int J Food Microbiol 119(3):334–339

Brigulla M, Hoffmann T, Krisp A, Völker A, Bremer E, Völker U (2003) Chill induction of the SigB-dependent general stress response in *Bacillus subtilis* and its contribution to low-temperature adaptation. J Bacteriol 185 (15):4305–4314

Broda DM, Saul DJ, Bell RG, Musgrave DR (2000a) *Clostridium algidixylanolyticum* sp. nov., a psychrotolerant, xylan-degrading, spore-forming bacterium. Int J Syst Evol Microbiol 50:623–631

Broda DM, Saul DJ, Lawson PA, Bell RG, Musgrave DR (2000b) *Clostridium gasigenes* sp. nov., a psychrophile causing spoilage of vacuum-packed meat. Int J Syst Evol Microbiol 50:107–118

Broeze RJ, Solomon CJ, Pope DH (1978) Effects of low temperature on in vivo and in vitro protein synthesis in *Escherichia coli* and Pseudomonas fluorescens. J Bacteriol 134(3):861–874

Budde I, Steil L, Scharf C, Völker U, Bremer E (2006) Adaptation of *Bacillus subtilis* to growth at low temperature: a combined transcriptomic and proteomic appraisal. Microbiology 152:831–853

Bylund GO, Wipemo LC, Lundberg LA, Wikstrom PM (1998) RimM and RbfA are essential for efficient processing of 16 S rRNA in *Escherichia coli*. J Bacteriol 180(1):73–82

Byun JS, Min JS, Kim IS, Kim JW, Chung MS, Lee M (2003) Comparison of indicators of microbial quality of meat during aerobic cold storage. J Food Prot 66(9):1733–1737

Cacace G, Mazzeo MF, Sorrentino A, Spada V, Malorni A, Siciliano RA (2010) Proteomics for the elucidation of cold adaptation mechanisms in *Listeria monocytogenes*. J Proteomics 73(10):2021–2030

Cairrão F, Cruz A, Mori H, Arraiano CM (2003) Cold shock induction of RNase R and its role in the maturation of the quality control mediator SsrA/tmRNA. Mol Microbiol 50(4):1349–1360

Camardella L, Di Fraia R, Antignani A, Ciardiello MA, Di Prisco G, Coleman JK, Buchon L, Guespin J, Russell NJ (2002) The Antarctic *Psychrobacter* sp. TAD1 has two cold-active glutamate dehydrogenases with different cofactor specificities. Characterisation of the NAD^+−dependent enzyme. Comp Biochem Physiol A Mol Integr Physiol 131(3):559–567

Carpenter EJ, Lin S, Capone DG (2000) Bacterial activity in South Pole snow. Appl Environ Microbiol 66(10):4514–4517

Carpentier B, Cerf O (2011) Review – persistence of *Listeria monocytogenes* in food industry equipment and premises. Int J Food Microbiol 145(1):1–8

Carty SM, Sreekumar KR, Raetz CR (1999) Effect of cold shock on lipid A biosynthesis in *Escherichia coli* Induction At 12 degrees C of an acyltransferase specific for palmitoleoyl-acyl carrier protein. J Biol Chem 274(14):9677–9685

Casanueva A, Tuffin M, Cary C, Cowan DA (2010) Molecular adaptations to psychrophily: the impact of "omic" technologies. Trends Microbiol 18(8):374–381

Cavicchioli R (2002) Extremophiles and the search for extraterrestrial life. Astrobiology 2(3):281–292

Cavicchioli R (2006) Cold-adapted archaea. Nat Rev Microbiol 4(5):331–343

Cavicchioli R (2011) Archaea–timeline of the third domain. Nat Rev Microbiol 9(1):51–61

Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low-temperature extremophiles and their applications. Curr Opin Biotechnol 13(3):253–261

Chablain PA, Philippe G, Groboillot A, Truffaut N, Guespin-Michel JF (1997) Isolation of a soil psychrotrophic toluene-degrading *Pseudomonas* strain: influence of temperature on the growth characteristics on different substrates. Res Microbiol 148(2):153–161

Champagne CP, Laing RR, Roy D, Mafu AA, Griffiths MW (1994) Psychrotrophs in dairy products: their effects and their control. Crit Rev Food Sci Nutr 34(1):1–30

Chan YC, Wiedmann M (2009) Physiology and genetics of *Listeria monocytogenes* survival and growth at cold temperatures. Crit Rev Food Sci Nutr 49(3):237–253

Chan YC, Raengpradub S, Boor KJ, Wiedmann M (2007) Microarray-based characterization of the *Listeria monocytogenes* cold regulon in log- and stationary-phase cells. Appl Environ Microbiol 73(20):6484–6498

Chassaing D, Auvray F (2007) The lmo1078 gene encoding a putative UDP-glucose pyrophosphorylase is involved in growth of *Listeria monocytogenes* at low temperature. FEMS Microbiol Lett 275(1):31–37

Chattopadhyay MK (2006) Mechanism of bacterial adaptation to low temperature. J Biosci 31(1):157–165

Chaturvedi P, Shivaji S (2006) *Exiguobacterium indicum* sp. nov., a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. Int J Syst Evol Microbiol 56:2765–2770

Chaturvedi P, Prabahar V, Manorama R, Pindi PK, Bhadra B, Begum Z, Shivaji S (2008) *Exiguobacterium soli* sp. nov., a psychrophilic bacterium from the McMurdo Dry Valleys, Antarctica. Int J Syst Evol Microbiol 58:2447–2453

Chihib NE, Ribeiro da Silva M, Delattre G, Laroche M, Federighi M (2003) Different cellular fatty acid pattern behaviours of two strains of Listeria monocytogenes Scott A and CNL 895807 under different temperature and salinity conditions. FEMS Microbiol Lett 218(1):155–160

Chin JP, Megaw J, Magill CL, Nowotarski K, Williams JP, Bhaganna P, Linton M, Patterson MF, Underwood GJ, Mswaka AY, Hallsworth JE (2010) Solutes determine the temperature windows for microbial survival and growth. Proc Natl Acad Sci USA 107(17):7835–7840

Chou M, Matsunaga T, Takada Y, Fukunaga N (1999) NH4+ transport system of a psychrophilic marine bacterium, *Vibrio* sp strain ABE-1. Extremophiles 3(2):89–95

Chowdhury S, Ragaz C, Kreuger E, Narberhaus F (2003) Temperature-controlled structural alterations of an RNA thermometer. J Biol Chem 278(48):47915–47921

Christner BC (2002) Incorporation of DNA and protein precursors into macromolecules by bacteria at −15 degrees C. Appl Environ Microbiol 68(12):6435–6438

Christner BC (2010) Bioprospecting for microbial products that affect ice crystal formation and growth. Appl Microbiol Biotechnol 85(3):481–489

Clarke DJ, Dowds BC (1994) The gene coding for polynucleotide phosphorylase in *Photorhabdus* sp strain K122 is induced at low temperatures. J Bacteriol 176(12):3775–3784

Claverie P, Vigano C, Ruysschaert JM, Gerday C, Feller G (2003) The precursor of a psychrophilic alpha-amylase: structural characterization and insights into cold adaptation. Biochim Biophys Acta 1649(2):119–122

Collins T, Meuwis MA, Stals I, Claeyssens M, Feller G, Gerday C (2002) A novel family 8 xylanase, functional and physicochemical characterization. J Biol Chem 277(38):35133–35139

Colquhoun DJ, Sorum H (2001) Temperature dependent siderophore production in *Vibrio salmonicida*. Microb Pathog 31(5):213–219

Cooley MB, Miller WG, Mandrell RE (2003) Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol 69(8):4915–4926

Cressy HK, Jerrett AR, Osborne CM, Bremer PJ (2003) A novel method for the reduction of numbers of *Listeria monocytogenes* cells by freezing in combination with an essential oil in bacteriological media. J Food Prot 66(3):390–395

Cronan JE, Rock CO (1996) Biosynthesis of membrane lipids. In: Neidhardt FC, Curtiss R, Ingraham JL, Lin ECC, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (eds) *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington DC

Cybulski LE, del Solar G, Craig PO, Espinosa M, de Mendoza D (2004) *Bacillus subtilis* DesR functions as a phosphorylation-activated switch to control membrane lipid fluidity. J Biol Chem 279(38):39340–39347

Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D (2010) Membrane thickness cue for cold sensing in a bacterium. Curr Biol 20(17):1539–1544

D'Amico S, Claverie P, Collins T, Georlette D, Gratia E, Hoyoux A, Meuwis MA, Feller G, Gerday C (2002) Molecular basis of cold adaptation. Philos Trans R Soc Lond B Biol Sci 357(1423):917–925

D'Amico S, Marx JC, Gerday C, Feller G (2003) Activity-stability relationships in extremophilic enzymes. J Biol Chem 278(10):7891–7896

D'Amico S, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7(4):385–389

Dalluge JJ, Hashizume T, Sopchik AE, McCloskey JA, Davis DR (1996) Conformational flexibility in RNA: the role of dihydrouridine. Nucleic Acids Res 24(6):1073–1079

Dalluge JJ, Hamamoto T, Horikoshi K, Morita RY, Stetter KO, McCloskey JA (1997) Posttranscriptional modification of tRNA in psychrophilic bacteria. J Bacteriol 179(6):1918–1923

Dame RT, Noom MC, Wuite GJ (2006) Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation. Nature 444(7117):387–390

Dammel CS, Noller HF (1995) Suppression of a cold-sensitive mutation in 16 S rRNA by overexpression of a novel ribosome-binding factor, RbfA. Genes Dev 9(5):626–637

Davlieva M, Shamoo Y (2009) Structure and biochemical characterization of an adenylate kinase originating from the psychrophilic organism *Marinibacillus marinus*. Acta Crystallogr Sect F Struct Biol Cryst Commun 65:751–756

De Jonghe V, Coorevits A, De Block J, Van Coillie E, Grijspeerdt K, Herman L, De Vos P, Heyndrickx M (2010) Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. Int J Food Microbiol 136(3):318–325

Delille D, Coulon F (2008) Comparative mesocosm study of biostimulation efficiency in two different oil-amended sub-antarctic soils. Microb Ecol 56(2):243–252

DiRita VJ, Engleberg NC, Heath A, Miller A, Crawford JA, Yu R (2000) Virulence gene regulation inside and outside. Philos Trans R Soc Lond B Biol Sci 355(1397):657–665

Dogan B, Boor KJ (2003) Genetic diversity and spoilage potentials among *Pseudomonas* spp isolated from fluid milk products and dairy processing plants. Appl Environ Microbiol 69(1):130–138

Dommel MK, Frenzel E, Strasser B, Blochinger C, Scherer S, Ehling-Schulz M (2010) Identification of the main promoter directing cereulide biosynthesis in emetic *Bacillus cereus* and its application for real-time monitoring of *ces* gene expression in foods. Appl Environ Microbiol 76(4):1232–1240

Drouin P, Prevost D, Antoun H (2000) Physiological adaptation to low temperatures of strains of *Rhizobium leguminosarum* bv *viciae* associated with *Lathyrus* spp(1). FEMS Microbiol Ecol 32(2):111–120

Duchaud E, Boussaha M, Loux V, Bernardet JF, Michel C, Kerouault B, Mondot S, Nicolas P, Bossy R, Caron C, Bessieres P, Gibrat JF, Claverol S, Dumetz F, Le Henaff M, Benmansour A (2007) Complete genome sequence of the fish pathogen *Flavobacterium psychrophilum*. Nat Biotechnol 25(7):763–769

Duilio A, Tutino ML, Matafora V, Sannia G, Marino G (2001) Molecular characterization of a recombinant replication protein (Rep) from the Antarctic bacterium *Psychrobacter* sp TA144. FEMS Microbiol Lett 198(1):49–55

Duilio A, Tutino ML, Marino G (2004) Recombinant protein production in Antarctic gram-negative bacteria. Methods Mol Biol 267:225–237

Duplantis BN, Osusky M, Schmerk CL, Ross DR, Bosio CM, Nano FE (2010) Essential genes from Arctic bacteria used to construct stable, temperature-sensitive bacterial vaccines. Proc Natl Acad Sci USA 107(30):13456–13460

Dussurget O, Dumas E, Archambaud C, Chafsey I, Chambon C, Hebraud M, Cossart P (2005) *Listeria monocytogenes* ferritin protects against multiple stresses and is required for virulence. FEMS Microbiol Lett 250(2):253–261

Dykes GA, Moorhead SM (2001) The role of L-carnitine and glycine betaine in the survival and sub-lethal injury of non-growing *Listeria monocytogenes* cells during chilled storage. Lett Appl Microbiol 32(4):282–286

Edgcomb MR, Sirimanne S, Wilkinson BJ, Drouin P, Morse RD (2000) Electron paramagnetic resonance studies of the membrane fluidity of the foodborne pathogenic psychrotroph *Listeria monocytogenes*. Biochim Biophys Acta 1463(1):31–42

Ehling-Schulz M, Knutsson R, Scherer S (2011) Bacillus cereus. In: Fratamico P, Liu Y, Kathariou S (eds) Genomes of foodborne and waterborne pathogens. ASM Press, Washington, DC, pp 147–164

El-Sharoud WM, Graumann PL (2007) Cold shock proteins aid coupling of transcription and translation in bacteria. Sci Prog 90:15–27

Eriksson S, Hurme R, Rhen M (2002) Low-temperature sensors in bacteria. Philos Trans R Soc Lond B Biol Sci 357(1423):887–893

Eriksson M, Sodersten E, Yu Z, Dalhammar G, Mohn WW (2003) Degradation of polycyclic aromatic hydrocarbons at low temperature under aerobic and nitrate-reducing conditions in enrichment cultures from northern soils. Appl Environ Microbiol 69(1):275–284

Ermolenko DN, Makhatadze GI (2002) Bacterial cold-shock proteins. Cell Mol Life Sci 59(11):1902–1913

Etchegaray JP, Inouye M (1999) CspA, CspB, and CspG, major cold shock proteins of *Escherichia coli*, are induced at low temperature under conditions that completely block protein synthesis. J Bacteriol 181(6):1827–1830

Fang L, Jiang W, Bae W, Inouye M (1997) Promoter-independent cold-shock induction of *cspA* and its derepression at 37 degrees C by mRNA stabilization. Mol Microbiol 23(2):355–364

Fang L, Hou Y, Inouye M (1998) Role of the cold-box region in the 5′ untranslated region of the *cspA* mRNA in its transient expression at low temperature in *Escherichia coli*. J Bacteriol 180(1):90–95

Feller G (2003) Molecular adaptations to cold in psychrophilic enzymes. Cell Mol Life Sci 60(4):648–662

Feller G (2007) Life at low temperatures: is disorder the driving force? Extremophiles 11(2):211–216

Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev 1(1):200–208

Feng Y, Huang H, Liao J, Cohen SN (2001) *Escherichia coli* poly(A)-binding proteins that interact with components of degradosomes or impede RNA decay mediated by polynucleotide phosphorylase and RNase E. J Biol Chem 276(34):31651–31656

Fernandez L, Marquez I, Guijarro JA (2004) Identification of specific in vivo-induced (ivi) genes in *Yersinia ruckeri* and analysis of ruckerbactin, a catecholate siderophore iron acquisition system. Appl Environ Microbiol 70(9):5199–5207

Fong NJ, Burgess ML, Barrow KD, Glenn DR (2001) Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress. Appl Microbiol Biotechnol 56(5–6):750–756

Forster J (1887) Über einige Eigenschaften leuchtender Bakterien. Centr Bakteriol Parasitenk 2:337–340

Francis KP, Stewart GSAB (1997) Detection and speciation of bacteria through PCR using universal major cold-shock protein primer oligomers. J Ind Microbiol Biotechnol 19:286–293

Franzmann PD, Hopfl P, Weiss N, Tindall BJ (1991) Psychrotrophic, lactic acid-producing bacteria from anoxic waters in Ace Lake, Antarctica; Carnobacterium funditum sp. nov. and Carnobacterium alterfunditum sp. nov. Arch Microbiol 156(4):255–262

Franzmann PD, Liu Y, Balkwill DL, Aldrich HC, de Conway Macario E, Boone DR (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H2-using methanogen from Ace Lake, Antarctica. Int J Syst Bacteriol 47(4):1068–1072

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biological invasions in the Antarctic: extent, impacts and implications. Biol Rev Camb Philos Soc 80(1):45–72

Fuchs TM, Bresolin G, Marcinowski L, Schachtner J, Scherer S (2008) Insecticidal genes of *Yersinia* spp taxonomical distribution, contribution to toxicity towards *Manduca sexta* and *Galleria mellonella*, and evolution. BMC Microbiol 8:21

Galvez A, Lopez RL, Abriouel H, Valdivia E, Omar NB (2008) Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. Crit Rev Biotechnol 28(2):125–152

Gao H, Yang ZK, Wu L, Thompson DK, Zhou J (2006) Global transcriptome analysis of the cold shock response of *Shewanella oneidensis* MR-1 and mutational analysis of its classical cold shock proteins. J Bacteriol 188(12):4560–4569

Gavaghan H (2002) Life in the deep freeze. Nature 415(6874):828–830

Georlette D, Blaise V, Collins T, D'Amico S, Gratia E, Hoyoux A, Marx JC, Sonan G, Feller G, Gerday C (2004) Some like it cold: biocatalysis at low temperatures. FEMS Microbiol Rev 28(1):25–42

Gerday C, Aittaleb M, Bentahir M, Chessa JP, Claverie P, Collins T, D'Amico S, Dumont J, Garsoux G, Georlette D, Hoyoux A, Lonhienne T, Meuwis MA, Feller G (2000) Cold-adapted enzymes: from fundamentals to biotechnology. Trends Biotechnol 18(3):103–107

Giangrossi M, Giuliodori AM, Gualerzi CO, Pon CL (2002) Selective expression of the beta-subunit of nucleoid-associated protein HU during cold shock in *Escherichia coli*. Mol Microbiol 44(1):205–216

Gilbert JA, Davies PL, Laybourn-Parry J (2005) A hyperactive, Ca2 + −dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 245(1):67–72

Giuliodori AM, Brandi A, Gualerzi CO, Pon CL (2004) Preferential translation of cold-shock mRNAs during cold adaptation. RNA 10(2):265–276

Giuliodori AM, Di Pietro F, Marzi S, Masquida B, Wagner R, Romby P, Gualerzi CO, Pon CL (2010) The *cspA* mRNA is a thermosensor that modulates translation of the cold-shock protein CspA. Mol Cell 37(1):21–33

Goldstein J, Pollitt NS, Inouye M (1990) Major cold shock protein of *Escherichia coli*. Proc Natl Acad Sci USA 87(1):283–287

Gonzalez B, Ceciliani F, Galizzi A (2003) Growth at low temperature suppresses readthrough of the UGA stop codon during the expression of *Bacillus subtilis flgM* gene in *Escherichia coli*. J Biotechnol 101(2):173–180

Goodchild A, Saunders NF, Ertan H, Raftery M, Guilhaus M, Curmi PM, Cavicchioli R (2004) A proteomic determination of cold adaptation in the Antarctic archaeon, *Methanococcoides burtonii*. Mol Microbiol 53(1):309–321

Gopal B, Haire LF, Gamblin SJ, Dodson EJ, Lane AN, Papavinasasundaram KG, Colston MJ, Dodson G (2001) Crystal structure of the transcription elongation/anti-termination factor NusA from *Mycobacterium tuberculosis* at 1.7 A resolution. J Mol Biol 314(5):1087–1095

Gounot AM, Russell NJ (1999) Physiology of cold-adapted microorganisms. In: Margesin R, Schinner F (eds) Cold-adapted organisms: ecology, physiology, enzymology and molecular biology. Springer, Berlin, pp 33–55

Goverde RL, Huis in't Veld JH, Kusters JG, Mooi FR (1998) The psychrotrophic bacterium *Yersinia enterocolitica* requires expression of *pnp*, the gene for polynucleotide phosphorylase, for growth at low temperature (5 degrees C). Mol Microbiol 28(3):555–569

Graham PH (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can J Microbiol 38(6):475–484

Granum PE, Lund T (1997) *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Lett 157(2):223–228

Gratia E, Weekers F, Margesin R, D'Amico S, Thonart P, Feller G (2009) Selection of a cold-adapted bacterium for bioremediation of wastewater at low temperatures. Extremophiles 13(5):763–768

Graumann P, Marahiel MA (1996) A case of convergent evolution of nucleic acid binding modules. Bioessays 18(4):309–315

Graumann PL, Marahiel MA (1998) A superfamily of proteins that contain the cold-shock domain. Trends Biochem Sci 23(8):286–290

Graumann PL, Marahiel MA (1999) Cold shock response in *Bacillus subtilis*. J Mol Microbiol Biotechnol 1(2):203–209

Graumann P, Schröder K, Schmid R, Marahiel MA (1996) Cold shock stress-induced proteins in *Bacillus subtilis*. J Bacteriol 178(15):4611–4619

Graumann P, Wendrich TM, Weber MH, Schröder K, Marahiel MA (1997) A family of cold shock proteins in *Bacillus subtilis* is essential for cellular growth and for efficient protein synthesis at optimal and low temperatures. Mol Microbiol 25(4):741–756

Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, Antranikian G (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 8(6):475–488

Gualerzi CO, Giuliodori AM, Pon CL (2003) Transcriptional and post-transcriptional control of cold-shock genes. J Mol Biol 331(3):527–539

Guillou C, Guespin-Michel JF (1996) Evidence for two domains of growth temperature for the psychrotrophic bacterium *Pseudomonas fluorescens* MF0. Appl Environ Microbiol 62(9):3319–3324

Hallsworth JE, Heim S, Timmis KN (2003) Chaotropic solutes cause water stress in *Pseudomonas putida*. Environ Microbiol 5(12):1270–1280

Hamasaki Y, Ayaki M, Fuchu H, Sugiyama M, Morita H (2003) Behavior of psychrotrophic lactic acid bacteria isolated from spoiling cooked meat products. Appl Environ Microbiol 69(6):3668–3671

Heath C, Hu XP, Cary SC, Cowan D (2009) Identification of a novel alkaliphilic esterase active at low temperatures by screening a metagenomic library from antarctic desert soil. Appl Environ Microbiol 75(13):4657–4659

Hebraud M, Guzzo J (2000) The main cold shock protein of *Listeria monocytogenes* belongs to the family of ferritin-like proteins. FEMS Microbiol Lett 190(1):29–34

Heermann R, Fuchs TM (2008) Comparative analysis of the *Photorhabdus luminescens* and the *Yersinia enterocolitica* genomes: uncovering candidate genes involved in insect pathogenicity. BMC Genomics 9:40

Herbst K, Bujara M, Heroven AK, Opitz W, Weichert M, Zimmermann A, Dersch P (2009) Intrinsic thermal sensing controls proteolysis of *Yersinia* virulence regulator RovA. PLoS Pathog 5(5):e1000435

Hilbi H, Weber SS, Ragaz C, Nyfeler Y, Urwyler S (2007) Environmental predators as models for bacterial pathogenesis. Environ Microbiol 9(3):563–575

Hinsa-Leasure SM, Bhavaraju L, Rodrigues JL, Bakermans C, Gilichinsky DA, Tiedje JM (2010) Characterization of a bacterial community from a Northeast Siberian seacoast permafrost sample. FEMS Microbiol Ecol 74(1):103–113

Hjerde E, Lorentzen MS, Holden MT, Seeger K, Paulsen S, Bason N, Churcher C, Harris D, Norbertczak H, Quail MA, Sanders S, Thurston S, Parkhill J, Willassen NP, Thomson NR (2008) The genome sequence of the fish pathogen *Aliivibrio salmonicida* strain LFI1238 shows extensive evidence of gene decay. BMC Genomics 9:616

Hoe NP, Goguen JD (1993) Temperature sensing in *Yersinia pestis*: translation of the LcrF activator protein is thermally regulated. J Bacteriol 175(24):7901–7909

Hogfors-Ronnholm E, Wiklund T (2010) Hemolytic activity in *Flavobacterium psychrophilum* is a contact-dependent, two-step mechanism and differently expressed in smooth and rough phenotypes. Microb Pathog 49(6):369–375

Horn G, Hofweber R, Kremer W, Kalbitzer HR (2007) Structure and function of bacterial cold shock proteins. Cell Mol Life Sci 64(12):1457–1470

Hu JM, Li H, Cao LX, Wu PC, Zhang CT, Sang SL, Zhang XY, Chen MJ, Lu JQ, Liu YH (2007) Molecular cloning and characterization of the gene encoding cold-active beta-galactosidase from a psychrotrophic and halotolerant *Planococcus* sp L4. J Agric Food Chem 55(6):2217–2224

Hunger K, Beckering CL, Wiegeshoff F, Graumann PL, Marahiel MA (2006) Cold-induced putative DEAD box RNA helicases CshA and CshB

are essential for cold adaptation and interact with cold shock protein B in *Bacillus subtilis*. J Bacteriol 188(1):240–248

Hurme R, Rhen M (1998) Temperature sensing in bacterial gene regulation–what it all boils down to. Mol Microbiol 30(1):1–6

Huston AL, Methe B, Deming JW (2004) Purification, characterization, and sequencing of an extracellular cold-active aminopeptidase produced by marine psychrophile *Colwellia psychrerythraea* strain 34 H. Appl Environ Microbiol 70(6):3321–3328

Ideno A, Yoshida T, Iida T, Furutani M, Maruyama T (2001) FK506-binding protein of the hyperthermophilic archaeum, *Thermococcus* sp. KS-1, a cold-shock-inducible peptidyl-prolyl cis-trans isomerase with activities to trap and refold denatured proteins. Biochem J 357:465–471

Inaba M, Suzuki I, Szalontai B, Kanesaki Y, Los DA, Hayashi H, Murata N (2003) Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in *Synechocystis*. J Biol Chem 278(14):12191–12198

Inniss WE (1975) Interaction of temperature and psychrophilic microorganisms. Annu Rev Microbiol 29:445–465

Inouye M, Phadtare S (2004) Cold shock response and adaptation at near-freezing temperature in microorganisms. Sci STKE 2004(237):pe26

Irwin JA (2010) Extremophiles and their application to veterinary medicine. Environ Technol 31(8–9):857–869

Irwin JA, Gudmundsson HM, Marteinsson VT, Hreggvidsson GO, Lanzetti AJ, Alfredsson GA, Engel PC (2001) Characterization of alanine and malate dehydrogenases from a marine psychrophile strain PA-43. Extremophiles 5(3):199–211

Jagannadham MV, Chattopadhyay MK, Subbalakshmi C, Vairamani M, Narayanan K, Rao CM, Shivaji S (2000) Carotenoids of an Antarctic psychrotolerant bacterium, *Sphingobacterium antarcticus*, and a mesophilic bacterium, *Sphingobacterium multivorum*. Arch Microbiol 173(5–6):418–424

Jäger S, Evguenieva-Hackenberg E, Klug G (2004) Temperature-dependent processing of the *cspA* mRNA in *Rhodobacter capsulatus*. Microbiology 150:687–695

Jahns T, Kaltwasser H (1993) Properties of the cold-labile NAD(+)-specific glutamate dehydrogenase from *Bacillus cereus* DSM 31. J Gen Microbiol 139:775–780

Janiyani KL, Ray MK (2002) Cloning, sequencing, and expression of the cold-inducible *hutU* gene from the antarctic psychrotrophic bacterium *Pseudomonas syringae*. Appl Environ Microbiol 68(1):1–10

Jay JM (2000) Low-temperature food preservation and characteristics of psychrotrophic microorganisms. In: Modern food microbiology, 6th edn. Aspen Publishers, Gaithersburg, pp 323–339

Jensen N, Varelis P, Whitfield FB (2001) Formation of guaiacol in chocolate milk by the psychrotrophic bacterium *Rahnella aquatilis*. Lett Appl Microbiol 33(5):339–343

Jeon JH, Kim JT, Kim YJ, Kim HK, Lee HS, Kang SG, Kim SJ, Lee JH (2009) Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. Appl Microbiol Biotechnol 81(5):865–874

Jiang W, Jones P, Inouye M (1993) Chloramphenicol induces the transcription of the major cold shock gene of *Escherichia coli*, *cspA*. J Bacteriol 175(18):5824–5828

Jiang W, Fang L, Inouye M (1996a) The role of the 5′-end untranslated region of the mRNA for CspA, the major cold-shock protein of *Escherichia coli*, in cold-shock adaptation. J Bacteriol 178(16):4919–4925

Jiang W, Fang L, Inouye M (1996b) Complete growth inhibition of *Escherichia coli* by ribosome trapping with truncated *cspA* mRNA at low temperature. Genes Cells 1(11):965–976

Jiang W, Hou Y, Inouye M (1997) CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J Biol Chem 272(1):196–202

Johansson PM, Wright SA (2003) Low-temperature isolation of disease-suppressive bacteria and characterization of a distinctive group of pseudomonads. Appl Environ Microbiol 69(11):6464–6474

Johansson J, Mandin P, Renzoni A, Chiaruttini C, Springer M, Cossart P (2002) An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. Cell 110(5):551–561

Jones PG, Inouye M (1994) The cold-shock response–a hot topic. Mol Microbiol 11(5):811–818

Jones PG, VanBogelen RA, Neidhardt FC (1987) Induction of proteins in response to low temperature in *Escherichia coli*. J Bacteriol 169:2092–2095

Jones PG, Cashel M, Glaser G, Neidhardt FC (1992a) Function of a relaxed-like state following temperature downshifts in *Escherichia coli*. J Bacteriol 174(12):3903–3914

Jones PG, Krah R, Tafuri SR, Wolffe AP (1992b) DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. J Bacteriol 174(18):5798–5802

Jones PG, Mitta M, Kim Y, Jiang W, Inouye M (1996) Cold shock induces a major ribosomal-associated protein that unwinds double-stranded RNA in *Escherichia coli*. Proc Natl Acad Sci USA 93(1):76–80

Jones CE, Shama G, Jones D, Roberts IS, Andrew PW (1997) Physiological and biochemical studies on psychrotolerance in *Listeria monocytogenes*. J Appl Microbiol 83(1):31–35

Jones SL, Drouin P, Wilkinson BJ, Morse PD II (2002) Correlation of long-range membrane order with temperature-dependent growth characteristics of parent and a cold-sensitive, branched-chain-fatty-acid-deficient mutant of *Listeria monocytogenes*. Arch Microbiol 177(3):217–222

Junge K, Eicken H, Deming JW (2003) Motility of *Colwellia psychrerythraea* strain 34 H at subzero temperatures. Appl Environ Microbiol 69(7):4282–4284

Junge K, Eicken H, Deming JW (2004) Bacterial Activity at −2 to −20 degrees C in Arctic wintertime sea ice. Appl Environ Microbiol 70(1):550–557

Kaan T, Jürgen B, Schweder T (1999) Regulation of the expression of the cold shock proteins CspB and CspC in *Bacillus subtilis*. Mol Gen Genet 262(2):351–354

Kaan T, Homuth G, Mader U, Bandow J, Schweder T (2002) Genome-wide transcriptional profiling of the *Bacillus subtilis* cold-shock response. Microbiology 148:3441–3455

Kalinin A, Rak A, Shcherbakov D, Bayer P (2002) ^{1}H, ^{13}C and ^{15}N resonance assignments of the ribosome-associated cold shock response protein Yfia of *Escherichia coli*. J Biomol NMR 23(4):335–336

Kämpfer P (1994) Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. Syst Appl Microbiol 17:86–98

Kandror O, DeLeon A, Goldberg AL (2002) Trehalose synthesis is induced upon exposure of *Escherichia coli* to cold and is essential for viability at low temperatures. Proc Natl Acad Sci USA 99(15):9727–9732

Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Rev 55(2):288–302

Karlsen C, Paulsen SM, Tunsjo HS, Krinner S, Sorum H, Haugen P, Willassen NP (2008) Motility and flagellin gene expression in the fish pathogen *Vibrio salmonicida*: effects of salinity and temperature. Microb Pathog 45(4):258–264

Karr EA, Sattley WM, Jung DO, Madigan MT, Achenbach LA (2003) Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. Appl Environ Microbiol 69(8):4910–4914

Kasai Y, Kishira H, Syutsubo K, Harayama S (2001) Molecular detection of marine bacterial populations on beaches contaminated by the *Nakhodka* tanker oil-spill accident. Environ Microbiol 3(4):246–255

Kasana RC, Yadav SK (2007) Isolation of a psychrotrophic *Exiguobacterium* sp. SKPB5 (MTCC 7803) and characterization of its alkaline protease. Curr Microbiol 54(3):224–229

Kasana RC, Kaur B, Yadav SK (2008) Isolation and identification of a psychrotrophic *Acinetobacter* sp. CR9 and characterization of its alkaline lipase. J Basic Microbiol 48(3):207–212

Katayama T, Kato T, Tanaka M, Douglas TA, Brouchkov A, Fukuda M, Tomita F, Asano K (2009) *Glaciibacter superstes* gen. nov., sp. nov., a novel member of the family Microbacteriaceae isolated from a permafrost ice wedge. Int J Syst Evol Microbiol 59:482–486

Katayama T, Kato T, Tanaka M, Douglas TA, Brouchkov A, Abe A, Sone T, Fukuda M, Asano K (2010) *Tomitella biformata* gen. nov., sp. nov., a new member of the suborder Corynebacterineae isolated from a permafrost ice wedge. Int J Syst Evol Microbiol 60:2803–2807

Kato T, Haruki M, Imanaka T, Morikawa M, Kanaya S (2001) Isolation and characterization of psychrotrophic bacteria from oil-reservoir water and oil sands. Appl Microbiol Biotechnol 55(6):794–800

Kawamoto J, Kurihara T, Kitagawa M, Kato I, Esaki N (2007) Proteomic studies of an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, for global identification of cold-inducible proteins. Extremophiles 11(6):819–826

Kazuoka T, Takigawa S, Arakawa N, Hizukuri Y, Muraoka I, Oikawa T, Soda K (2003) Novel psychrophilic and thermolabile L-threonine dehydrogenase from psychrophilic *Cytophaga* sp. strain KUC-1. J Bacteriol 185(15):4483–4489

Kempler G, Ray B (1978) Nature of freezing damage on the lipopolysaccharide molecule of *Escherichia coli* B. Cryobiology 15(5):578–584

Kendall MM, Wardlaw GD, Tang CF, Bonin AS, Liu Y, Valentine DL (2007) Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of *Methanogenium boonei* sp. nov. Appl Environ Microbiol 73(2):407–414

Kennedy J, Flemer B, Jackson SA, Lejon DP, Morrissey JP, O'Gara F, Dobson AD (2010) Marine metagenomics: new tools for the study and exploitation of marine microbial metabolism. Mar Drugs 8(3):608–628

Khmelenina VN, Makutina VA, Kalyuzhnaya MG, Rivkina EM, Gilichinsky DA, Trotsenko Y (2002) Discovery of viable methanotrophic bacteria in permafrost sediments of northeast Siberia. Dokl Biol Sci 384:235–237

Kim HJ, Park S, Lee JM, Park S, Jung W, Kang JS, Joo HM, Seo KW, Kang SH (2008) *Moritella dasanensis* sp. nov., a psychrophilic bacterium isolated from the Arctic ocean. Int J Syst Evol Microbiol 58:817–820

Kirchman DL, Moran XA, Ducklow H (2009) Microbial growth in the polar oceans – role of temperature and potential impact of climate change. Nat Rev Microbiol 7(6):451–459

Kjelleberg S (1993) Starvation in bacteria. Kluwer, Dordrecht

Klein W, Weber MH, Marahiel MA (1999) Cold shock response of *Bacillus subtilis*: isoleucine-dependent switch in the fatty acid branching pattern for membrane adaptation to low temperatures. J Bacteriol 181(17):5341–5349

Klinkert B, Narberhaus F (2009) Microbial thermosensors. Cell Mol Life Sci 66(16):2661–2676

Knoblauch C, Jørgensen BB (1999) Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate-reducing bacteria from Arctic sediments. Environ Microbiol 1(5):457–467

Knoblauch C, Sahm K, Jorgensen BB (1999) Psychrophilic sulfate-reducing bacteria isolated from permanently cold arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov. Int J Syst Bacteriol 49:1631–1643

Koh EY, Atamna-Ismaeel N, Martin A, Cowie RO, Beja O, Davy SK, Maas EW, Ryan KG (2010) Proteorhodopsin-bearing bacteria in Antarctic sea ice. Appl Environ Microbiol 76(17):5918–5925

Könneke M, Widdel F (2003) Effect of growth temperature on cellular fatty acids in sulphate-reducing bacteria. Environ Microbiol 5(11):1064–1070

Konstantinidis KT, Braff J, Karl DM, DeLong EF (2009) Comparative metagenomic analysis of a microbial community residing at a depth of 4,000 meters at station ALOHA in the North Pacific subtropical gyre. Appl Environ Microbiol 75(16):5345–5355

Krispin O, Allmansberger R (1995) Changes in DNA supertwist as a response of *Bacillus subtilis* towards different kinds of stress. FEMS Microbiol Lett 134(2–3):129–135

LaFrentz BR, LaPatra SE, Call DR, Wiens GD, Cain KD (2009) Proteomic analysis of *Flavobacterium psychrophilum* cultured in vivo and in iron-limited media. Dis Aquat Organ 87(3):171–182

Lanoil B, Skidmore M, Priscu JC, Han S, Foo W, Vogel SW, Tulaczyk S, Engelhardt H (2009) Bacteria beneath the West Antarctic ice sheet. Environ Microbiol 11(3):609–615

Larsen MH, Koch AG, Ingmer H (2010) *Listeria monocytogenes* efficiently invades Caco-2 cells after low-temperature storage in broth and on deli meat. Foodborne Pathog Dis 7(9):1013–1018

Leblanc L, Leboeuf C, Leroi F, Hartke A, Auffray Y (2003) Comparison between NaCl tolerance response and acclimation to cold temperature in *Shewanella putrefaciens*. Curr Microbiol 46(3):157–162

Lechner S, Mayr R, Francis K, Prüß B, Kaplan T, Wießner-Gunkel E, Stewart GS, Scherer S (1998) *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group. Int J Syst Bacteriol 48(Pt 4):1373–1378

Lee SJ, Xie A, Jiang W, Etchegaray JP, Jones PG, Inouye M (1994) Family of the major cold-shock protein, CspA (CS7.4), of *Escherichia coli*, whose members show a high sequence similarity with the eukaryotic Y-box binding proteins. Mol Microbiol 11(5):833–839

Lee JH, Cho MH, Lee J (2010) 3-indolylacetonitrile decreases *Escherichia coli* O157:H7 biofilm formation and *Pseudomonas aeruginosa* virulence. Environ Microbiol 13(1):62–73

Lettinga G, Rebac S, Zeeman G (2001) Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol 19(9):363–370

Li J, Kolling GL, Matthews KR, Chikindas ML (2003) Cold and carbon dioxide used as multi-hurdle preservation do not induce appearance of viable but non-culturable *Listeria monocytogenes*. J Appl Microbiol 94(1):48–53

Lillard JW Jr, Bearden SW, Fetherston JD, Perry RD (1999) The haemin storage (Hms+) phenotype of *Yersinia pestis* is not essential for the pathogenesis of bubonic plague in mammals. Microbiology 145:197–209

Lin C, Yu RC, Chou CC (2004) Susceptibility of *Vibrio parahaemolyticus* to various environmental stresses after cold shock treatment. Int J Food Microbiol 92(2):207–215

Lipponen MT, Suutari MH, Martikainen PJ (2002) Occurrence of nitrifying bacteria and nitrification in Finnish drinking water distribution systems. Water Res 36(17):4319–4329

Liu S, Graham JE, Bigelow L, Morse PD 2nd, Wilkinson BJ (2002) Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl Environ Microbiol 68(4):1697–1705

Liu S, Bayles DO, Mason TM, Wilkinson BJ (2006) A cold-sensitive *Listeria monocytogenes* mutant has a transposon insertion in a gene encoding a putative membrane protein and shows altered (p)ppGpp levels. Appl Environ Microbiol 72(6):3955–3959

Loepfe C, Raimann E, Stephan R, Tasara T (2010) Reduced host cell invasiveness and oxidative stress tolerance in double and triple csp gene family deletion mutants of *Listeria monocytogenes*. Foodborne Pathog Dis 7(7):775–783

Lonhienne T, Gerday C, Feller G (2000) Psychrophilic enzymes: revisiting the thermodynamic parameters of activation may explain local flexibility. Biochim Biophys Acta 1543(1):1–10

Lonhienne T, Zoidakis J, Vorgias CE, Feller G, Gerday C, Bouriotis V (2001) Modular structure, local flexibility and cold-activity of a novel chitobiase from a psychrophilic Antarctic bacterium. J Mol Biol 310(2):291–297

Lopez-Garcia P, Forterre P (1999) Control of DNA topology during thermal stress in hyperthermophilic archaea: DNA topoisomerase levels, activities and induced thermotolerance during heat and cold shock in *Sulfolobus*. Mol Microbiol 33(4):766–777

Los DA (2004) The effect of low-temperature-induced DNA supercoiling on the expression of the desaturase genes in *Synechocystis*. Cell Mol Biol (Noisy-le-Grand) 50(5):605–612

Los DA, Murata N (1999) Responses to cold shock in cyanobacteria. J Mol Microbiol Biotechnol 1(2):221–230

Lundheim R (2002) Physiological and ecological significance of biological ice nucleators. Philos Trans R Soc Lond B Biol Sci 357(1423):937–943

Luttinger A, Hahn J, Dubnau D (1996) Polynucleotide phosphorylase is necessary for competence development in *Bacillus subtilis*. Mol Microbiol 19(2):343–356

Lynch WH, Franklin M (1978) Effect of temperature on the uptake of glucose, gluconate, and 2-ketogluconate by *Pseudomonas fluorescens*. Can J Microbiol 24(1):56–62

Madan Babu M, Teichmann SA (2003) Evolution of transcription factors and the gene regulatory network in *Escherichia coli*. Nucleic Acids Res 31(4):1234–1244

Madigan MT, Jung DO, Woese CR, Achenbach LA (2000) *Rhodoferax antarcticus* sp. nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an Antarctic microbial mat. Arch Microbiol 173(4):269–277

Männistö MK, Puhakka JA (2001) Temperature- and growth-phase-regulated changes in lipid fatty acid structures of psychrotolerant groundwater Proteobacteria. Arch Microbiol 177(1):41–46

Mansfield BE, Dionne MS, Schneider DS, Freitag NE (2003) Exploration of host-pathogen interactions using *Listeria monocytogenes* and *Drosophila melanogaster*. Cell Microbiol 5(12):901–911

Mansilla MC, de Mendoza D (2005) The *Bacillus subtilis* desaturase: a model to understand phospholipid modification and temperature sensing. Arch Microbiol 183(4):229–235

Maoz A, Mayr R, Bresolin G, Neuhaus K, Francis KP, Scherer S (2002) Sensitive in situ monitoring of a recombinant bioluminescent *Yersinia enterocolitica* reporter mutant in real time on Camembert cheese. Appl Environ Microbiol 68(11):5737–5740

Maraki S, Samonis G, Marnelakis E, Tselentis Y (1994) Surgical wound infection caused by *Rahnella aquatilis*. J Clin Microbiol 32(11):2706–2708

Marceau M (2005) Transcriptional regulation in *Yersinia*: an update. Curr Issues Mol Biol 7(2):151–177

Margesin R (2007) Alpine microorganisms: useful tools for low-temperature bioremediation. J Microbiol 45(4):281–285

Margesin R (2009) Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. Extremophiles 13(2):257–262

Margesin R, Fell JW (2008) *Mrakiella cryoconiti* gen. nov., sp. nov., a psychrophilic, anamorphic, basidiomycetous yeast from alpine and arctic habitats. Int J Syst Evol Microbiol 58:2977–2982

Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Res Microbiol 162(3):346–361

Margesin R, Schinner F (1999) Biodegradation of diesel oil by cold-adapted microorganisms in presence of sodium dodecyl sulfate. Chemosphere 38(15):3463–3472

Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl Microbiol Biotechnol 56(5–6):650–663

Margesin R, Labbe D, Schinner F, Greer CW, Whyte LG (2003) Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine Alpine soils. Appl Environ Microbiol 69(6):3085–3092

Margesin R, Fauster V, Fonteyne PA (2005) Characterization of cold-active pectate lyases from psychrophilic *Mrakia frigida*. Lett Appl Microbiol 40(6):453–459

Margesin R, Neuner G, Storey KB (2007) Cold-loving microbes, plants, and animals–fundamental and applied aspects. Naturwissenschaften 94(2):77–99

Martínez-Antonio A, Collado-Vides J (2003) Identifying global regulators in transcriptional regulatory networks in bacteria. Curr Opin Microbiol 6(5):482–489

Marx JC, Collins T, D'Amico S, Feller G, Gerday C (2007) Cold-adapted enzymes from marine Antarctic microorganisms. Mar Biotechnol (NY) 9(3):293–304

Mascarenhas J, Weber MH, Graumann PL (2001) Specific polar localization of ribosomes in *Bacillus subtilis* depends on active transcription. EMBO Rep 2(8):685–689

Mastronicolis SK, German JB, Megoulas N, Petron E, Foka P, Smith GM (1998) Influence of cold shock on the fatty acid composition of different classes of the food-borne pathogen *Listeria monocytogenes*. Food Microbiol 15:299–306

Mastronicolis SK, Boura A, Karaliota A, Magiatis P, Arvanitis N, Litos C, Tsakirakis A, Paraskevas P, Moustaka H, Heropoulos G (2006) Effect of cold temperature on the composition of different lipid classes of the foodborne pathogen *Listeria monocytogenes*: focus on neutral lipids. Food Microbiol 23(2):184–194

Mathy N, Jarrige AC, Robert-Le Meur M, Portier C (2001) Increased expression of *Escherichia coli* polynucleotide phosphorylase at low temperatures is linked to a decrease in the efficiency of autocontrol. J Bacteriol 183(13):3848–3854

Maxwell A, Howells AJ (1999) Overexpression and purification of bacterial DNA gyrase. Methods Mol Biol 94:135–144

Medigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin PN, Cheung F, Cruveiller S, D'Amico S, Duilio A, Fang G, Feller G, Ho C, Mangenot S, Marino G, Nilsson J, Parrilli E, Rocha EP, Rouy Z, Sekowska A, Tutino ML, Vallenet D, von Heijne G, Danchin A (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res 15(10):1325–1335

Mendez MB, Orsaria LM, Philippe V, Pedrido ME, Grau RR (2004) Novel roles of the master transcription factors Spo0A and sigmaB for survival and sporulation of *Bacillus subtilis* at low growth temperature. J Bacteriol 186(4):989–1000

Mendum ML, Smith LT (2002) Characterization of glycine betaine porter I from *Listeria monocytogenes* and its roles in salt and chill tolerance. Appl Environ Microbiol 68(2):813–819

Michaux C, Massant J, Kerff F, Frere JM, Docquier JD, Vandenberghe I, Samyn B, Pierrard A, Feller G, Charlier P, Van Beeumen J, Wouters J (2008) Crystal structure of a cold-adapted class C beta-lactamase. FEBS J 275(8):1687–1697

Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M, Ono A, Yanagawa H (1999) Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. Am J Epidemiol 150(8):787–796

Mikami K, Kanesaki Y, Suzuki I, Murata N (2002) The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp PCC 6803. Mol Microbiol 46(4):905–915

Mikucki JA, Pearson A, Johnston DT, Turchyn AV, Farquhar J, Schrag DP, Anbar AD, Priscu JC, Lee PA (2009) A contemporary microbially maintained subglacial ferrous "ocean". Science 324(5925):397–400

Minakhin L, Severinov K (2003) On the role of the *Escherichia coli* RNA polymerase sigma 70 region 4.2 and alpha-subunit C-terminal domains in promoter complex formation on the extended −10 galP1 promoter. J Biol Chem 278(32):29710–29718

Miteva VI, Brenchley JE (2005) Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. Appl Environ Microbiol 71(12):7806–7818

Miteva VI, Sheridan PP, Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 70(1):202–213

Miyazaki M, Nogi Y, Fujiwara Y, Horikoshi K (2008) *Psychromonas japonica* sp. nov., *Psychromonas aquimarina* sp. nov., *Psychromonas macrocephali* sp. nov. and *Psychromonas ossibalaenae* sp. nov., psychrotrophic bacteria isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. Int J Syst Evol Microbiol 58:1709–1714

Mondino LJ, Asao M, Madigan MT (2009) Cold-active halophilic bacteria from the ice-sealed Lake Vida, Antarctica. Arch Microbiol 191(10):785–790

Monedero V, Maze A, Boel G, Zuniga M, Beaufils S, Hartke A, Deutscher J (2007) The phosphotransferase system of *Lactobacillus casei*: regulation of carbon metabolism and connection to cold shock response. J Mol Microbiol Biotechnol 12(1–2):20–32

Moran MA, Buchan A, Gonzalez JM, Heidelberg JF, Whitman WB, Kiene RP, Henriksen JR, King GM, Belas R, Fuqua C, Brinkac L, Lewis M, Johri S, Weaver B, Pai G, Eisen JA, Rahe E, Sheldon WM, Ye W, Miller TR, Carlton J, Rasko DA, Paulsen IT, Ren Q, Daugherty SC, Deboy RT, Dodson RJ, Durkin AS, Madupu R, Nelson WC, Sullivan SA, Rosovitz MJ, Haft DH, Selengut J, Ward N (2004) Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. Nature 432(7019):910–913

Moreno JM, Sorensen HP, Mortensen KK, Sperling-Petersen HU (2000) Macromolecular mimicry in translation initiation: a model for the initiation factor IF2 on the ribosome. IUBMB Life 50(6):347–354

Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39(2):144–167

Morita RY, Albright LJ (1965) Cell yields of *Vibrio marinus*, an obligate psychrophile, at low temperature. Can J Microbiol 11:221–227

Morita MT, Tanaka Y, Kodama TS, Kyogoku Y, Yanagi H, Yura T (1999) Translational induction of heat shock transcription factor sigma32: evidence for a built-in RNA thermosensor. Genes Dev 13(6):655–665

Mountfort DO, Rainey FA, Burghardt J, Kaspar HF, Stackebrandt E (1998) Psychromonas antarcticus gen. nov., sp. nov., A new aerotolerant anaerobic, halophilic psychrophile isolated from pond sediment of the McMurdo ice shelf, antarctica. Arch Microbiol 169(3):231–238

Mountfort DO, Kaspar HF, Asher RA, Sutherland D (2003) Influences of pond geochemistry, temperature, and freeze-thaw on terminal anaerobic processes occurring in sediments of six ponds of the McMurdo Ice Shelf, near Bratina Island, Antarctica. Appl Environ Microbiol 69(1):583–592

Mujacic M, Cooper KW, Baneyx F (1999) Cold-inducible cloning vectors for low-temperature protein expression in *Escherichia coli*: application to the production of a toxic and proteolytically sensitive fusion protein. Gene 238(2):325–332

Murakawa T, Yamagata H, Tsuruta H, Aizono Y (2002) Cloning of cold-active alkaline phosphatase gene of a psychrophile, *Shewanella* sp., and

expression of the recombinant enzyme. Biosci Biotechnol Biochem 66(4):754–761

Nara T, Lee L, Imae Y (1991) Thermosensing ability of Trg and tap chemoreceptors in *Escherichia coli*. J Bacteriol 173(3):1120–1124

Nara T, Kawagishi I, Nishiyama S, Homma M, Imae Y (1996) Modulation of the thermosensing profile of the *Escherichia coli* aspartate receptor tar by covalent modification of its methyl-accepting sites. J Biol Chem 271(30):17932–17936

Narberhaus F, Waldminghaus T, Chowdhury S (2006) RNA thermometers. FEMS Microbiol Rev 30(1):3–16

Nedwell DB (1999) Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiol Ecol 30(2):101–111

Neuhaus KM (2000) Characterization of major cold shock protein genes of the psychrotolerant food pathogens *Bacillus weihenstephanensis* and *Yersinia enterocolitica*. Univ. Diss, Technical University Munich Hieronymus München. ISBN 3-89791-124-8

Neuhaus K, Rapposch S, Francis KP, Scherer S (2000) Restart of exponential growth of cold-shocked *Yersinia enterocolitica* occurs after down-regulation of cspA1/A2 mRNA. J Bacteriol 182(11):3285–3288

Neuhaus K, Anastasov N, Kaberdin VR, Francis K, Miller VL, Scherer S (2003) The AGUAAA motif in *cspA1/A2* mRNA is important for adaptation of *Yersinia enterocolitica* to grow at low temperature. Mol Microbiol 50(5):1629–1645

Nicodeme M, Perrin C, Hols P, Bracquart P, Gaillard JL (2004) Identification of an iron-binding protein of the Dps family expressed by *Streptococcus thermophilus*. Curr Microbiol 48(1):51–56

Nishiyama SI, Umemura T, Nara T, Homma M, Kawagishi I (1999) Conversion of a bacterial warm sensor to a cold sensor by methylation of a single residue in the presence of an attractant. Mol Microbiol 32(2):357–365

Nozhevnikova AN, Nekrasova VK, Kevbrina MV, Kotsyurbenko OR (2001a) Production and oxidation of methane at low temperature by the microbial population of municipal sludge checks situated in north-east Europe. Water Sci Technol 44(4):89–95

Nozhevnikova AN, Simankova MV, Parshina SN, Kotsyurbenko OR (2001b) Temperature characteristics of methanogenic archaea and acetogenic bacteria isolated from cold environments. Water Sci Technol 44(8):41–48

Nozhevnikova AN, Zepp K, Vazquez F, Zehnder AJ, Holliger C (2003) Evidence for the existence of psychrophilic methanogenic communities in anoxic sediments of deep lakes. Appl Environ Microbiol 69(3):1832–1835

O'Connell KP, Thomashow MF (2000) Transcriptional organization and regulation of a polycistronic cold shock operon in *Sinorhizobium meliloti* RM1021 encoding homologs of the *Escherichia coli* major cold shock gene *cspA* and ribosomal protein gene *rpsU*. Appl Environ Microbiol 66(1):392–400

O'Connell KP, Gustafson AM, Lehmann MD, Thomashow MF (2000) Identification of cold shock gene loci in *Sinorhizobium meliloti* by using a *luxAB* reporter transposon. Appl Environ Microbiol 66(1):401–405

O'Malley MA (2008) 'Everything is everywhere: but the environment selects': ubiquitous distribution and ecological determinism in microbial biogeography. Stud Hist Philos Biol Biomed Sci 39(3):314–325

Ochiai T, Fukunaga N, Sasaki S (1979) Purification and some properties of two $NADP^+$ −specific isocitrate dehydrogenases from an obligately psychrophilic marine bacterium, *Vibrio* sp., strain ABE-1. J Biochem (Tokyo) 86(2):377–384

Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc Natl Acad Sci USA 99(11):7663–7668

Ozcan N, Ejsing CS, Shevchenko A, Lipski A, Morbach S, Kramer R (2007) Osmolality, temperature, and membrane lipid composition modulate the activity of betaine transporter BetP in *Corynebacterium glutamicum*. J Bacteriol 189(20):7485–7496

Panoff JM, Thammavongs B, Gueguen M, Boutibonnes P (1998) Cold stress responses in mesophilic bacteria. Cryobiology 36(2):75–83

Papa R, Rippa V, Sannia G, Marino G, Duilio A (2007) An effective cold inducible expression system developed in *Pseudoalteromonas haloplanktis* TAC125. J Biotechnol 127(2):199–210

Patel GB, Sprott GD (1999) Archaeobacterial ether lipid liposomes (archaeosomes) as novel vaccine and drug delivery systems. Crit Rev Biotechnol 19(4):317–357

Pearce DA, Bridge PD, Hughes KA, Sattler B, Psenner R, Russell NJ (2009) Microorganisms in the atmosphere over Antarctica. FEMS Microbiol Ecol 69(2):143–157

Perrot F, Hebraud M, Junter GA, Jouenne T (2000) Protein synthesis in *Escherichia coli* at 4 degrees C. Electrophoresis 21(8):1625–1629

Perrot F, Hebraud M, Charlionet R, Junter GA, Jouenne T (2001) Cell immobilization induces changes in the protein response of *Escherichia coli* K-12 to a cold shock. Electrophoresis 22(10):2110–2119

Pfennig PL, Flower AM (2001) BipA is required for growth of *Escherichia coli* K12 at low temperature. Mol Genet Genomics 266(2):313–317

Phadtare S (2004) Recent developments in bacterial cold-shock response. Curr Issues Mol Biol 6(2):125–136

Phadtare S, Inouye M (1999) Sequence-selective interactions with RNA by CspB, CspC and CspE, members of the CspA family of *Escherichia coli*. Mol Microbiol 33(5):1004–1014

Phadtare S, Inouye M (2004) Genome-wide transcriptional analysis of the cold shock response in wild-type and cold-sensitive, quadruple-*csp*-deletion strains of *Escherichia coli*. J Bacteriol 186(20):7007–7014

Phadtare S, Severinov K (2010) RNA remodeling and gene regulation by cold shock proteins. RNA Biol 7(6)

Polissi A, De Laurentis W, Zangrossi S, Briani F, Longhi V, Pesole G, Deho G (2003) Changes in *Escherichia coli* transcriptome during acclimatization at low temperature. Res Microbiol 154(8):573–580

Ponder MA, Gilmour SJ, Bergholz PW, Mindock CA, Hollingsworth R, Thomashow MF, Tiedje JM (2005) Characterization of potential stress responses in ancient Siberian permafrost psychroactive bacteria. FEMS Microbiol Ecol 53(1):103–115

Ponder MA, Thomashow MF, Tiedje JM (2008) Metabolic activity of Siberian permafrost isolates, *Psychrobacter arcticus* and *Exiguobacterium sibiricum*, at low water activities. Extremophiles 12(4):481–490

Potier P, Drevet P, Gounot AM, Hipkiss AR (1990) Temperature-dependent changes in proteolytic activities and protein composition in the psychrotrophic bacterium *Arthrobacter globiformis* S_1-55. J Gen Microbiol 136:283–291

Prakash JS, Sinetova M, Zorina A, Kupriyanova E, Suzuki I, Murata N, Los DA (2009) DNA supercoiling regulates the stress-inducible expression of genes in the cyanobacterium *Synechocystis*. Mol Biosyst 5(12):1904–1912

Prévost, D, Drouin P, Antoun H (1999) The potential use of cold-adapted rhizobia to improve symbiotic nitrogen fixation in legumes cultivated in temperate regions. In: Margesin R, Schinner F (eds) Biotechnological applications of cold-adapted organisms. Springer, Berlin, pp 161–176

Price PB (2009) Microbial genesis, life and death in glacial ice. Can J Microbiol 55(1):1–11

Price PB, Sowers T (2004) Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc Natl Acad Sci USA 101(13):4631–4636

Prud'homme-Genereux A, Beran RK, Iost I, Ramey CS, Mackie GA, Simons RW (2004) Physical and functional interactions among RNase E, polynucleotide phosphorylase and the cold-shock protein, CsdA: evidence for a 'cold shock degradosome'. Mol Microbiol 54(5):1409–1421

Prüß BM, Francis KP, von Stetten F, Scherer S (1999) Correlation of 16 S ribosomal DNA signature sequences with temperature- dependent growth rates of mesophilic and psychrotolerant strains of the *Bacillus cereus* group. J Bacteriol 181(8):2624–2630

Purdy KJ, Nedwell DB, Embley TM (2003) Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting Antarctic sediments. Appl Environ Microbiol 69(6):3181–3191

Qing G, Ma LC, Khorchid A, Swapna GV, Mal TK, Takayama MM, Xia B, Phadtare S, Ke H, Acton T, Montelione GT, Ikura M, Inouye M (2004) Cold-shock induced high-yield protein production in *Escherichia coli*. Nat Biotechnol 22(7):877–882

Qoronfleh MW, Debouck C, Keller J (1992) Identification and characterization of novel low-temperature-inducible promoters of *Escherichia coli*. J Bacteriol 174(24):7902–7909

Quillaguaman J, Delgado O, Mattiasson B, Hatti-Kaul R (2004) *Chromohalobacter sarecensis* sp. nov., a psychrotolerant moderate halophile isolated from the saline Andean region of Bolivia. Int J Syst Evol Microbiol 54:1921–1926

Rajkumari K, Gowrishankar J (2001) In vivo expression from the RpoS-dependent P1 promoter of the osmotically regulated *proU* operon in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium: activation by *rho* and *hns* mutations and by cold stress. J Bacteriol 183(22):6543–6550

Rak A, Kalinin A, Shcherbakov D, Bayer P (2002) Solution structure of the ribosome-associated cold shock response protein Yfia of *Escherichia coli*. Biochem Biophys Res Commun 299(5):710–714

Ramstein J, Hervouet N, Coste F, Zelwer C, Oberto J, Castaing B (2003) Evidence of a thermal unfolding dimeric intermediate for the *Escherichia coli* histone-like HU proteins: thermodynamics and structure. J Mol Biol 331(1):101–121

Rasche F, Trondl R, Naglreiter C, Reichenauer TG, Sessitsch A (2006) Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum anuum* L.). Can J Microbiol 52(11):1036–1045

Ray MK, Sitaramamma T, Seshu Kumar G, Shivaji S (1999) Transcriptional activity at supraoptimal temperature growth in the antarctic psychrotrophic bacterium *Pseudomonas syringae*. Curr Microbiol 38:143–150

Reddy GS, Pradhan S, Manorama R, Shivaji S (2010) *Cryobacterium roopkundense* sp. nov., a psychrophilic bacterium isolated from glacial soil. Int J Syst Evol Microbiol 60:866–870

Repoila F, Gottesman S (2003) Temperature sensing by the *dsrA* promoter. J Bacteriol 185(22):6609–6614

Reva ON, Weinel C, Weinel M, Böhm K, Stjepandic D, Hoheisel JD, Tümmler B (2006) Functional genomics of stress response in *Pseudomonas putida* KT2440. J Bacteriol 188(11):4079–4092

Riley M, Staley JT, Danchin A, Wang TZ, Brettin TS, Hauser LJ, Land ML, Thompson LS (2008) Genomics of an extreme psychrophile, *Psychromonas ingrahamii*. BMC Genomics 9:210

Ritz M, Jugiau F, Federighi M, Chapleau N, de Lamballerie M (2008) Effects of high pressure, subzero temperature, and pH on survival of *Listeria monocytogenes* in buffer and smoked salmon. J Food Prot 71(8):1612–1618

Riva A, Delorme MO, Chevalier T, Guilhot N, Henaut C, Henaut A (2004) Characterization of the GATC regulatory network in *E. coli*. BMC Genomics 5(1):48

Rivkina EM, Friedmann EI, McKay CP, Gilichinsky DA (2000) Metabolic activity of permafrost bacteria below the freezing point. Appl Environ Microbiol 66(8):3230–3233

Rivkina E, Laurinavichius K, McGrath J, Tiedje J, Shcherbakova V, Gilichinsky D (2004) Microbial life in permafrost. Adv Space Res 33(8):1215–1221

Rivkina E, Shcherbakova V, Laurinavichius K, Petrovskaya L, Krivushin K, Kraev G, Pecheritsina S, Gilichinsky D (2007) Biogeochemistry of methane and methanogenic archaea in permafrost. FEMS Microbiol Ecol 61(1):1–15

Rotert KR, Toste AP, Steiert JG (1993) Membrane fatty acid analysis of Antarctic bacteria. FEMS Microbiol Lett 114(3):253–257

Rudolph C, Wanner G, Huber R (2001) Natural communities of novel archaea and bacteria growing in cold sulfurous springs with a string-of-pearls-like morphology. Appl Environ Microbiol 67(5):2336–2344

Russell NJ (1990) Cold adaptation of microorganisms. Philos Trans R Soc Lond B Biol Sci 326(1237):595–608, discussion 608–611

Russell NJ (1997) Psychrophilic bacteria–molecular adaptations of membrane lipids. Comp Biochem Physiol A Physiol 118(3):489–493

Russell NJ (1998) Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications. Adv Biochem Eng Biotechnol 61:1–21

Russell NJ (2000) Toward a molecular understanding of cold activity of enzymes from psychrophiles. Extremophiles 4(2):83–90

Russell NJ (2002) Bacterial membranes: the effects of chill storage and food processing. An overview. Int J Food Microbiol 79(1–2):27–34

Saito R, Nakayama A (2004) Differences in malate dehydrogenases from the obligately piezophilic deep-sea bacterium *Moritella* sp. strain 2D2 and the psychrophilic bacterium *Moritella* sp. strain 5710. FEMS Microbiol Lett 233(1):165–172

Sakamoto T, Murata N (2002) Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress. Curr Opin Microbiol 5(2):208–210

Sakamoto T, Higashi S, Wada H, Murata N, Bryant DA (1997) Low-temperature-induced desaturation of fatty acids and expression of desaturase genes in the cyanobacterium *Synechococcus* sp PCC 7002. FEMS Microbiol Lett 152(2):313–320

Sardesai N, Babu CR (2000) Cold stress induces switchover of respiratory pathway to lactate glycolysis in psychrotrophic *Rhizobium* strains. Folia Microbiol (Praha) 45(2):177–182

Sato N (1994) A cold-regulated cyanobacterial gene cluster encodes RNA-binding protein and ribosomal protein S21. Plant Mol Biol 24(5):819–823

Sato Y, Watanabe S, Yamaoka N, Takada Y (2008) Gene cloning of cold-adapted isocitrate lyase from a psychrophilic bacterium, *Colwellia psychrerythraea*, and analysis of amino acid residues involved in cold adaptation of this enzyme. Extremophiles 12(1):107–117

Sattley WM, Madigan MT (2010) Temperature and nutrient induced responses of Lake Fryxell sulfate-reducing prokaryotes and description of *Desulfovibrio lacusfryxellense*, sp. nov., a pervasive, cold-active, sulfate-reducing bacterium from Lake Fryxell, Antarctica. Extremophiles 14(4):357–366

Saunders NF, Thomas T, Curmi PM, Mattick JS, Kuczek E, Slade R, Davis J, Franzmann PD, Boone D, Rusterholtz K, Feldman R, Gates C, Bench S, Sowers K, Kadner K, Aerts A, Dehal P, Detter C, Glavina T, Lucas S, Richardson P, Larimer F, Hauser L, Land M, Cavicchioli R (2003) Mechanisms of thermal adaptation revealed from the genomes of the Antarctic Archaea *Methanogenium frigidum* and *Methanococcoides burtonii*. Genome Res 13(7):1580–1588

Scheyhing CH, Hörmann S, Ehrmann MA, Vogel RF (2004) Barotolerance is inducible by preincubation under hydrostatic pressure, cold-, osmotic- and acid-stress conditions in *Lactobacillus sanfranciscensis* DSM 20451 T. Lett Appl Microbiol 39(3):284–289

Schiefner A, Breed J, Bösser L, Kneip S, Gade J, Holtmann G, Diederichs K, Welte W, Bremer E (2004) Cation-pi interactions as determinants for binding of the compatible solutes glycine betaine and proline betaine by the periplasmic ligand-binding protein ProX from *Escherichia coli*. J Biol Chem 279(7):5588–5596

Schikora A, Carreri A, Charpentier E, Hirt H (2008) The dark side of the salad: *Salmonella typhimurium* overcomes the innate immune response of *Arabidopsis thaliana* and shows an endopathogenic lifestyle. PLoS One 3(5):e2279

Schlösser A, Lipski A, Schmalfuss J, Kugler F, Beckmann G (2008) *Oceaniserpentilla haliotis* gen. nov., sp. nov., a marine bacterium isolated from haemolymph serum of blacklip abalone. Int J Syst Evol Microbiol 58:2122–2125

Schmid B, Klumpp J, Raimann E, Loessner MJ, Stephan R, Tasara T (2009) Role of cold shock proteins in growth of *Listeria monocytogenes* under cold and osmotic stress conditions. Appl Environ Microbiol 75(6):1621–1627

Schmidt M, Prieme A, Stougaard P (2006) *Rhodonellum psychrophilum* gen. nov., sp. nov., a novel psychrophilic and alkaliphilic bacterium of the phylum bacteroidetes isolated from Greenland. Int J Syst Evol Microbiol 56:2887–2892

Schumann W (2007) Thermosensors in eubacteria: role and evolution. J Biosci 32(3):549–557

Secades P, Alvarez B, Guijarro JA (2003) Purification and properties of a new psychrophilic metalloprotease (Fpp 2) in the fish pathogen *Flavobacterium psychrophilum*. FEMS Microbiol Lett 226(2):273–279

Shahjee HM, Banerjee K, Ahmad F (2002) Comparative analysis of naturally occurring L-amino acid osmolytes and their D-isomers on protection of *Escherichia coli* against environmental stresses. J Biosci 27(5):515–520

Shivaji S, Prakash JS (2010) How do bacteria sense and respond to low temperature? Arch Microbiol 192(2):85–95

Shivers RP, Dineen SS, Sonenshein AL (2006) Positive regulation of *Bacillus subtilis* ackA by CodY and CcpA: establishing a potential hierarchy in carbon flow. Mol Microbiol 62(3):811–822

Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–433

Sifri CD, Begun J, Ausubel FM (2005) The worm has turned–microbial virulence modeled in *Caenorhabditis elegans*. Trends Microbiol 13(3):119–127

Simankova MV, Kotsyurbenko OR, Lueders T, Nozhevnikova AN, Wagner B, Conrad R, Friedrich MW (2003) Isolation and characterization of new strains of methanogens from cold terrestrial habitats. Syst Appl Microbiol 26(2):312–318

Simon C, Wiezer A, Strittmatter AW, Daniel R (2009) Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. Appl Environ Microbiol 75(23):7519–7526

Sinensky M (1974) Homeoviscous adaptation–a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci USA 71(2):522–525

Smirnova A, Li H, Weingart H, Aufhammer S, Burse A, Finis K, Schenk A, Ullrich MS (2001) Thermoregulated expression of virulence factors in plant-associated bacteria. Arch Microbiol 176(6):393–399

Smirnova AV, Wang L, Rohde B, Budde I, Weingart H, Ullrich MS (2002) Control of temperature-responsive synthesis of the phytotoxin coronatine in *Pseudomonas syringae* by the unconventional two-component system CorRPS. J Mol Microbiol Biotechnol 4(3):191–196

Smith B, Oliver JD (2006) In situ and in vitro gene expression by *Vibrio vulnificus* during entry into, persistence within, and resuscitation from the viable but nonculturable state. Appl Environ Microbiol 72(2):1445–1451

Smyth CP, Lundback T, Renzoni D, Siligardi G, Beavil R, Layton M, Sidebotham JM, Hinton JC, Driscoll PC, Higgins CF, Ladbury JE (2000) Oligomerization of the chromatin-structuring protein H-NS. Mol Microbiol 36(4):962–972

Soares A, Guieysse B, Delgado O, Mattiasson B (2003) Aerobic biodegradation of nonylphenol by cold-adapted bacteria. Biotechnol Lett 25(9):731–738

Srikumar S, Fuchs TM (2011) Ethanolamine utilization contributes to proliferation of *Salmonella enterica* serovar Typhimurium in food and in nematodes. Appl Environ Microbiol 77(1):281–290

Stasiewicz MJ, Wiedmann M, Bergholz TM (2010) The combination of lactate and diacetate synergistically reduces cold growth in brain heart infusion broth across *Listeria monocytogenes* lineages. J Food Prot 73(4):631–640

Stefanidi E, Vorgias CE (2008) Molecular analysis of the gene encoding a new chitinase from the marine psychrophilic bacterium *Moritella marina* and biochemical characterization of the recombinant enzyme. Extremophiles 12(4):541–552

Straley SC, Perry RD (1995) Environmental modulation of gene expression and pathogenesis in *Yersinia*. Trends Microbiol 3(8):310–317

Stülke J (2002) Control of transcription termination in bacteria by RNA-binding proteins that modulate RNA structures. Arch Microbiol 177(6):433–440

Suetin SV, Shcherbakova VA, Chuvilskaya NA, Rivkina EM, Suzina NE, Lysenko AM, Gilichinsky DA (2009) *Clostridium tagluense* sp. nov., a psychrotolerant, anaerobic, spore-forming bacterium from permafrost. Int J Syst Evol Microbiol 59:1421–1426

Suutari M, Laakso S (1992) Unsaturated and branched chain-fatty acids in temperature adaptation of *Bacillus subtilis* and *Bacillus megaterium*. Biochim Biophys Acta 1126(2):119–124

Suzuki I, Los DA, Kanesaki Y, Mikami K, Murata N (2000) The pathway for perception and transduction of low-temperature signals in *Synechocystis*. EMBO J 19(6):1327–1334

Suzuki Y, Haruki M, Takano K, Morikawa M, Kanaya S (2004) Possible involvement of an FKBP family member protein from a psychrotrophic bacterium *Shewanella* sp. SIB1 in cold-adaptation. Eur J Biochem 271(7):1372–1381

Takeuchi S, Mandai Y, Otsu A, Shirakawa T, Masuda K, Chinami M (2003) Differences in properties between human alphaA- and alphaB-crystallin proteins expressed in *Escherichia coli* cells in response to cold and extreme pH. Biochem J 375:471–475

Tanabe H, Goldstein J, Yang M, Inouye M (1992) Identification of the promoter region of the *Escherichia coli* major cold shock gene, *cspA*. J Bacteriol 174(12):3867–3873

Taormina PJ (2010) Implications of salt and sodium reduction on microbial food safety. Crit Rev Food Sci Nutr 50(3):209–227

Tasaka Y, Gombos Z, Nishiyama Y, Mohanty P, Ohba T, Ohki K, Murata N (1996) Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis*: evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis. EMBO J 15(23):6416–6425

Tendeng C, Krin E, Soutourina OA, Marin A, Danchin A, Bertin PN (2003) A Novel H-NS-like protein from an antarctic psychrophilic bacterium reveals a crucial role for the N-terminal domain in thermal stability. J Biol Chem 278(21):18754–18760

Thomas DN, Dieckmann GS (2002) Antarctic Sea ice–a habitat for extremophiles. Science 295(5555):641–644

Ting L, Williams TJ, Cowley MJ, Lauro FM, Guilhaus M, Raftery MJ, Cavicchioli R (2010) Cold adaptation in the marine bacterium, *Sphingopyxis alaskensis*, assessed using quantitative proteomics. Environ Microbiol 12 (10):2658–2676

Tobe T, Yoshikawa M, Mizuno T, Sasakawa C (1993) Transcriptional control of the invasion regulatory gene *virB* of *Shigella flexneri*: activation by *virF* and repression by H-NS. J Bacteriol 175(19):6142–6149

Trotsenko YA, Khmelenina VN (2002) Biology of extremophilic and extremotolerant methanotrophs. Arch Microbiol 177(2):123–131

Trotsenko YA, Khmelenina VN (2005) Aerobic methanotrophic bacteria of cold ecosystems. FEMS Microbiol Ecol 53(1):15–26

Tse-Dinh YC, Qi H, Menzel R (1997) DNA supercoiling and bacterial adaptation: thermotolerance and thermoresistance. Trends Microbiol 5(8):323–326

Tsuruta H, Tamura J, Yamagata H, Aizono Y (2004) Specification of amino acid residues essential for the catalytic reaction of cold-active protein-tyrosine phosphatase of a psychrophile, *Shewanella* sp. Biosci Biotechnol Biochem 68(2):440–443

Tutino ML, Duilio A, Parrilli R, Remaut E, Sannia G, Marino G (2001) A novel replication element from an Antarctic plasmid as a tool for the expression of proteins at low temperature. Extremophiles 5(4):257–264

Tyler HL, Triplett EW (2008) Plants as a habitat for beneficial and/or human pathogenic bacteria. Annu Rev Phytopathol 46:53–73

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428(6978):37–43

Vallet-Gely I, Lemaitre B, Boccard F (2008) Bacterial strategies to overcome insect defences. Nat Rev Microbiol 6(4):302–313

Van Petegem F, Collins T, Meuwis MA, Gerday C, Feller G, Van Beeumen J (2003) The structure of a cold-adapted family 8 xylanase at 1.3 A resolution. Structural adaptations to cold and investgation of the active site. J Biol Chem 278(9):7531–7539

VanBogelen RA, Neidhardt FC (1990) Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc Natl Acad Sci USA 87(15):5589–5593

Vandieken V, Mussmann M, Niemann H, Jorgensen BB (2006) *Desulfuromonas svalbardensis* sp. nov. and *Desulfuromusa ferrireducens* sp. nov., psychrophilic, Fe(III)-reducing bacteria isolated from Arctic sediments, Svalbard. Int J Syst Evol Microbiol 56:1133–1139

Varkonyi Z, Masamoto K, Debreczeny M, Zsiros O, Ughy B, Gombos Z, Domonkos I, Farkas T, Wada H, Szalontai B (2002) Low-temperature-induced accumulation of xanthophylls and its structural consequences in the photosynthetic membranes of the cyanobacterium *Cylindrospermopsis raciborskii*: an FTIR spectroscopic study. Proc Natl Acad Sci USA 99(4):2410–2415

Veldhuizen EJ, Creutzberg TO, Burt SA, Haagsman HP (2007) Low temperature and binding to food components inhibit the antibacterial activity of carvacrol against *Listeria monocytogenes* in steak tartare. J Food Prot 70(9):2127–2132

Vidovic S, Mangalappalli-Illathu AK, Korber DR (2011) Prolonged cold stress response of *Escherichia coli* O157 and the role of *rpoS*. Int J Food Microbiol 146(2):163–169

Vila-Sanjurjo A, Schuwirth BS, Hau CW, Cate JH (2004) Structural basis for the control of translation initiation during stress. Nat Struct Mol Biol 11(11):1054–1059

Violot S, Aghajari N, Czjzek M, Feller G, Sonan GK, Gouet P, Gerday C, Haser R, Receveur-Brechot V (2005) Structure of a full length psychrophilic cellulase from *Pseudoalteromonas haloplanktis* revealed by X-ray diffraction and small angle X-ray scattering. J Mol Biol 348(5):1211–1224

von Stetten F, Francis K, Lechner S, Neuhaus K, Scherer S (1998) Rapid discrimination of psychrotolerant and mesophilic strains of the *Bacillus cereus* group by PCR targeting of 16 S rDNA. J Microbiol Methods 34:99–106

von Stetten F, Mayr R, Scherer S (1999) Climatic influence on mesophilic *Bacillus cereus* and psychrotolerant *Bacillus weihenstephanensis* populations in tropical, temperate and alpine soil. Environ Microbiol 1(6):503–515

Vorachek-Warren MK, Carty SM, Lin S, Cotter RJ, Raetz CR (2002) An *Escherichia coli* mutant lacking the cold shock-induced palmitoleoyltransferase of lipid

A biosynthesis: absence of unsaturated acyl chains and antibiotic hypersensitivity at 12 degrees C. J Biol Chem 277(16):14186–14193

Walker D, Rolfe M, Thompson A, Moore GR, James R, Hinton JC, Kleanthous C (2004) Transcriptional profiling of colicin-induced cell death of *Escherichia coli* MG1655 identifies potential mechanisms by which bacteriocins promote bacterial diversity. J Bacteriol 186(3):866–869

Walter EH, Kabuki DY, Esper LM, Sant'Ana AS, Kuaye AY (2009) Modelling the growth of *Listeria monocytogenes* in fresh green coconut (*Cocos nucifera* L.) water. Food Microbiol 26(6):653–657

Wang JY, Syvanen M (1992) DNA twist as a transcriptional sensor for environmental changes. Mol Microbiol 6(14):1861–1866

Wang W, Sun M, Liu W, Zhang B (2008) Purification and characterization of a psychrophilic catalase from Antarctic *Bacillus*. Can J Microbiol 54(10):823–828

Watanabe S, Yamaoka N, Takada Y, Fukunaga N (2002) The cold-inducible *icl* gene encoding thermolabile isocitrate lyase of a psychrophilic bacterium, *Colwellia maris*. Microbiology 148(Pt 8):2579–2589

Waterfield NR, Wren BW, Ffrench-Constant RH (2004) Invertebrates as a source of emerging human pathogens. Nat Rev Microbiol 2(10):833–841

Weber MH, Marahiel MA (2003) Bacterial cold shock responses. Sci Prog 86:9–75

Weber MH, Klein W, Müller L, Niess UM, Marahiel MA (2001) Role of the *Bacillus subtilis* fatty acid desaturase in membrane adaptation during cold shock. Mol Microbiol 39(5):1321–1329

Wemekamp-Kamphuis HH, Sleator RD, Wouters JA, Hill C, Abee T (2004a) Molecular and physiological analysis of the role of osmolyte transporters BetL, Gbu, and OpuC in growth of *Listeria monocytogenes* at low temperatures. Appl Environ Microbiol 70(5):2912–2918

Wemekamp-Kamphuis HH, Wouters JA, de Leeuw PP, Hain T, Chakraborty T, Abee T (2004b) Identification of sigma factor sigma B-controlled genes and their impact on acid stress, high hydrostatic pressure, and freeze survival in *Listeria monocytogenes* EGD-e. Appl Environ Microbiol 70(6):3457–3466

White-Ziegler CA, Um S, Perez NM, Berns AL, Malhowski AJ, Young S (2008) Low temperature (23 degrees C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. Microbiology 154:148–166

Wick LM, Egli T (2004) Molecular components of physiological stress responses in *Escherichia coli*. Adv Biochem Eng Biotechnol 89:1–45

Williams RM, Rimsky S (1997) Molecular aspects of the *E. coli* nucleoid protein, H-NS: a central controller of gene regulatory networks. FEMS Microbiol Lett 156(2):175–185

Wouters JA, Kamphuis HH, Hugenholtz J, Kuipers OP, de Vos WM, Abee T (2000a) Changes in glycolytic activity of *Lactococcus lactis* induced by low temperature. Appl Environ Microbiol 66(9):3686–3691

Wouters JA, Rombouts FM, Kuipers OP, de Vos WM, Abee T (2000b) The role of cold-shock proteins in low-temperature adaptation of food-related bacteria. Syst Appl Microbiol 23(2):165–173

Xia B, Etchegaray JP, Inouye M (2001a) Nonsense mutations in *cspA* cause ribosome trapping leading to complete growth inhibition and cell death at low temperature in *Escherichia coli*. J Biol Chem 276(38):35581–35588

Xia B, Ke H, Inouye M (2001b) Acquirement of cold sensitivity by quadruple deletion of the *cspA* family and its suppression by PNPase S1 domain in *Escherichia coli*. Mol Microbiol 40(1):179–188

Xia B, Ke H, Jiang W, Inouye M (2002) The Cold Box stem-loop proximal to the 5′-end of the *Escherichia coli cspA* gene stabilizes its mRNA at low temperature. J Biol Chem 277(8):6005–6011

Xia B, Ke H, Shinde U, Inouye M (2003) The role of RbfA in 16 S rRNA processing and cell growth at low temperature in *Escherichia coli*. J Mol Biol 332 (3):575–584

Xing W, Zhao Y, Zuo JE (2010) Microbial activity and community structure in a lake sediment used for psychrophilic anaerobic wastewater treatment. J Appl Microbiol 109(5):1829–1837

Xu Y, Feller G, Gerday C, Glansdorff N (2003a) Metabolic enzymes from psychrophilic bacteria: challenge of adaptation to low temperatures in ornithine carbamoyltransferase from *Moritella abyssi*. J Bacteriol 185(7):2161–2168

Xu Y, Feller G, Gerday C, Glansdorff N (2003b) *Moritella* cold-active dihydrofolate reductase: are there natural limits to optimization of catalytic efficiency at low temperature? J Bacteriol 185(18):5519–5526

Yamanaka K, Fang L, Inouye M (1998) The CspA family in *Escherichia coli*: multiple gene duplication for stress adaptation. Mol Microbiol 27(2):247–255

Yamanaka K, Inouye M, Inouye S (1999a) Identification and characterization of five *cspA* homologous genes from *Myxococcus xanthus*. Biochim Biophys Acta 1447(2–3):357–365

Yamanaka K, Mitta M, Inouye M (1999b) Mutation analysis of the 5′ untranslated region of the cold shock cspA mRNA of *Escherichia coli*. J Bacteriol 181(20):6284–6291

Yang SH, Kwon KK, Lee HS, Kim SJ (2006) *Shewanella spongiae* sp. nov., isolated from a marine sponge. Int J Syst Evol Microbiol 56:2879–2882

Yang SH, Lee JH, Ryu JS, Kato C, Kim SJ (2007) *Shewanella donghaensis* sp. nov., a psychrophilic, piezosensitive bacterium producing high levels of polyunsaturated fatty acid, isolated from deep-sea sediments. Int J Syst Evol Microbiol 57:208–212

Yang X, Lin X, Fan T, Bian J, Huang X (2008) Cloning and expression of *lipP*, a gene encoding a cold-adapted lipase from *Moritella* sp.2-5-10-1. Curr Microbiol 56(2):194–198

Ygberg SE, Clements MO, Rytkonen A, Thompson A, Holden DW, Hinton JC, Rhen M (2006) Polynucleotide phosphorylase negatively controls *spv* virulence gene expression in *Salmonella enterica*. Infect Immun 74(2):1243–1254

Yi H, Chun J (2006) *Flavobacterium weaverense* sp. nov. and *Flavobacterium segetis* sp. nov., novel psychrophiles isolated from the Antarctic. Int J Syst Evol Microbiol 56:1239–1244

Yu Y, Xin YH, Liu HC, Chen B, Sheng J, Chi ZM, Zhou PJ, Zhang DC (2008) *Sporosarcina antarctica* sp. nov., a psychrophilic bacterium isolated from the Antarctic. Int J Syst Evol Microbiol 58:2114–2117

Zangrossi S, Briani F, Ghisotti D, Regonesi ME, Tortora P, Deho G (2000) Transcriptional and post-transcriptional control of polynucleotide phosphorylase during cold acclimation in *Escherichia coli*. Mol Microbiol 36:1470–1480

Zhang DC, Wang HX, Liu HC, Dong XZ, Zhou PJ (2006a) *Flavobacterium glaciei* sp. nov., a novel psychrophilic bacterium isolated from the China No.1 glacier. Int J Syst Evol Microbiol 56:2921–2925

Zhang DC, Yu Y, Chen B, Wang HX, Liu HC, Dong XZ, Zhou PJ (2006b) *Glaciecola psychrophila* sp. nov., a novel psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 56:2867–2869

Zhang DC, Li HR, Xin YH, Chi ZM, Zhou PJ, Yu Y (2008a) *Marinobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1463–1466

Zhang DC, Li HR, Xin YH, Liu HC, Chi ZM, Zhou PJ, Yu Y (2008b) *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. Int J Syst Evol Microbiol 58:1384–1387

Zhang DC, Liu HC, Xin YH, Yu Y, Zhou PJ, Zhou YG (2008c) *Salinibacterium xinjiangense* sp. nov., a psychrophilic bacterium isolated from the China No. 1 glacier. Int J Syst Evol Microbiol 58:2739–2742

Zhang GI, Hwang CY, Kang SH, Cho BC (2009) *Maribacter antarcticus* sp. nov., a psychrophilic bacterium isolated from a culture of the Antarctic green alga *Pyramimonas gelidicola*. Int J Syst Evol Microbiol 59:1455–1459

Zhang DC, Busse HJ, Liu HC, Zhou YG, Schinner F, Margesin R (2010a) *Sphingomonas glacialis* sp. nov., a psychrophilic bacterium isolated from alpine glacier cryoconite. Int J Syst Evol Microbiol 61:587–591

Zhang DC, Liu HC, Xin YH, Zhou YG, Schinner F, Margesin R (2010b) *Dyadobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from soil. Int J Syst Evol Microbiol 60:1640–1643

Zhang DC, Liu HC, Xin YH, Zhou YG, Schinner F, Margesin R (2010c) *Luteimonas terricola* sp. nov., a psychrophilic bacterium isolated from soil. Int J Syst Evol Microbiol 60:1581–1584

Zhang DC, Liu HC, Xin YH, Zhou YG, Schinner F, Margesin R (2010d) *Sphingopyxis bauzanensis* sp. nov., a psychrophilic bacterium isolated from soil. Int J Syst Evol Microbiol 60:2618–2622

Zhang DC, Redzic M, Schinner F, Margesin R (2010e) *Glaciimonas immobilis* gen. nov., sp. nov., a novel member of the family Oxalobacteraceae isolated from alpine glacier cryoconite. Int J Syst Evol Microbiol 61(Pt 9):2186–2190

Zhang DC, Schumann P, Liu HC, Xin YH, Zhou YG, Schinner F, Margesin R (2010f) *Arthrobacter alpinus* sp. nov., a psychrophilic bacterium isolated from alpine soil. Int J Syst Evol Microbiol 60:2149–2153

Zhang DC, Busse HJ, Liu HC, Zhou YG, Schinner F, Margesin R (2011) *Hymenobacter psychrophilus* sp. nov., a psychrophilic bacterium isolated from soil. Int J Syst Evol Microbiol 61:859–863

Zhao JS, Manno D, Thiboutot S, Ampleman G, Hawari J (2007) *Shewanella canadensis* sp. nov. and *Shewanella atlantica* sp. nov., manganese dioxide- and hexahydro-1,3,5-trinitro-1,3,5-triazine-reducing, psychrophilic marine bacteria. Int J Syst Evol Microbiol 57:2155–2162

Zhao JS, Deng Y, Manno D, Hawari J (2010) *Shewanella* spp. genomic evolution for a cold marine lifestyle and in-situ explosive biodegradation. PLoS One 5(2):e9109

19 Life at High Salt Concentrations

Aharon Oren
Division of Microbial and Molecular Ecology, The Institute of Life Sciences, and The Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

Introduction

A great variety of prokaryotes, Bacteria as well as Archaea, can be found in saline and hypersaline environments. These microorganisms are adapted to life at high salt concentrations and to the high osmotic pressure of their environment resulting from the high salinity. This chapter presents a general overview of the hypersaline environments as biotopes for prokaryotic life, the types of organisms encountered in them, and the mechanisms the different groups of prokaryotes have developed to cope with the special requirements of life in the presence of molar concentrations of salt. More detailed information on the variety of halophilic organisms can be found in the specific chapters that deal with the different taxonomic groups.

Saline and Hypersaline Habitats

The greatest part of the biosphere is saline. The waters of the oceans and seas that cover most of the earth's surface contain around 35 g dissolved salts per liter. Higher salt concentrations are often encountered in near-shore environments such as salt marshes, sabkhas, and lagoons, under conditions in which evaporation is rapid and water exchange with the open sea is slow. Still higher concentrations of salts, up to saturation of NaCl and beyond, exist in natural inland salt lakes such as the Dead Sea on the border between Israel and Jordan (currently about 346 g/l total dissolved salts), Great Salt Lake, Utah (270–300 g/l salt in the North Arm – 2008 values), and many others. Gradients of increasing salt concentrations are found in the man-made evaporation ponds and crystallizer basins of multi-pond solar saltern systems near tropical and subtropical shores worldwide. All these environments, from seawater salinity to NaCl-saturated brines, are potential habitats for prokaryotic life (Grant 2004; Oren 2002b, 2007; Rodriguez-Valera 1988, 1993). Additional hypersaline environments inhabited by salt-tolerating (halotolerant) and salt-loving (halophilic) microorganisms are salted food products such as salted fish, animal hides treated with salt for their preservation, saline soils, subterranean brines that are often associated with oil fields, and mural paintings and rock paintings in prehistoric caves (Saiz-Jimenez and Laiz 2000). Unusual hypersaline environments recently explored for their prokaryote diversity include salt glands in the nostrils of seabirds (Brito-Echeverría et al. 2009), polar glaciers, and brines on the deep-sea floor (Boetius and Joye 2009; Daffonchio et al. 2006; van der Wielen et al. 2005).

The properties of hypersaline environments as habitats for halophilic and halotolerant prokaryotes are primarily defined according to the total salt concentration. However, also the ionic composition is a key factor determining the properties of the environment as a biotope. Brines that originated by evaporation of seawater (so-called thalassohaline brines) reflect the ionic composition of the sea, at least during the first stages of evaporation (*Fig. 19.1*). The ionic composition starts to change significantly when evaporation proceeds to the stage at which the solubility limit of $CaSO_4$ is reached and gypsum precipitates (at a total salt concentration above 100–120 g/l). The brines that enter saltern crystallizer ponds in multi-pond salterns are thus depleted in calcium and to a minor extent in sulfate. During the subsequent precipitation of NaCl as halite, the ionic composition changes again, and the relative concentrations of K^+ and Mg^{2+} increase. Great Salt Lake, Utah, though since long detached from the world ocean, still reflects in its ionic composition the seawater that contributed its salt, and therefore, its waters can still be classified as thalassohaline. Thalassohaline brines are characterized by neutral or slightly alkaline pH values (7–8).

In other hypersaline environments, the ionic composition may greatly differ from that of seawater ("athalassohaline environments"). The Dead Sea is a prime example of an athalassohaline lake. Here, divalent cations dominate, with concentrations of Mg^{2+} (1.98 M) and Ca^{2+} (0.47 M) exceeding those of Na^+ (1.54 M) and K^+ (0.21 M) (2007 values). As a result of the high Ca^{2+} concentration, the solubility of sulfate is low and

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_57,

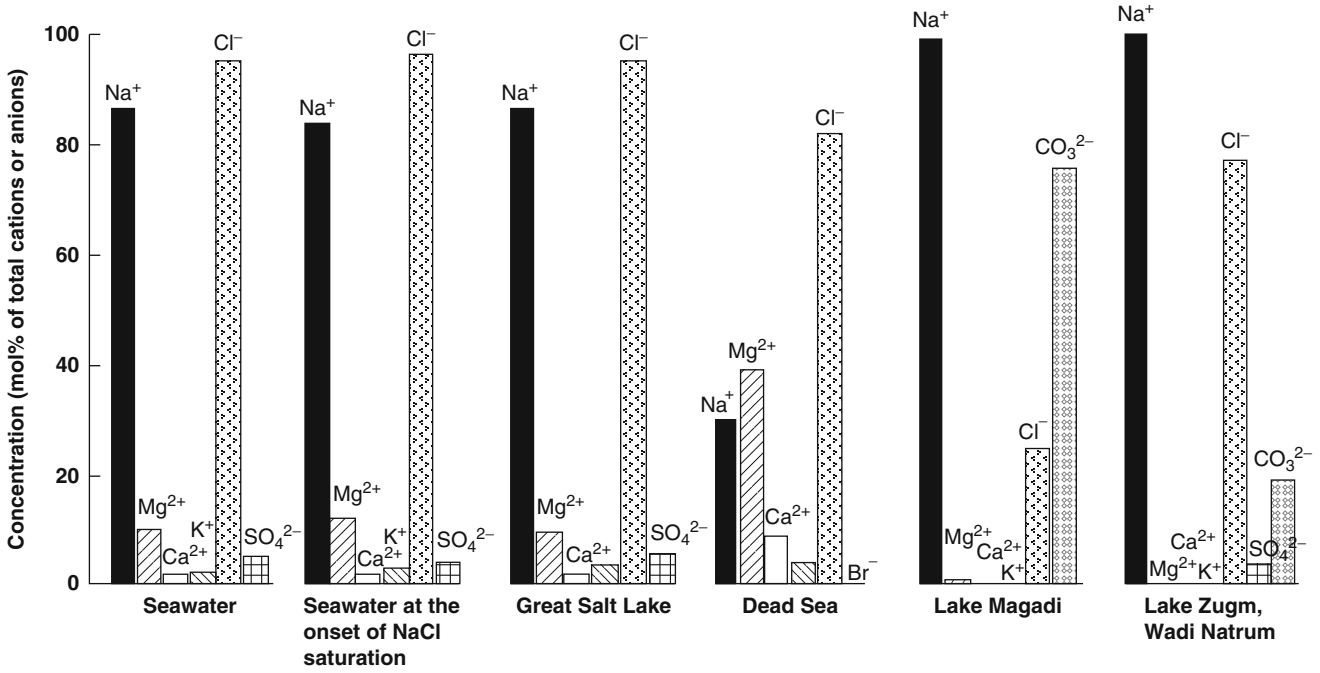


Fig. 19.1

The ionic composition of seawater and selected hypersaline environments. The *bars* represent the mol fraction of different cations and anions in the brines. Data for seawater, saltern brines, and Great Salt Lake were derived from Javor (1989), and data on Lake Magadi (analyses for 1976) and on Lake Zugm, Wadi Natrun, Egypt, were from Grant and Tindall (1986) and from Grant et al. (1998a), respectively. Dead Sea data (average composition for 2007) were obtained from Dr. Ittai Gavrieli (the Geological Survey of Israel, personal communication)

monovalent anions (Cl^- and Br^-) make up more than 99.9 % of the anion sum (*Fig. 19.1*). The pH of the Dead Sea brine is relatively low, around 6.0.

Alkaline athalassohaline "soda lakes" brines are present in diverse geographic locations such as in East Africa (Lake Magadi and other lakes in Kenya and Tanganyika), in the Wadi Natrun in Egypt, and in California, Nevada, India, Tibet, China, and elsewhere. Here, the salt composition is dominated by monovalent cations. Because of the high pH (up to 10–11 and higher), the solubility of the divalent cations Mg^{2+} and Ca^{2+} is very low, and the concentrations of these ions may be below the detection limit. Carbonate and bicarbonate ions contribute a significant part of the anion sum in such lakes, in addition to chloride and sulfate.

Classification and Phylogeny of Prokaryotes Living at High Salt Concentrations

Microorganisms adapted to life at high salt concentrations are widespread, both within the bacterial and archaeal domains. Highly diverse prokaryote communities can be found at all salt concentrations from seawater up to about 340–350 g/l (brines saturated with NaCl) in both thalassohaline and athalassohaline environments (Oren 2002a, b, 2007, 2010a, b). A few microorganisms can adapt to life over the whole salt concentration range from near fresh water to halite saturation. *Halomonas elongata* is a well-known example of such a bacterium (Vreeland et al. 1980). In most cases, however, each organism has a relatively restricted salt concentration range enabling growth. Some bacteria are adapted to life in saturated and near-saturated brines, being unable to grow and even survive at NaCl concentrations below 15–20 %. Most representatives of the halophilic Archaea of the order Halobacteriales show such a behavior (Oren 2006a); *Salinibacter ruber* (Bacteroidetes) is another example (Antón et al. 2002; Oren 2006c; Oren et al. 2004). Others thrive at an intermediate salt concentration range. Salt requirement and tolerance may be temperature dependent, salt tolerance and requirement being enhanced at increased temperatures (see e.g., Mullakhanbhai and Larsen 1975; Novitsky and Kushner 1975).

Different classification schemes have been designed to define the salt relationships of microorganisms. All such schemes are artificial to some extent. Because of the continuum of properties found within the prokaryote world, there will always be organisms that cannot unequivocally be classified within any of the groups defined. The most widely accepted classification according to salt dependence and salt tolerance is that of Kushner (1978, 1985), given in a slightly modified form in *Table 19.1*. This scheme recognizes different degrees of salt dependence (slightly, moderately, and extremely halophilic). In addition, halotolerant microorganisms exist that, while not requiring high salt concentrations for growth, are able to grow at often high concentrations of NaCl and other salts.

Table 19.1
Classification of microorganisms according to their response to salt

Category	Properties	Examples
Non-halophilic	Grows best in media containing less than 0.2 M salt	Most freshwater bacteria
Slight halophile	Grows best in media containing 0.2–0.5 M salt	Most marine bacteria
Moderate halophile	Grows best in media containing 0.5–2.5 M salt	*Salinivibrio costicola*
		Halomonas elongata
Borderline extreme halophile	Grows best in media containing 1.5–4.0 M salt	*Halorhodospira halophila*
Extreme halophile	Grows best in media containing 2.5–5.2 M salt	*Halobacterium salinarum*
		Salinibacter ruber
Halotolerant	Non-halophile which can tolerate salt; if the growth range extends above 2.5 M salt, it may be considered extremely halotolerant	*Staphylococcus aureus*

The table is based on classification schemes proposed by Kushner (1978, 1985)

Staphylococcus species present a good example for this category, as they grow well both in the absence of salt and at NaCl concentrations as high as 10–15 % and even higher, a property often exploited in the design of selective and diagnostic growth media.

It should be noted that classification should be based not only on the behavior toward NaCl but to other ions as well, especially for organisms adapted to life in athalassohaline environments (Edgerton and Brimblecombe 1981). The Dead Sea is a high magnesium and high calcium, relatively low sodium environment. Some deep-sea brines are also exceedingly high in magnesium, a chaotropic (destabilizing) ion which at high concentrations is hostile to life. A recent study of the brines of Discovery Basin, a hypersaline brine pool on the bottom of the Mediterranean Sea, defined the upper limit of $MgCl_2$ concentration compatible to life at 2.3 M (in the absence of stabilizing "kosmotropic" ions such as sodium) (Hallsworth et al. 2007). Chloride is specifically required by some halophiles, Archaea (Halobacteriaceae) as well as Bacteria (*Salinibacter ruber*, *Halobacillus halophilus*) (Müller and Oren 2003; Roeßler and Müller 2002).

Halophilic behavior is found all over the phylogenetic tree of the prokaryotes, both within the Archaea and the Bacteria (Oren 2002a, 2008, 2010a). Within the archaeal domain, growth at salt concentrations above 15–20 % has been documented not only in the Halobacteriales but also in the methanogenic genus *Methanohalophilus* (family *Methanosarcinaceae*). Most halophilic Bacteria characterized belong to the Gammaproteobacteria, but moderate halophiles can also be found in other subgroups of the Proteobacteria, the low G+C and the high G+C Firmicutes, the cyanobacterial branch, the Bacteroidetes branch, and the Spirochaetes (*Fig. 19.2*) (Ventosa et al. 1998).

The archaeal order of the Halobacteriales with a single family, the Halobacteriaceae, contains the extreme halophiles par excellence. These are highly specialized microorganisms, most of which will not grow at total salt concentrations below 2.5–3 M. When suspended in solutions containing less than 1–2 M salt, cells of most species are irreversibly damaged, and in many cases, lysis occurs. Some of the early studies on this unique group of prokaryotes were summarized in Larsen's classic essay on "the halobacteria's confusion to biology" (Larsen 1973), and a full account of their properties can be found elsewhere (e.g., Kushner 1985; Oren 1994, 2006a; Tindall and Trüper 1986). The first genome sequence of a member of the Halobacteriaceae, *Halobacterium* sp. NRC-1, was released in 2000 (Ng et al. 2000), and at least ten species of the family have already been sequenced, including *Haloferax volcanii* (Hartman et al. 2010), *Haloarcula marismortui* (Baliga et al. 2004), the square flat-celled *Haloquadratum walsbyi* (Bolhuis et al. 2006), and the haloalkaliphilic *Natronomonas pharaonis* (Falb et al. 2005); see http://halo4.umbi.umd.edu/ for an overview. Analysis of the genomic information enabled a reconstruction of the metabolic patterns used by the Halobacteriaceae (Falb et al. 2008).

One of the most interesting advances in recent years was the cultivation of the elusive flat, square Archaea first observed by Walsby (1980) in a brine pool on the Sinai Peninsula, Egypt, and since then found abundantly in saltern crystallizer ponds worldwide. The organism was first isolated in 2004 (Bolhuis et al. 2004; Burns et al. 2004a; see also Walsby 2005) and described as *Haloquadratum walsbyi* (Burns et al. 2007).

In the past, it was assumed that the Halobacteriaceae all require salt concentrations above 15–20 % for growth and do not survive at lower concentrations. However, less salt-requiring representatives of the group are increasingly found in a variety of environments, including some unexpected niches. A low-salt (< 1.2 %) sulfidic spring in Oklahoma yielded isolates such as *Haladaptatus paucihalophilus*, *Halosarcina pallida*, and *Haloferax sulfurifontis* organisms growing at NaCl concentrations as low as 4.7–6 % (Elshahed et al. 2004a, b; Savage et al. 2007, 2008). Low-salt-requiring isolates were also obtained from a marine salt marsh (Purdy et al. 2004). Even more unexpected was the isolation of *Haloarcula* sp. from steam vent waters of fumaroles in Kamchatka, Hawaii, New Mexico, California, and Wyoming (Ellis et al. 2008). The recent finding of 16S rRNA sequences associated with the Halobacteriaceae in intestinal mucosa of human patients with inflammatory bowel disease

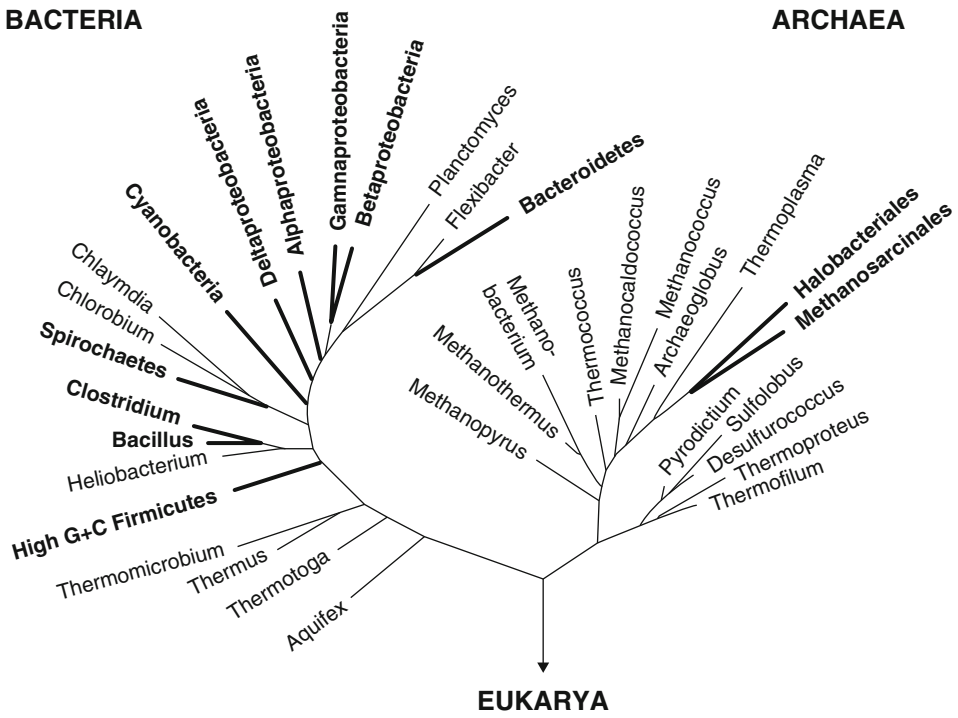


Fig. 19.2

Phylogenetic tree of the Bacteria and the Archaea, based on 16S rRNA sequence comparisons, indicating the distribution of halophilism. *Bold lines* indicate branches containing representatives able to grow at or near optimal rates at NaCl concentrations exceeding 15 %

also deserves a more in-depth study of the nature of the organisms involved (Oxley et al. 2010).

Halophilic Archaea may survive for prolonged periods within halite crystals. This property has aroused considerable interest in recent years, following the isolation of viable halophilic Archaea from salt collected from salt mines dating from the Triassic (195–225 million years B.P.) and Permian (225–270 million years B.P.) periods (Norton et al. 1993) and the isolation of a strain closely related to *Virgibacillus marismortui* from a 250 million-year-old Permian Salado Formation, Carlsbad, New Mexico (Vreeland et al. 2000). The latter study was criticized on account of the unexpectedly high similarity of the 16S rRNA gene sequence of the isolate with its modern relatives (Graur and Pupko 2001; Nickle et al. 2002). The heterogeneous 16S rRNA genes in *Haloarcula* isolates from ancient salt deposits have been compared with those of modern strains, assuming that fewer differences would be expected between the genes of truly ancient *Haloarcula* than in modern strains if the gene multiplicity originated by duplication. No indications were found that the genes from present-day strains are indeed more divergent than the ancient ones (Grant et al. 1998b). Controversies still exist whether indeed these prokaryotes were trapped within the crystals at the time the salt deposits were formed and had retained their viability ever since or whether these cells may have entered the salt more recently during disturbances of the salt layer, either due to natural phenomena or caused by human activity.

The presence of dense communities of members of the Halobacteriales in hypersaline environments can often be observed with the unaided eye, thanks to the bright red, orange, or purple coloration of most representatives of the group and to the extremely high community densities at which these Archaea may develop. The occurrence of red hues has been documented for the North Arm of Great Salt Lake (Post 1977), the Dead Sea (Oren 1988a), and hypersaline alkaline lakes such as Lake Magadi, Kenya (Grant and Tindall 1986). Red-colored brines are also typically present during the final stages of the evaporation of seawater in solar saltern crystallizer ponds (Borowitzka 1981; Javor 1989, 2002; Oren 1993, 1994, 2009) (*Figs. 19.3–19.5*).

Sometimes, other types of microorganisms may also contribute to the color of the brine, such as the β-carotene-rich green halophilic alga *Dunaliella salina* in saltern ponds (*Fig. 19.5*) or photosynthetic purple bacteria of the genus *Ectothiorhodospira* or *Halorhodospira* that may be responsible for at least part of the red coloration of the brines in the alkaline lakes of the Wadi Natrun, Egypt (Jannasch 1957).

Salinibacter ruber, a member of the Bacteroidetes, is recent addition to the list of extremely halophilic red prokaryotes that may contribute to the color of saltern crystallizer brines (Oren and Rodríguez-Valera 2001) and other hypersaline waters with salinities approaching saturation (Antón et al. 2002). This organism requires and tolerates as much salt as the most halophilic among the members of the Halobacteriaceae, and it shares many other properties

Fig. 19.3
Saltern crystallizer pond of the Israel Salt Company at the Red Sea coast near Eilat at a total dissolved salt concentration of about 340 g/l, colored red by halophilic Archaea

Fig. 19.4
Saltern ponds of the Cargill Solar Salt Works (Newark, CA), showing a crystallizer pond colored orange-red by halophilic Archaea and other halophilic microorganisms (Courtesy of Carol D. Litchfield, George Mason University, Fairfax, VA)

Fig. 19.5
Brines in crystallizer pond of the Cargill Solar Salt Works (Newark, CA), colored in part red due to dense communities of halophilic Archaea (*foreground*) and in part showing a more orange color imparted by the β-carotene-rich unicellular green alga *Dunaliella salina*. The total dissolved salt concentration when the picture was taken (February 1997, following a period of heavy rains) was about 250 g/l

with its archaeal counterparts (Oren 2006c; Oren et al. 2004), as also shown by the analysis of its genome sequence (Mongodin et al. 2005).

Other taxonomically coherent groups consisting solely or mainly of halophilic microorganisms are the order Halanaerobiales and the family Halomonadaceae (Gammaproteobacteria). The Halanaerobiales (Oren 2006b) form an order of moderately halophilic anaerobic bacteria within the low G+C branch of the Firmicutes (Rainey et al. 1995). As discussed below and elsewhere (Oren 2006b), this group is of special interest as the mechanism of salt adaptation used by its members resembles that of the aerobic halophilic Archaea rather than that of most other halophilic or halotolerant Bacteria. The family of the Halomonadaceae (Arahal and Ventosa 2006) contains some of the most versatile prokaryotes with respect to their adaptability to a wide range of salt concentrations. The adaptations of some of its representatives to salt have been extensively studied (Ventosa et al. 1998), and the genome sequences of two representatives, *Halomonas elongata* and *Chromohalobacter salexigens*, have been published (Csonka et al. 2005; Oren et al. 2005; Schwibbert et al. 2010). An alkaliphilic *Halomonas* isolate from Mono Lake, CA, recently became famous for its alleged ability to incorporate arsenic instead of phosphorus into its DNA and other macromolecules (Wolfe-Simon et al. 2011). However, this ability was not confirmed in later experiments.

Other phylogenetically unrelated branches of the 16S rRNA gene-based tree of the prokaryotes harbor halophiles as well. An interesting example is the wall-less anaerobe *Haloplasma contractile*, isolated from a brine-filled deep of the Red Sea and growing at 1.5–18 % salt. A new order, the Haloplasmatales, was established to classify this organism, which phylogenetically branches between the Firmicutes and the Mollicutes (Antunes et al. 2007).

The true diversity of halophilic prokaryotes in nature is much larger than the diversity known from organism studied in culture. Methods of environmental genomics/metagenomics have been applied to different hypersaline environments, including Great Salt Lake (Parnell et al. 2010), the Dead Sea (Bodaker et al. 2010), saltern evaporation and crystallizer ponds (Benlloch et al. 2002), and deep-sea brine pools (Daffonchio et al. 2006; van der Wielen et al. 2005). These studies and others clearly show that many more types of halophilic prokaryotes are awaiting isolation and characterization. However, with the appropriate skill and a lot of patience, it may be possible to cultivate most of these yet

unknown organisms, as shown in a study of Australian saltern crystallizer ponds (Burns et al. 2004b).

Thermophilic, Psychrophilic, and Alkaliphilic Halophiles

Among the halophilic prokaryotes, some are adapted to other forms of environmental stress in addition to salt stress. Thus, thermophilic, psychrophilic, and alkaliphilic halophiles are known. No acidophilic halophiles have been described as yet. The Dead Sea with a pH of about 6.0 is probably the most acidic environment in which mass development of halophilic Archaea has been reported (Oren 1988a).

Most aerobic halophilic Archaea of the order Halobacteriales have rather high temperature optima, in the range between 35 °C and 50 °C and sometimes even higher. Growth at high temperatures may be an adaptation to the often relatively high temperatures of salt lakes in tropical areas.

Within the anaerobic Bacteria of the order Halanaerobiales, several moderately thermophilic representatives were described. *Halothermothrix orenii*, the first truly thermophilic halophile discovered, was isolated from Chott El Guettar, a warm saline lake in Tunisia. It grows optimally at 60 °C and up to 68 °C at salt concentrations as high as 200 g/l (Cayol et al. 1994). Its genome sequence was recently published (Mavromatis et al. 2009). Another halophile with thermophilic properties is *Acetohalobium arabaticum* strain Z-7492 which has a temperature optimum of 55 °C (Kevbrin et al. 1995).

Recent studies in the alkaline hypersaline lakes of the Wadi Natrun, Egypt, have yielded a number of interesting anaerobic "polyextremophilic" prokaryotes that show halophilic, alkaliphilic, as well as thermophilic properties. These include *Natranaerobius thermophilus*, *Natranaerobius trueperi*, and *Natronovirga wadinatrunensis*, which all grow optimally at pH 9.5–10.5, 3.7–3.9 M Na^+, and 51–53 °C. Phylogenetically these belong to a new order, the Natranaerobiales within the class Clostridia (Bowers et al. 2009; Mesbah and Wiegel 2008, 2009).

Cold-adapted halophiles also occur. The halophilic archaeon *Halorubrum lacusprofundi* was isolated from Deep Lake, Antarctica, a hypersaline lake in which the water temperature varies according to the season between below zero and +11.5 °C. The isolate grows optimally at 31–37 °C, but slow growth does occur down to temperatures as low as 4 °C (Franzmann et al. 1988). In addition, a variety of halophilic and halotolerant Bacteria was isolated from different salt lakes in Antarctica (Franzmann 1991; McMeekin et al. 1993).

Halophilic Archaea of the order Halobacteriales are abundant in hypersaline soda lakes such as Lake Magadi (Kenya) (Grant and Tindall 1986; Tindall and Trüper 1986; Tindall et al. 1980, 1984), the Wadi Natrun lakes (Egypt) (Imhoff et al. 1978, 1979; Soliman and Trüper 1982), and soda lakes in China and India. They may impart a red color to such lakes. These environments are characterized by a salinity at or close to halite saturation and contain in addition high concentrations of carbonates. The pH values are around 10–11 (Grant and Tindall 1986). Also, anaerobic halophilic alkaliphiles occur in such environments. Lake Magadi harbors a varied anaerobic community, including cellulolytic, proteolytic, saccharolytic, and homoacetogenic bacteria (Zhilina and Zavarzin 1994; Zhilina et al. 1996). The homoacetogen *Natroniella acetigena* was isolated from this environment. Its pH optimum is 9.8–10.0, and it can grow up to pH 10.7 (Zhilina et al. 1996).

Metabolic Diversity of Halophilic Microorganisms

A survey of the halophilic microorganisms for metabolic diversity shows that many but not all types of dissimilatory metabolism known within the prokaryotic world can also function in hypersaline environments. *Figure 19.6* presents an overview of the functional diversity of halophilic prokaryotes, based both on laboratory experiments with isolated cultures and on measurements of the processes as they occur in nature.

Oxygenic photosynthesis by cyanobacteria can occur almost up to NaCl saturation. While the main planktonic primary producers in most hypersaline environments are eukaryotic algae of the genus *Dunaliella* (Javor 1989; Oren 1988a, 1994; Post 1977), cyanobacteria such as *Aphanothece halophytica* [*Halothece*/*Euhalothece*; for a discussion of the problems in the taxonomy and nomenclature of the "Halothece" group, see Garcia-Pichel et al. (1998)] are often found abundantly in benthic microbial mats that cover the shallow sediments of salt lakes and saltern ponds, especially in the salinity range between 150 and 250 g/l (Oren 2000, 2009). *Figure 19.7* shows an example of a dense benthic community of unicellular cyanobacteria living within a gypsum crust of a saltern evaporation pond.

Anoxygenic photosynthetic prokaryotes also abound up to the highest salt concentrations. Examples are representatives of the genus *Halorhodospira*, which contains species such as *Halorhodospira halochloris* and *Halorhodospira halophila* that can be classified as borderline extreme halophiles (see *Table 19.1*). *Halorhodospira* cells were documented to impart a bright red color to the alkaline hypersaline lakes of Wadi Natrun, Egypt (Jannasch 1957). Additional halophilic purple bacteria have been characterized, such as *Halochromatium* and *Thiohalocapsa*.

Most halophilic prokaryotes that have been isolated and studied are aerobic chemoorganotrophs. Aerobic breakdown of organic compounds is possible at salinities up to NaCl saturation. Both halophilic Archaea of the order Halobacteriales and different types of Bacteria may be involved in the breakdown of organic compounds in hypersaline environments. In addition to simple compounds such as sugars, amino acids, etc., also a number of unusual substrates can be degraded at high salt concentrations. Aliphatic and aromatic hydrocarbons, including even and odd carbon number saturated hydrocarbons, saturated isoprenoid alkanes (pristane), different aromatic compounds including benzoate, cinnamate, and phenylpropionate, and also long-chain fatty acids such as palmitic acid have been

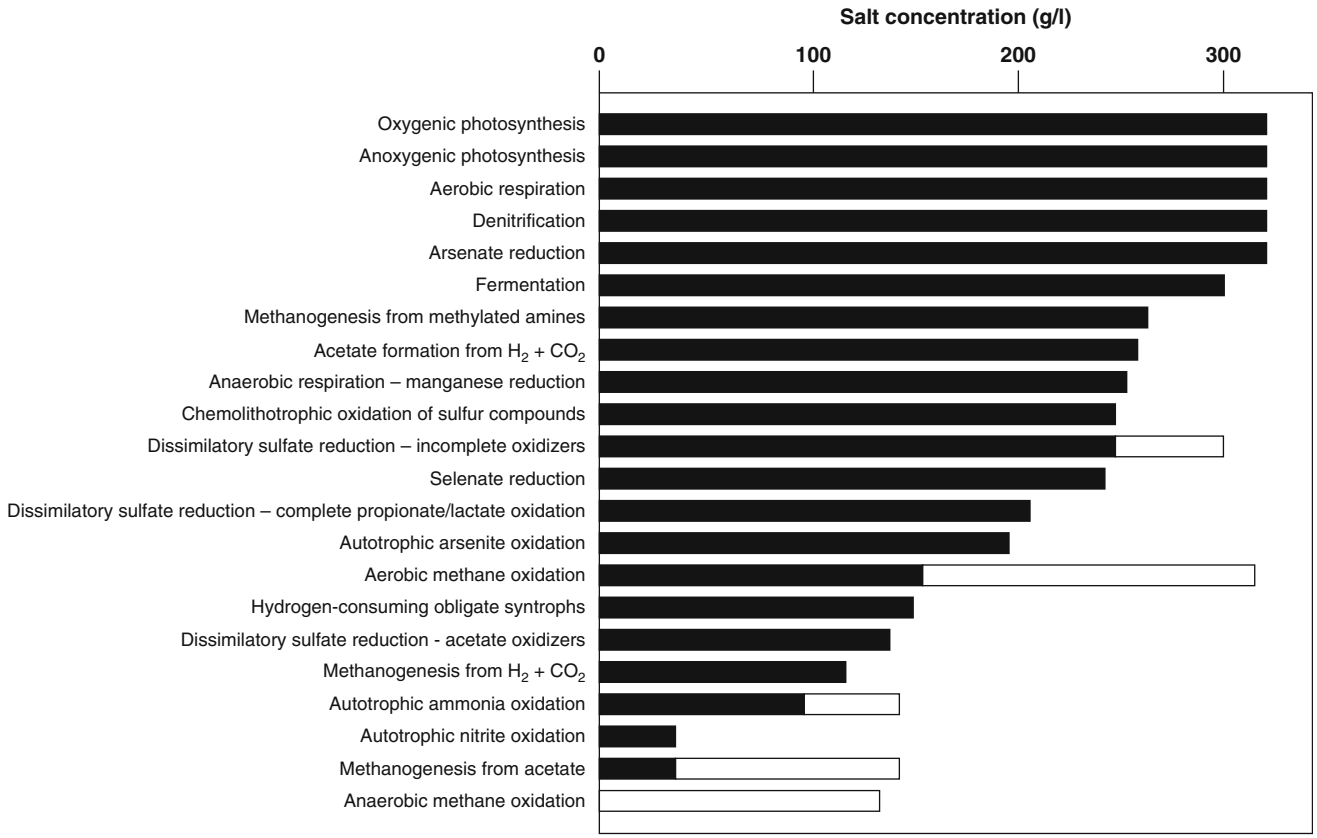


Fig. 19.6

Approximate upper salt concentration limits for the occurrence of selected microbial processes. Values presented are based in part on laboratory studies of pure cultures (*black bars*) and on activity measurements of natural microbial communities in hypersaline environments (*white bars*) (Adapted from data presented in Oren 2011)

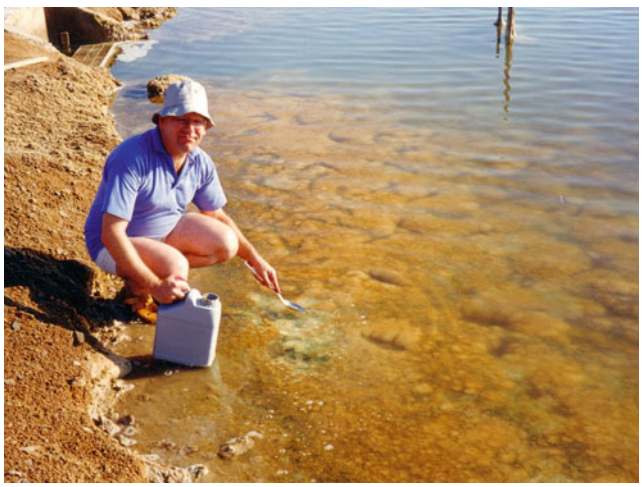


Fig. 19.7

A crust of gypsum densely populated with cyanobacteria (*Aphanothece halophytica*/*Halothece* and others) in a saltern evaporation pond of the Israel Salt Company at the Red Sea coast near Eilat at a total dissolved salt concentration of 286 g/l. The author is sampling the carotenoid-rich upper unicellular cyanobacterial layer and the *green layer* of filamentous cyanobacteria below

shown to serve as sole carbon and energy sources to certain isolates of halophilic Archaea (Bertrand et al. 1990; Tapilatu et al. 2010). Degradation of hexadecane was shown in Great Salt Lake only up to a salinity of 172 g/l (Ward and Brock 1978). Degradation of hydrocarbons by halophiles was recently reviewed by McGenity (2010), and Oren et al. (1992) presented an overview of the potential for breakdown of other unusual compounds, including industrial pollutants, at high salt concentrations.

Oxygen is poorly soluble in concentrated brines, and therefore, it is not surprising to find a considerable variety of anaerobic halophilic heterotrophs. Many representatives of the aerobic halophilic Archaea of the order Halobacteriales can grow anaerobically by using nitrate as electron acceptor (Mancinelli and Hochstein 1986; Oren 2006a). Other potential electron acceptors used by many species are dimethyl sulfoxide, trimethylamine *N*-oxide, and fumarate.

Halobacterium salinarum, but none of the many other aerobic halophilic Archaea tested, is able to grow fermentatively on L-arginine (Hartmann et al. 1980). However, the group of halophilic microorganisms that has specialized in anaerobic fermentative growth is that of the Halanaerobiales (low G+C branch of the Firmicutes). Different sugars and in some cases also amino acids are fermented to products such as

acetate, ethanol, butyrate, hydrogen, and carbon dioxide (Lowe et al. 1993; Mermelstein and Zeikus 1998; Oren 2006b; Rainey et al. 1995).

In low-salt anaerobic environments, breakdown of organic compounds is completed by the cooperative action of a variety of microbial processes, including fermentation, dissimilatory sulfate reduction, methanogenesis, and possibly also activity of proton-reducing acetogens that degrade compounds such as ethanol, butyrate, and others to hydrogen and acetate. Not all these processes have yet been identified in anaerobic hypersaline environments (Oren 1988b, 1999, 2001, 2011).

Dissimilatory sulfate reduction occurs up to quite high salt concentrations. Black, sulfide-containing sediments are often found on the bottom of salt lakes and saltern ponds almost up to NaCl saturation. The most salt-tolerant sulfate reducer isolated thus far is *Desulfohalobium retbaense*, isolated from Lake Retba in Senegal and documented to grow at NaCl concentrations up to 24 % (Ollivier et al. 1991). Other halophilic isolates such as *Desulfovibrio halophilus* and *Desulfovibrio oxyclinae* tolerate NaCl concentrations up to 18–22.5 % only (Caumette 1993; Krekeler et al. 1997; Ollivier et al. 1994). Most halophilic and halotolerant sulfate reducers isolated are incomplete oxidizers that grow on lactate and produce acetate. The most halotolerant acetate-oxidizing sulfate-reducing bacterium known is *Desulfobacter halotolerans*, obtained from the bottom sediments of the Great Salt Lake (Brandt and Ingvorsen 1997). This organism has a rather restricted salt range, being unable to grow above 13 % NaCl. It has been suggested that bioenergetic constraints may define the upper salinity limit at which the different dissimilatory processes can occur (Oren 1999, 2001, 2011).

All these halophilic sulfate reducers are Proteobacteria that use organic compatible solutes to provide osmotic balance, which is energetically much more expensive than the use of inorganic ions for that purpose (see below). Accumulation of trehalose and glycine betaine was documented in *Desulfovibrio halophilus* (Welsh et al. 1996). Dissimilatory sulfate reduction provides relatively little energy, and therefore, the need to spend a substantial part of the available energy for the production of organic osmotic solutes may set the upper limit to the salt concentration at which these bacteria can grow. The oxidation of lactate to acetate and CO_2 yields much more energy ($2\ Lactate^- + SO_4^{2-} \rightarrow 2\ Acetate^- + 2\ HCO_3^- + HS^- + H^+$; $\Delta G^{o\prime} = -160.1$ kJ) than the oxidation of acetate with sulfate as electron acceptor ($Acetate^- + SO_4^{2-} \rightarrow 2\ HCO_3^- + HS^-$; $\Delta G^{o\prime} = -47.7$ kJ). This difference may possibly explain the apparent lack of complete oxidizers at the highest salt concentration range (Oren 1999, 2001, 2011).

The main methanogenic processes in freshwater environments are the reduction of CO_2 with hydrogen and the aceticlastic split. Neither of these reactions has been shown to occur at high salt concentrations. Solar Lake (Sinai) sediments (70–74 g/l salt) did not show any methanogenesis from acetate or from $H_2 + CO_2$ (Giani et al. 1984). The highest salt concentration at which methanogenesis from $H_2 + CO_2$ was demonstrated in nature was 88 g/l (Mono Lake, CA) (Oremland and King 1989). The most halotolerant isolate that grows on $H_2 + CO_2$ is *Methanocalculus halotolerans* obtained from an oil well. This organism grows up to 12 % NaCl with an optimum at 5 % (Ollivier et al. 1998). The upper salinity boundary for the use of acetate as methanogenic substrate is probably even lower, but available data are few. To my knowledge, no cultures of aceticlastic methanogens are extant that grow above 4–5 % NaCl.

Energetic constraints may explain the apparent lack of truly halophilic methanogens that grow on $H_2 + CO_2$ or on acetate. In contrast to the aerobic halophilic Archaea of the order Halobacteriales which contain inorganic ions for osmotic stabilization, the methanogens use the energetically more expensive option of synthesizing organic osmotic solutes (see *Table 19.5*). The aceticlastic split yields very little energy ($\Delta G^{o\prime} = -31.1$ kJ per mol acetate). The free energy yield during growth on hydrogen is −34 kJ per mol of hydrogen, not much more than that on acetate.

Methanogenesis does occur, however, at much higher salt concentrations. The most salt-tolerant methanogen known in culture is *Methanohalobium evestigatum* which grows up to 30 % NaCl (Zhilina and Zavarzin 1987). Additional moderately halophilic methanogens have been isolated, growing optimally at 4–12 % salt (e.g., *Methanohalophilus mahii*, *Methanohalophilus halophilus*, *Methanohalophilus portucalensis*, and *Methanohalophilus zhilinae*). The energy sources used by these methanogens are methylated amines, methanol, and dimethyl sulfide (Boone et al. 1993; Lai and Gunsalus 1992; Oremland and King 1989; Zhilina and Zavarzin 1987, 1990; see also the review paper by Ollivier et al. 1994). The substrates used by these bacteria in their natural environment may be derived from microbial degradation of methylated compounds that serve as organic osmotic solutes in many halophilic microorganisms, such as glycine betaine and dimethylsulfoniopropionate (Oremland and King 1989; Zhilina and Zavarzin 1990, see also *Table 19.5*). Thermodynamic calculations show that the energy yield on methylated amines is relatively large (between −92 and −191 kJ per mol of substrate transformed), and this may at least in part explain why growth of methanogenic Archaea on methylated amines may occur up to high salt concentrations (see Oren 1999, 2001).

While, as discussed above, methanogens growing on $H_2 + CO_2$ appear to be absent in hypersaline environments, halophilic homoacetogenic bacteria that use the same substrates for the production of acetate have been isolated (Zavarzin et al. 1994; Zhilina and Zavarzin 1990). *Acetohalobium arabaticum* grows between 10 % and 25 % NaCl with an optimum at 15–18 % (Zhilina and Zavarzin 1990). At first sight reaction, thermodynamics do not explain why halophilic homoacetogenic bacteria do occur when CO_2-reducing methanogens do not, as the acetogenic reaction yields even less energy than the methanogenic reaction (−26.1 and −31.1 kJ per hydrogen oxidized, respectively). However, the halophilic homoacetogens belong to the order Halanaerobiales (Rainey et al. 1995; Zhilina et al. 1996), a group that uses the energetically cheaper option of

accumulating inorganic ions to establish osmotic balance (Oren 1986; Oren et al. 1997; Rengpipat et al. 1988).

Halophilic aerobic chemoautotrophic bacteria that obtain their energy from the oxidation of reduced sulfur compounds are known. *Halothiobacillus halophilus*, isolated from a hypersaline lake in Western Australia, grows up to about 24 % NaCl (Wood and Kelly 1991). However, autotrophic oxidation of NH_4^+ to NO_2^- was never demonstrated above 150 g/l salt, and the salt limit for the oxidation of NO_2^- to NO_3^- may be even lower (Rubentschik 1929). To my knowledge, no halophilic or halotolerant ammonia- or nitrite-oxidizing bacteria have been documented to grow at salinities significantly exceeding those of seawater. "Nitrosococcus halophilus," with an optimum at 4 % NaCl and a maximum at 9.4 %, may be the most halophilic strain isolated to date (Koops et al. 1990). Lack of energy source is probably not the main reason: ammonia, and not nitrate, is the dominant inorganic nitrogen species in most or all hypersaline water bodies, and it generally occurs in quite high concentrations. Energetic constraints may be the cause for the apparent lack of halophilic nitrifying bacteria in nature, as only very small amounts of energy are gained from the oxidation of ammonia and of nitrite (Oren 1999, 2001).

Thermodynamic constraints cannot explain the apparent lack of aerobic methane oxidation in hypersaline environments. Methane oxidation is a highly exergonic process ($CH_4 + 2\,O_2 \rightarrow HCO_3^- + H_2O$; $\Delta G^{o\prime} = -813.1$ kJ). However, even in an environment with a relatively low salinity such as the epilimnion of Solar Lake, Sinai, Egypt, during winter stratification (about 9 % salt), no methane oxidation could be measured in spite of the availability of both methane and oxygen (Conrad et al. 1995). Reports on the occurrence of methane oxidation in sediments of hypersaline reservoirs in Ukraine and Tuva (up to 330 g/l total dissolved salts) and the isolation of halophilic methanotrophs from these environments (Heyer et al. 2005; Khmelenina et al. 1997; Sokolov and Trotsenko 1995) indicate that the existence of halophilic methane oxidizers is at least thermodynamically feasible and that the earlier reported lack of methane oxidation in other hypersaline environments (Conrad et al. 1995) should have other reasons. The most halotolerant aerobic methane oxidizer reported is *Methylohalobius crimeensis*, isolated from hypersaline lakes in the Crimean Peninsula, Ukraine. It grows optimally at 58–87 g/l NaCl; very slow growth is still possible at 120–150 g/l (Heyer et al. 2005).

Mechanisms of Salt Adaptation in Halophilic Prokaryotes

As biological membranes are permeable to water, any microorganism living at high salt concentrations has to maintain its intracellular environment at least isosmotic with the salt concentration in its environment and even hyperosmotic when a turgor pressure has to be maintained (Brown 1976, 1990; Csonka 1989; Grant 2004; Vreeland 1987).

Two fundamentally different strategies exist that enable halophilic and halotolerant prokaryotes to cope with the osmotic stress exerted by the high ionic strength of their hypersaline environment. The option used by the aerobic Archaea of the order Halobacteriales (Oren 2006a), by the aerobic *Salinibacter ruber* (Bacteroidetes) (Oren 2006c; Oren et al. 2004), and by the anaerobic Bacteria of the order Halanaerobiales (Oren 2006b) is based on the accumulation of high concentrations of inorganic ions in the cytoplasm. In most cases, K^+ rather than Na^+ is the dominant intracellular cation, and Cl^- is the dominant anion. Presence of molar concentrations of inorganic ions requires special adaptations of the entire intracellular enzymatic machinery. The "salt-in" strategy permits little flexibility and adaptability to changing conditions, as many salt-adapted enzymes and structural proteins require the continuous presence of high salt for activity and stability (Dennis and Shimmin 1997; Mevarech et al. 2000).

The second strategy is to prevent high salt concentrations from reaching the cytoplasm and maintaining "conventional" enzymes and other proteins, not specifically designed to function at high ionic strength. Low intracellular ionic concentrations are maintained by active pumping of ions out of the cells. Osmotic equilibrium is provided by organic solutes that are either produced by the cells or accumulated from the medium (Kempf and Bremer 1998). Such "compatible" solutes are low-molecular-weight organic compounds, soluble in water at high concentrations, and not inhibitory to enzymatic activities even in the molar concentration range. The intracellular concentrations of the organic solutes are regulated according to the salinity of the external medium. Thus, the use of organic osmotic solutes provides a great deal of flexibility and adaptability to an often wide range of salt concentrations, with the possibility of rapid adaptation to changes in the salinity of the medium. The strategy of maintaining isosmotic concentrations of organic osmotic solutes is used by most halophilic and halotolerant Bacteria (with the exception of *Salinibacter* and the Halanaerobiales, as stated above) and by the halophilic methanogenic Archaea. Halophilic eukaryotic microorganisms also use organic compatible solutes for osmotic stabilization. Under certain conditions do the alkaliphilic members of the Halobacteriales also make use of an organic osmoticum (2-sulfotrehalose) to aid in the achievement of osmotic equilibrium with the environment (Desmarais et al. 1997; Martin et al. 1999).

The "Salt-in" Strategy

Analyses of intracellular ionic concentrations in different aerobic halophilic Archaea show that these microorganisms maintain extremely high salt concentrations inside their cells. Moreover, the ionic composition of their intracellular milieu differs greatly from that of the outside medium, with K^+ being the main intracellular cation (*Table 19.2*).

The representatives of the order Halanaerobiales (low G+C Firmicutes) (Oren 2006b) display a number of physiological and

biochemical properties that are characteristic for the halophilic aerobic Archaea, rather than for the moderately halophilic aerobic Bacteria which use the organic solute strategy. No organic osmotic solutes have yet been found in this group of anaerobic halophilic fermentative Bacteria (Mermelstein and Zeikus 1998; Oren 1986; Oren et al. 1997; Rengpipat et al. 1988). High concentrations of Na^+, K^+, and Cl^- were measured inside the cells of *Halanaerobium praevalens*, *Halanaerobium acetethylicum*, and *Halobacteroides halobius*, high enough to be at least isotonic with the medium (*Table 19.3*). In exponentially growing cells, K^+ was the major cation.

The huge potassium concentration gradient over the cytoplasmic membrane (often up to three orders of magnitude) and also the generally large sodium gradient present can only be created and maintained at the expense of energy. Also, the chloride ion is far from thermodynamic equilibrium as the presence of an inside negative membrane potential would tend to expel Cl^- from the cell. The peculiar ionic composition of the cells' cytoplasm and the concentration gradients over the cell membrane are the result of the cooperative action of different ion pumps, antiporters, and other transport proteins (Schäfer et al. 1999). The most important ones are summarized in *Fig. 19.8*, and the numbers in square brackets in the explanation below refer to the different parts of that figure.

In the Halobacteriales, respiratory electron transport with oxygen or other electron acceptors (see Oren 2006a) is accompanied by the extrusion of protons [1], generating a primary proton electrochemical gradient (acidic outside, alkaline inside, positive outside, negative inside). Species that contain the retinal protein bacteriorhodopsin in their membranes (Lanyi 2003; Oren 2006a; Schäfer et al. 1999) may also use light energy for the direct generation of the proton electrochemical gradient [2]. The primary proton gradient is the driving force for all energy-requiring processes within the cell. Thus, ATP formation is mediated by the membrane-bound ATP synthase that couples phosphorylation of ADP with an inward flux of H^+ [3]. The membrane ATP synthase may also be used in the reverse direction, the buildup of a proton electrochemical gradient at the expense of ATP. This process is relevant in cases in which ATP formation by substrate-level phosphorylation is the primary energy-yielding process in the cell. This is the case, e.g., in *Halobacterium salinarum*, when growing anaerobically by fermentation of arginine (Hartmann et al. 1980), or in the anaerobic Bacteria of the order Halanaerobiales which obtain their energy by fermentation of sugars or amino acids (Oren 2006b).

The membranes of all halophiles investigated possess high activities of Na^+/H^+ antiporters which use the proton electrochemical gradient as the driving force for the extrusion of Na^+ from the cell [4] (Hamaide et al. 1983; Lanyi and MacDonald 1976; Luisi et al. 1980). In *Halobacterium salinarum*, the antiporter was shown to be electrogenic and probably has a stoichiometry of 2 H^+/Na^+ (Lanyi and Silverman 1979). In addition to its function of keeping intracellular Na^+ concentrations at the desired low levels, the Na^+/H^+ antiporter activity plays an important role in the regulation of the intracellular pH.

The sodium gradient thus established can in its turn be used to drive certain endergonic processes. Thus, many of the membrane transport systems for amino acids and other compounds in the aerobic halophilic Archaea are energized by cotransport with Na^+ ions [5]. The same is true for many moderately halophilic Bacteria, which also maintain a relatively low intracellular Na^+ concentration (Shindler et al. 1977; Ventosa et al. 1998). The Na^+ gradient thus serves to some extent as an energy reserve.

It is generally accepted that the negative-inside membrane potential is the driving force for the massive K^+ accumulation. The membranes of halophilic Archaea were found to be highly permeable to potassium. K^+ ions probably enter the cells via a uniport system in response to the membrane potential (Wagner et al. 1978) [6]. K^+ enters the cell as Na^+ is ejected

Table 19.2
Estimates of intracellular ionic concentrations in aerobic halophilic Archaea of the order Halobacteriales

	Medium concentration				Intracellular concentration			
Species	Na^+	K^+	Mg^{2+}	Cl^-	Na^+	K^+	Mg^{2+}	Cl^-
Halobacterium salinarum	4.0	0.032			1.37	4.57		3.61
Halobacterium salinarum[a]	3.7	0.013	0.1		1.63	2.94		
Halobacterium salinarum[a]	3.33	0.05	0.13		0.80	5.32	0.12	
Haloarcula marismortui[b]	3.9	0.004–0.007	0.15	3.9	1.2–3.0	3.77–5.5		2.3–4.2
Haloarcula marismortui[a]	3.9	0.001–0.004	0.15	3.9	1.6–2.1	3.7–4.0		3.2–4.1
Haloarcula marismortui[c]	3.9	0.0075	0.15	3.9	0.5–0.7	3.7–4.0		2.3–2.9
Halococcus morrhuae	4.0	0.032		3.17	2.03			3.66

[a]Late exponential growth phase cells
[b]Early exponential growth phase cells
[c]Stationary growth phase cells
For additional information, see text. Data were derived from Christian and Waltho (1962), Ginzburg et al. (1970), Lanyi and Silverman (1972), and Matheson et al. (1976). All concentrations are in molar units, except those relating to *Haloarcula marismortui*, which are expressed in molal units

Table 19.3

Intracellular ionic concentrations of halophilic anaerobic Bacteria of the order Halanaerobiales

	Medium concentration			Intracellular concentration		
Species	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
Halanaerobium praevalens	0.99	0.013	1.07	0.44	0.96	
	2.22	0.013	2.3	1.52	1.59	2.24
Halanaerobium praevalens[a]	2.22	0.013	2.3	0.44	1.14	1.26
	3.08	0.013	3.16	2.63	2.05	3.28
Halanaerobium acetethylicum	1.16	0.032	1.4	0.92	0.24	1.2
	2.52	0.034	2.7	1.50	0.78	2.5
Halobacteroides halobius	1.56	0.013		0.54	0.92	

[a]Data obtained by X-ray microanalysis in the electron microscope. Values probably underestimate the true values. Data were derived from Oren (1986), Oren et al. (1997), and Rengpipat et al. (1988)

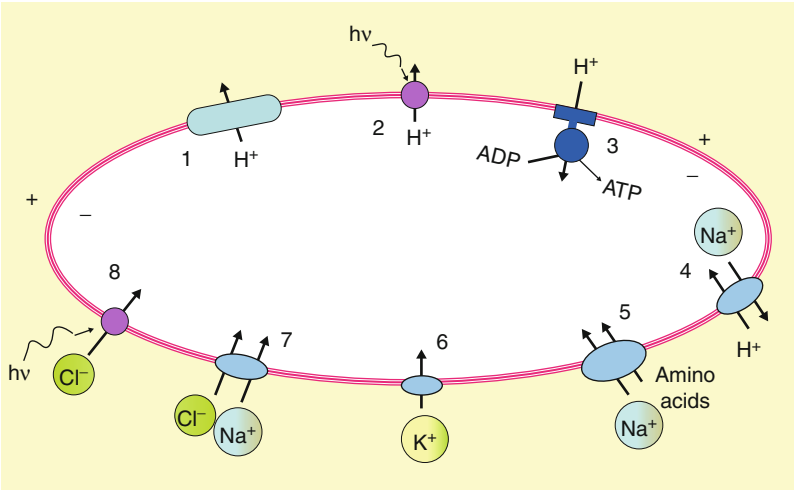


Fig. 19.8

Ion movements in the aerobic halophilic Archaea (order Halobacteriales). [1], proton extrusion via respiratory electron transport; [2], light-driven proton extrusion mediated by bacteriorhodopsin; [3], ATP formation by ATP synthase, driven by the proton gradient. Alternatively, this system can serve to generate a proton gradient at the expense of ATP during fermentative growth on arginine; [4], electrogenic sodium/proton antiporter; [5], sodium gradient-driven inward amino acids transport; [6], potassium uniport, driven by the membrane potential; [7], light-independent chloride transport system, probably coupled with inward transport of sodium; [8], halorhodopsin, the primary, light-driven chloride pump. For details, see text

by the electrogenic Na^+/H^+ antiporter, thus maintaining electroneutrality. A K^+ transport system analogous to the Kdp system of *Escherichia coli* that requires ATP for activation was detected in *Haloferax volcanii* (Meury and Kohiyama 1989) and in *Halobacterium salinarum*, where its expression is induced by K^+ limitation (Strahl and Greie 2008).

The high internal Cl^- concentration is not in equilibrium with the large negative-inside electrical potential that accompanies the H^+ circulation and the Na^+ efflux. Thus, electrical potential-driven passive chloride movement can result only in a loss of chloride from the cells, rather than in the required uptake. An increase in the amount of intracellular Cl^- is essential if the cells should increase their volume during growth and cell division. It has been suggested that during growth, the net flux of ions should result in K^+ uptake in excess of Na^+ loss and that Cl^- uptake should be equal to the difference, so as to provide net gain of intracellular KCl commensurate with the gain in intracellular volume (Lanyi 1986).

Two energy-dependent inward chloride pumps have been identified in *Halobacterium* and in other halophilic Archaea. The first is a light-independent transport system, which is probably driven by symport with Na^+ (Duschl and Wagner 1986) [7]. The second is light driven and is based on the retinal protein halorhodopsin, a primary inward Cl^- pump (Lanyi 1986; Schäfer et al. 1999) [8].

The presence of molar concentrations of salts is generally devastating to proteins and other macromolecules. It causes aggregation or collapse of the protein structure because of

enhancement of hydrophobic interactions, it interferes with essential electrostatic interactions within or between macromolecules due to charge shielding, and because of salt ion hydration, it reduces the availability of free water below the level required to sustain essential biological processes (Dennis and Shimmin 1997; Zaccai and Eisenberg 1991). The presence of high intracellular salt concentrations thus requires special adaptations of the whole enzymatic machinery of the cell. Cells thus adapted are able to function in the presence of high salt. However, these adaptations make the cells strictly dependent on the continuous presence of high salt concentrations for the maintenance of structural integrity and viability (Ebel et al. 1999; Eisenberg and Wachtel 1987; Eisenberg et al. 1992; Lanyi 1974). As a result, the aerobic halophilic Archaea display little flexibility and adaptability to changes in the external salt concentration.

Most enzymes and other proteins of the Halobacteriales denature when suspended in solutions containing less than 1–2 M salt. Many enzymes are more active in the presence of KCl than of NaCl, agreeing well with the finding that K^+ is intracellularly the dominating cation. "Salting-out" salts stabilize, while "salting-in" salts inactivate halophilic enzymes. The behavior of different salts coincides with the lyotropic Hofmeister series (Lanyi 1974). Similarly, intracellular enzymes from the fermentative anaerobic Bacteria (order Halanaerobiales) generally function better in the presence of molar concentrations of salts than in salt-free medium, and they can be expected to be fully active at the actual salt concentrations present in the cytoplasm (Oren and Gurevich 1993; Rengpipat et al. 1988; Zavarzin et al. 1994).

Most proteins of the Halobacteriales contain a large excess of the acidic amino acids glutamate and aspartate and a low content of the basic amino acids lysine and arginine. The high content of acidic side groups was first recognized during analyses of the bulk protein of *Halobacterium* and *Halococcus* (Reistad 1970). The malate dehydrogenase of *Haloarcula marismortui* has a 10.4 mol percent excess of acidic residues and the cell envelope glycoprotein of *Halobacterium salinarum* even 19–20 mol %. Analysis of the genome sequences now available for more than 10 species of the Halobacteriaceae (see http://halo4.umbi.umd.edu/) fully confirmed the highly acidic nature of most of the proteins encoded. The same is true for the genome of *Salinibacter ruber*, an organism that, while phylogenetically belonging to the Bacteroidetes, shares many properties with the members of the Halobacteriaceae (Mongodin et al. 2005; Oren 2006c).

The bulk cellular protein of the members of the Halanaerobiales tested (*Halanaerobium praevalens*, *Halanaerobium saccharolyticum*, *Halobacteroides halobius*, *Sporohalobacter lortetii*, *Natroniella acetigena*) is also highly acidic (Detkova and Boltyanskaya 2006; Oren 1986). No genome sequences of any of these organisms are yet available for further analysis. Surprisingly, most proteins encoded by the genome of the thermophilic halophilic anaerobe *Halothermothrix orenii* do not show any unusually high excess of acidic amino acids (Mavromatis et al. 2009).

The excess of acidic residues may be a major factor determining the halophilic character of the proteins: excess of negative charges on the protein surface makes the structure unstable because of the mutual repulsion of the side groups. Only when high concentrations of cations are added to shield the negative charges can the protein maintain its proper conformation required for structural stability and enzymatic activity.

Shielding of negative charges by cations undoubtedly plays an important part in the effects of salt on the enzymes and other proteins of the halophiles. A theoretical analysis of the contribution of electrostatic interactions in *Haloarcula marismortui* ferredoxin and malate dehydrogenase shows that the repulsive interactions between the acidic residues at the protein surface are a major factor in the destabilization of halophilic proteins in low-salt conditions (Elcock and McCammon 1998). However, Lanyi (1974) and Lanyi and Stevenson (1970) stated that all the effects of salts cannot be due to charge-shielding action alone, as the concentrations required are too high. Maximal electrostatic charge shielding would be achieved already in about 0.1 M salt or 0.5 M at most and in even much lower concentrations of divalent cations. However, a high content of especially glutamate may be favorable as glutamate has the greatest water binding ability of any amino acid residue. This may have important implications when considering the need of any functional protein to maintain a proper hydration shell.

Another prominent feature of the proteins of the Halobacteriales is their low content of hydrophobic amino acid residues, generally offset by an increased content of the borderline hydrophobic amino acids serine and threonine (Lanyi 1974). The requirement for extremely high salt concentrations for structural stability of the proteins can probably to a large extent be attributed to the low content of hydrophobic residues and the accordingly weak hydrophobic interactions within the protein molecules. High salt is then needed to maintain the weak hydrophobic interactions. Entropy increases when nonpolar groups turn away from the water phase and interact with each other to form hydrophobic interactions. These interactions seem to be driven more by an avoidance of water than by an active attraction between the nonpolar molecules (Lanyi 1974). At higher salt concentrations, new hydrophobic interactions are formed which have insufficient stability in water, and the molecule assumes a more tightly folded conformation. The possible involvement of the weak hydrophobic interactions in the salt requirement of the halophilic proteins is supported by the finding that certain enzymes from halophilic Archaea (e.g., threonine deaminase, aspartate carbamoyltransferase, and alanine dehydrogenase) show cold lability: their maximal stability is reached at temperatures greater than 0 °C and decreases at lower temperatures. The effect may be considered in terms of water structure: at lower temperature, the size of the cluster of water molecules is increased, and hydrophobic groups can interact more easily, breaking the hydrophobic interactions (Lanyi 1974).

Detailed studies of the malate dehydrogenase of *Haloarcula marismortui* have contributed much valuable information on the possible mechanisms involved in the halophilic behavior of proteins. Techniques such as velocity sedimentation,

Table 19.4
Intracellular ionic concentrations of selected aerobic halophilic Bacteria

	Medium concentration			Intracellular concentration		
Species	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
Halomonas elongata	1.38	0.02		0.31	0.02	
	3.4	0.01		0.63	0.02	
Chromohalobacter canadensis	4.4	0.04		0.62	0.58	
Halomonas halodenitrificans	1.0	0.04	1.0	0.31	0.47	0.055
	3.0		3.0	1.07	0.12	
"Pseudomonas halosaccharolytica"	2.0	0.006	2.0	1.15	0.89	0.98
	3.0	0.006	3.0	1.04	0.67	0.70
Salinivibrio costicola	2.0	0.008		0.90	0.57	

All data relate to exponentially growing cells. Data were derived from Christian and Waltho (1962), Masui and Wada (1973), Matheson et al. (1976), Shindler et al. (1977), and Vreeland et al. (1983). For more extensive data see *Table 19.5* in Ventosa et al. (1998)

light scattering, neutron scattering, and circular dichroism measurements have been used to obtain information on the structural changes occurring as a function of changing salt concentrations and the hydration properties of the protein (Eisenberg and Wachtel 1987; Mevarech and Neumann 1977; Pundak and Eisenberg 1981; Pundak et al. 1981). These studies showed that the halophilic properties of the enzyme are related to its capacity of associating with unusually high amounts of salts, and led to a thermodynamic "solvation-stabilization model," in which the halophilic protein has adapted to bind hydrated ions cooperatively via a network of acidic groups on its surface (Ebel et al. 1999; Madern et al. 2000; Mevarech et al. 2000).

X-ray diffraction studies on crystals of the halophilic malate dehydrogenase and the ferredoxin of *Haloarcula marismortui* and the dihydrofolate reductase of *Haloferax volcanii* have added much important information (Dym et al. 1995; Frolow et al. 1996; Pieper et al. 1998). These studies showed how the carboxylic groups on the acidic residues are used to sequester, organize, and arrange a tight network of water and hydrated K^+ ions at the surface of the protein and to form an unusually large number of internal salt bridges with strategically located basic amino acid residues to provide internal structural rigidity of the protein. These salt bridges appear to be important determinants in the stabilization of the three-dimensional structure of halophilic proteins. Intervening solvent molecules shield the negative charges of the carboxylic acid groups on the protein surface from each other.

Comparison of the *Haloarcula marismortui* ferredoxin with the plant-type 2Fe-2S ferredoxin showed that the surface of the halophilic protein is coated with acidic residues except for the vicinity of the iron-sulfur cluster and that it contains two additional helices near the N-terminus which form a separate hyperacidic domain, postulated to provide extra surface carboxylates for solvation. Bound water molecules on the protein surface have on the average 40 % more hydrogen bonds than in a typical non-halophilic protein crystal structure. These water molecules are thus tightly bound within the hydration shell by protein-water and water-water hydrogen bonds and by hydration of interspersed K^+ ions (Frolow et al. 1996).

A recent study of the glutamate dehydrogenase of *Halobacterium salinarum* showed the surface of the molecule being covered with acidic residues and displaying a significant reduction in exposed hydrophobic character as compared to non-halophilic counterparts. The low lysine content helps to increase the overall negative charge on the protein surface but also serves to decrease the hydrophobic fraction of the solvent-accessible surface (Britton et al. 1998).

Site-directed mutagenesis studies now enable modification of the halophilic character of proteins, leading to a better understanding of the link between amino acid composition and salt requirement for stability and activity (Tadeo et al. 2009).

Although the "salt-in" strategy, with its associated acidic proteome, is known mainly from prokaryotes living at salt concentrations of 150 g/l and higher, it may be more widespread in nature. Metagenomic analysis of a microbial mat found in a lagoon in Guerrero Negro, Baja California, Mexico, already showed strongly acid-shifted isoelectric points for the predicted proteins encoded by the community growing in at the relatively low salt concentration of 90 g/l (Kunin et al. 2008).

The "Low Salt-in" Strategy

The second option, realized in most halophilic and halotolerant representatives of the Bacteria and also in the halophilic methanogenic Archaea, involves the maintenance of a cytoplasm much lower in salt than the outside medium. *Table 19.4* summarizes estimates of intracellular salt concentrations in a number of aerobic halophilic Bacteria. While in some cases, the apparent intracellular ionic concentrations are in the molar range (possibly in part due to technical difficulties connected to the exact assessment of the intracellular water volumes in cell pellets) (Ventosa et al. 1998), it is clear that the intracellular salt concentrations are generally insufficient to provide osmotic balance. Generally, the intracellular Na^+ concentrations are kept

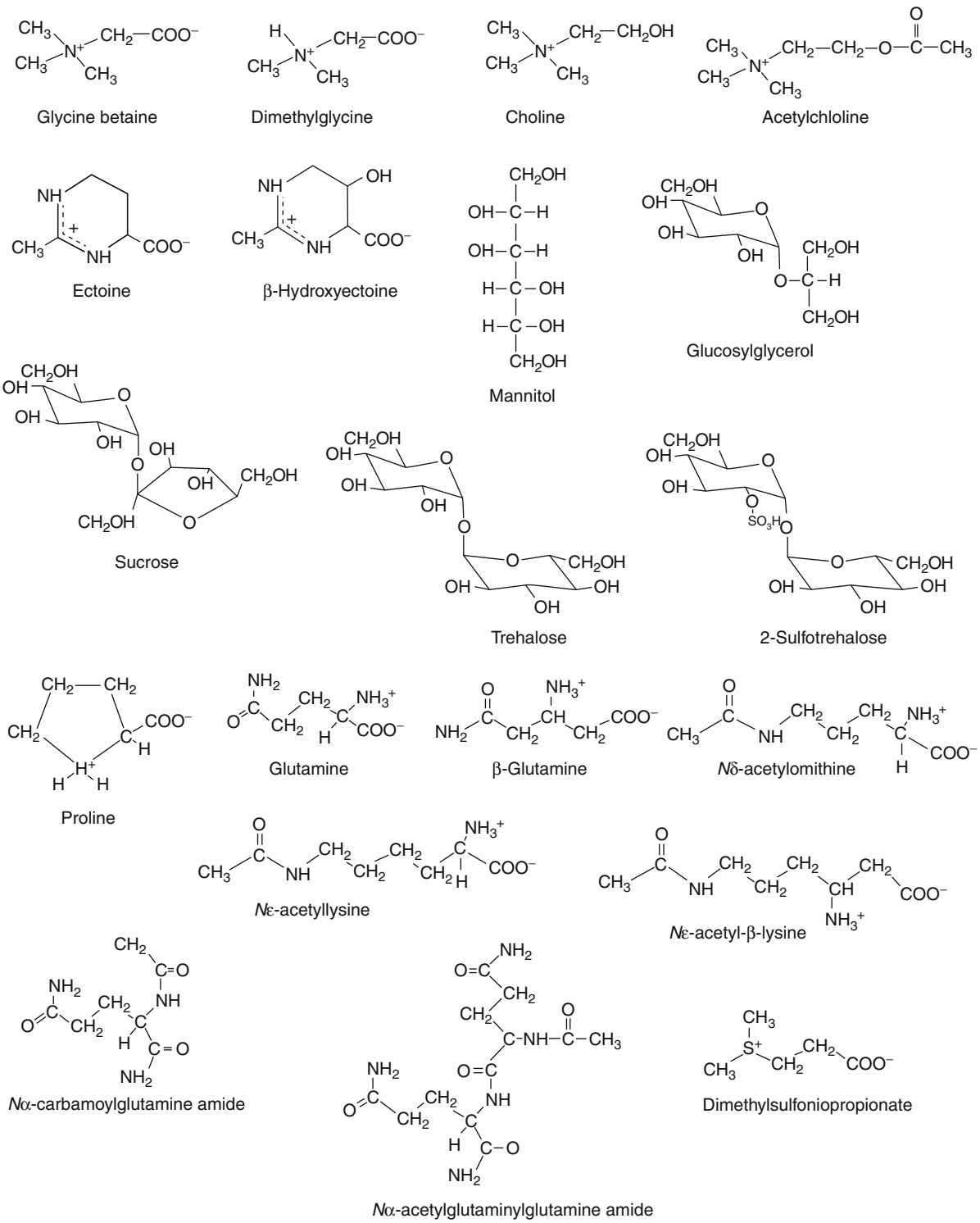


Fig. 19.9
Organic osmotic solutes documented to occur in halophilic and halotolerant Bacteria and Archaea

low. Outward-directed sodium transporters in the cytoplasmic membrane (in most cases electrogenic Na^+/H^+ antiporters) are highly important both in maintaining the proper intracellular ionic environment and in pH regulation (Hamaide et al. 1983; Ventosa et al. 1998).

The "low salt-in" strategy does not require far-going modification of the intracellular proteome, and the bulk protein of prokaryotes that use this strategy does not show a high excess of acid over basic amino acids. There is an exception for proteins that are exposed to the high salt

Table 19.5
Distribution of selected organic osmotic solutes within the bacterial and the archaeal domains

Solute	Distribution
Glycine betaine	Cyanobacteria; anoxygenic phototrophic bacteria; methanogenic Archaea; *Actinopolyspora halophila*; is taken up by many heterotrophic bacteria and used as osmotic solute
Dimethylglycine	Methanogenic Archaea
Choline, acetylcholine	*Lactobacillus plantarum*
Ectoine, hydroxyectoine	Heterotrophic Gammaproteobacteria; *Halorhodospira* spp., *Rhodovulum sulfidophilum*; *Micrococcus* spp.; *Bacillus* spp.; *Marinococcus* spp.; *Halobacillus halophilus*; *Brevibacterium*
Proline	*Bacillus* spp.; *Planococcus citreus*; *Salinicoccus* sp.
Glutamine	Corynebacteria
β-Glutamine	Methanogenic Archaea
Nε-acetyllysine	*Halobacillus halophilus*, other bacilli
Nδ-acetylornithine	*Halobacillus halophilus*, other bacilli
Nε-acetyl-β-lysine	Methanogenic Archaea
Nα-carbamoyl-glutamine amide	*Ectothiorhodospira marismortui* (a later heterotypic synonym of *Ectothiorhodospira mobilis*)
Nα-acetylglutaminyl-glutamine amide	*Halochromatium*; *Thiohalocapsa*; *Rhodopseudomonas* sp.; *Azospirillum brasilense*; *Ensifer meliloti*; *Pseudomonas aeruginosa*
Sucrose	Cyanobacteria
Trehalose	Cyanobacteria; *Halorhodospira* spp.
2-Sulfotrehalose	Alkaliphilic members of the Halobacteriales
Mannitol	*Pseudomonas putida*
Glucosylglycerol	Cyanobacteria; *Rhodovulum sulfidophilum*; *Pseudomonas mendocina*

For additional information, see Desmarais et al. (1997), Galinski (1993, 1995), Hagemann (2011), Hagemann et al. (1999), Imhoff (1993), Roberts (2005, 2006), Trüper et al. (1991), Ventosa et al. (1998), and Wohlfarth et al. (1990)

concentrations outside the cells. Thus, analysis of the genome of the moderately halophilic bacterium *Chromohalobacter salexigens* (Gammaproteobacteria) showed a distinctive halophilic signature of predicted periplasmic proteins, such as the substrate binding proteins of ABC-type transport systems located external to the cytoplasmic membrane (Oren et al. 2005).

Organic compatible solutes make up the major part of the osmotically active compounds in the cells' cytoplasm. Compatible solutes are polar, highly soluble molecules, most of them uncharged or zwitterionic at the physiological pH. The list of compounds known to be synthesized as compatible solutes by halophilic microorganisms is steadily growing (Galinski 1993, 1995; Galinski and Trüper 1994; Reed 1986; Roberts 2005, 2006; Trüper et al. 1991; Ventosa et al. 1998; Wohlfarth et al. 1990). *Figure 19.9* shows the main osmotic solutes identified thus far in prokaryotes, and *Table 19.5* provides information on the taxonomic groups in which the different solutes have been detected.

The accumulation of "compatible" osmotic solutes achieves osmotic equilibrium while still enabling activity of "conventional," non-salt-adapted enzymes (Galinski 1993, 1995). Many prokaryotic cells contain cocktails of different compatible solutes rather than relying on a single compound (Galinski 1995). The concentrations of the osmotic solutes are regulated according to the salt concentration in which the cells are found (Galinski and Louis 1999) and can be rapidly adjusted as required when the outside salinity is changed (by synthesis or uptake from the medium upon salt upshock, by degradation, transformation into osmotically inactive forms, or excretion following dilution stress) (Trüper and Galinski 1990). The use of organic osmotic solutes thus bestows a high degree of flexibility and adaptability.

Compatible solutes are strong water structure formers, and as such, they are probably excluded from the hydration shell of proteins. This "preferential exclusion" probably explains their function as effective stabilizers of the hydration shell of proteins. This phenomenon of nonspecific exclusion is often described in terms of increased surface tension of water, with the presence of solutes affecting the forces of cohesion between water molecules, minimization of entropy, and reinforcement of the hydrophobic effect. Compatible solutes display a general stabilizing effect by preventing the unfolding and denaturation of proteins caused by heating, freezing, and drying (Galinski 1993, 1995) and have already found a number of interesting biotechnological applications (Lentzen and Schwarz 2006; Margesin and Schinner 2001; Oren 2002a, 2010c).

Concluding Remarks

A comparison of the two strategies of adaptation to high salt concentrations ("salt-in" vs. use of organic osmotic solutes) shows that the salt-in strategy is energetically much less costly than the synthesis of organic compatible solutes (Oren 1999, 2011). However, it requires a far-going adaptation of the whole intracellular machinery to the presence of high ionic concentrations. This energetically relatively cheap solution of balancing "salt-out" with "salt-in" is not widely used in nature. Evolutionary processes toward such adaptation, as described by Dennis and Shimmin (1997), have led to the establishment of a small number of specialized groups: the aerobic extremely halophilic Archaea (Oren 2006a), the physiologically similar but phylogenetically unrelated *Salinibacter* (Bacteroidetes), and the fermentative obligatory anaerobic Bacteria (Oren 1999).

The use of organic compatible solutes allows much more flexibility with respect to the range of salt concentrations tolerated and does not require a high degree of adaptation of the intracellular enzymes. The enzymes do not greatly differ from those of non-halophilic prokaryotes, although they may have a somewhat increased content of acidic amino acids (Oren et al. 2005). Many taxonomic groups, displaying a great metabolic diversity, use this strategy. Thus, many of the dissimilatory processes identified in freshwater environments can also take place at high salinity. Certain metabolic types, however, such as methanogenesis from $H_2 + CO_2$ or from acetate, autotrophic nitrification, and others appear to be absent above 10–15 % salt. It is tempting to speculate that it is the too high energetic cost connected with adaptation to life at the highest salt concentrations that has prevented the evolution of halophiles performing these reactions (Oren 1999, 2011). In any case, the prokaryotes inhabiting hypersaline environments display an amazing diversity, and much progress has been made toward the understanding of their metabolism, phylogeny, and the molecular mechanisms of their adaptation to high salt (Ma et al. 2010).

References

Antón J, Oren A, Benlloch S, Rodríguez-Valera F, Amann R, Rosselló-Mora R (2002) *Salinibacter ruber* gen. nov., sp. nov., a novel extreme halophilic member of the Bacteria from saltern crystallizer ponds. Int J Syst Evol Microbiol 52:485–491

Antunes A, Rainey FA, Wanner G, Patzold J, Nobre MF, da Costa MS, Huber R (2007) A new lineage of halophilic, wall-less, contractile bacteria from a brine-filled deep of the Red Sea. J Bacteriol 190:3580–3587

Arahal DR, Ventosa A (2006) The family *Halomonadaceae*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 6, 3rd edn. Springer, New York, pp 811–835

Baliga NS, Bonneau R, Facciotti MT, Pan M, Glusman G, Deutsch EW, Shannon P, Chiu Y, Weng RS, Gan RR, Hung P, Date SV, Marcotte E, Hood L, Ng WV (2004) Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. Genome Res 14:2221–2234

Benlloch S, López-López A, Casamajor EO, Øvreås L, Goddard V, Dane L, Smerdon G, Massana R, Joint I, Thingstad F, Pedrós-Alió C, Rodríguez-Valera F (2002) Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environ Microbiol 4:349–360

Bertrand JC, Almallah M, Aquaviva M, Mille G (1990) Biodegradation of hydrocarbons by an extremely halophilic archaebacterium. Lett Appl Microbiol 11:260–263

Bodaker I, Sharon I, Suzuki MT, Reingersch R, Shmoish M, Andreishcheva F, Sogin ML, Rosenberg M, Belkin S, Oren A, Béjà O (2010) Comparative community genomics in the Dead Sea: an increasingly extreme environment. ISME J 4:399–407

Boetius A, Joye S (2009) Thriving in salt. Science 324:1523–1525

Bolhuis H, te Poele EM, Rodríguez-Valera F (2004) Isolation and cultivation of Walsby's square archaeon. Environ Microbiol 6:1287–1291

Bolhuis H, Palm P, Wende A, Farb M, Rampp M, Rodriguez-Valera F, Pfeiffer F, Oesterhelt D (2006) The genome of the square archaeon "Haloquadratum walsbyi": life at the limits of water activity. BMC Genomics 7:169

Boone DR, Mathrani IM, Liu Y, Menaia JAGF, Mah RA, Boone JE (1993) Isolation and characterization of *Methanohalophilus portucalensis* sp. nov. and DNA-reassociation study of the genus *Methanohalophilus*. Int J Syst Bacteriol 43:430–437

Borowitzka LJ (1981) The microflora. Adaptations to life in extremely saline lakes. Hydrobiologia 81:33–46

Bowers KJ, Mesbah NM, Wiegel J (2009) Biodiversity of poly-extremophilic bacteria: does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life? Sal Syst 5:9

Brandt KK, Ingvorsen K (1997) *Desulfobacter halotolerans* sp. nov., a halotolerant acetate-oxidizing sulfate-reducing bacterium isolated from sediments of Great Salt Lake. Utah Syst Appl Microbiol 20:366–373

Brito-Echeverría J, López-López A, Yarza P, Antón J, Rosselló-Móra R (2009) Occurrence of *Halococcus* spp. in the nostrils salt glands of the seabird *Calonectris diomedea*. Extremophiles 13:557–565

Britton KL, Stillman TJ, Yip KSP, Forterre P, Engel PC, Rice DW (1998) Insights into the molecular basis of salt tolerance from the study of glutamate dehydrogenase from *Halobacterium salinarum*. J Biol Chem 273:9023–9030

Brown AD (1976) Microbial water stress. Bacteriol Rev 40:803–846

Brown AD (1990) Microbial water stress physiology. Principles and perspectives. Wiley, Chichester

Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML (2004a) Cultivation of Walsby's square haloarchaeon. FEMS Microbiol Lett 238:469–473

Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML (2004b) Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. Appl Environ Microbiol 70:5258–5265

Burns DG, Janssen PH, Itoh T, Kamekura M, Li Z, Jensen G, Rodríguez-Valera F, Bolhuis H, Dyall-Smith ML (2007) *Haloquadratum walsbyi* gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. Int J Syst Evol Microbiol 57:387–392

Caumette P (1993) Ecology and physiology of phototrophic bacteria and sulfate-reducing bacteria in marine salterns. Experientia 49:473–481

Cayol JL, Ollivier B, Patel BKC, Prensier G, Guezennec J, Garcia J-L (1994) Isolation and characterization of *Halothermothrix orenii* gen. nov., sp. nov., a halophilic, thermophilic, fermentative strictly anaerobic bacterium. Int J Syst Bacteriol 44:534–540

Christian JHB, Waltho JA (1962) Solute concentrations within cells of halophilic and non-halophilic bacteria. Biochim Biophys Acta 65:506–508

Conrad R, Frenzel P, Cohen Y (1995) Methane emission from hypersaline microbial mats: lack of aerobic methane oxidation activity. FEMS Microbiol Ecol 16:297–305

Csonka LN (1989) Physiological and genetic response of bacteria to osmotic stress. Microbiol Rev 53:121–147

Csonka LN, O'Connor K, Larimer F, Richardson P, Lapidus A, Ewing AD, Goodner BW, Oren A (2005) What we can deduce about metabolism in the moderate halophile *Chromohalobacter salexigens* from its genomic sequence. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya. Springer, Dordrecht, pp 267–285

Daffonchio D, Borin S, Brusa T, Brusetti L, van der Wielen PWJJ, Bolhuis H, Yakimov MM, D'Auria G, Giulianio L, Marty D, Tamburini C, McGenity TJ,

Hallsworth JE, Sass AM, Timmis KN, Tselepides A, de Lange GJ, Hubner A, Thomson J, Varnavas SP, Gasparoni F, Gerber HW, Malinverno E, Corselli C, Biodeep Scientific Party (2006) Stratified prokaryote network in the oxic-anoxic transition of a deep-sea halocline. Nature 440:203–207

Dennis PP, Shimmin LC (1997) Evolutionary divergence and salinity-mediated selection in halophilic archaea. Microbiol Mol Biol Rev 61:90–104

Desmarais D, Jablonski PE, Fedarko NS, Roberts MF (1997) 2-Sulfotrehalose, a novel osmolyte in haloalkaliphilic archaea. J Bacteriol 179:3146–3153

Detkova EN, Boltyanskaya YV (2006) Relationships between the osmoadaptation strategy, amino acid composition of bulk protein, and properties of certain enzymes of haloalkaliphilic bacteria. Microbiologia (Russ) 75:259–265

Duschl A, Wagner G (1986) Primary and secondary chloride transport in *Halobacterium halobium*. J Bacteriol 168:548–552

Dym O, Mevarech M, Sussman JL (1995) Structural features that stabilize halophilic malate dehydrogenase from an archaebacterium. Science 267:1344–1346

Ebel C, Faou P, Franzetti B, Kernel B, Madern D, Pascu M, Pfister C, Richard S, Zaccai G (1999) Molecular interactions in extreme halophiles – the solvation-stabilization hypothesis for halophilic proteins. In: Oren A (ed) Microbiology and biogeochemistry of hypersaline environments. CRC Press, Boca Raton, pp 227–237

Edgerton ME, Brimblecombe P (1981) Thermodynamics of halobacterial environments. Can J Microbiol 27:899–909

Eisenberg H, Wachtel EJ (1987) Structural studies of halophilic proteins, ribosomes, and organelles of bacteria adapted to extreme salt concentrations. Ann Rev Biophys Biophys Chem 16:69–92

Eisenberg H, Mevarech M, Zaccai G (1992) Biochemical, structural, and molecular genetic aspects of halophilism. Adv Prot Chem 43:1–62

Elcock AH, McCammon JA (1998) Electrostatic contribution to the stability of halophilic proteins. J Mol Biol 280:731–748

Ellis DG, Bizzoco RW, Kelley ST (2008) Halophilic archaea isolated from geothermal steam vent aerosols. Environ Microbiol 10:1582–1590

Elshahed MS, Najar FZ, Roe BA, Oren A, Dewers TA, Krumholz LR (2004a) Survey of archaeal diversity reveals abundance of halophilic *Archaea* in a low-salt, sulfide- and sulfur-rich spring. Appl Environ Microbiol 70:2230–2239

Elshahed MS, Savage KN, Oren A, Gutierrez MC, Ventosa A, Krumholz LR (2004b) *Haloferax sulfurifontis* sp. nov., a halophilic archaeon isolated from a sulfide and sulfur-rich spring. Int J Syst Evol Microbiol 54:2275–2279

Falb M, Pfeiffer F, Palm P, Rodewald K, Hickmann V, Tittor J, Oesterhelt D (2005) Living with two extremes: conclusions from the genome sequence of *Natronomonas pharaonis*. Genome Res 15:1336–1343

Falb M, Müller K, Königsmaier L, Oberwinkler T, Horn P, von Gronau S, Gonzalez O, Pfeiffer F, Bornberg-Bauer E, Oesterhelt D (2008) Metabolism of halophilic archaea. Extremophiles 12:177–196

Franzmann PD (1991) The microbiota of saline lakes of the Vestvold Hills, Antarctica. In: Rodriguez-Valera F (ed) General and applied aspects of halophilic microorganisms. Plenum, New York, pp 9–14

Franzmann PD, Stackebrandt E, Sanderson K, Volkman JK, Cameron DE, Stevenson PL, McMeekin TA, Burton HR (1988) *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolated from Deep Lake Antarctica. Syst Appl Microbiol 11:20–27

Frolow F, Harel M, Sussman JL, Mevarech M, Shoham M (1996) Insights into protein adaptation to a saturated salt environment from the crystal structure of a halophilic 2Fe-2S ferredoxin. Nat Struct Biol 3:452–457

Galinski EA (1993) Compatible solutes of halophilic eubacteria: molecular principles, water-solute interactions, stress protection. Experientia 49:487–496

Galinski EA (1995) Osmoadaptation in bacteria. Adv Microb Physiol 37:273–328

Galinski EA, Louis P (1999) Compatible solutes: ectoine production and gene expression. In: Oren A (ed) Microbiology and biogeochemistry of hypersaline environments. CRC Press, Boca Raton, pp 187–202

Galinski EA, Trüper HG (1994) Microbial behaviour in salt stressed ecosystems. FEMS Microbiol Rev 15:95–108

Garcia-Pichel F, Nübel U, Muyzer G (1998) The phylogeny of unicellular, extremely halotolerant cyanobacteria. Arch Microbiol 169:469–482

Giani D, Giani L, Cohen Y, Krumbein WE (1984) Methanogenesis in the hypersaline Solar Lake (Sinai). FEMS Microbiol Lett 25:219–224

Ginzburg M, Sachs L, Ginzburg BZ (1970) Ion metabolism in a *Halobacterium*.I. Influence of age of culture on intracellular concentrations. J Gen Physiol 55:187–207

Grant WD (2004) Life at low water activity. Phil Trans R Soc B 359:1249–1267

Grant WD, Tindall BJ (1986) The alkaline saline environment. In: Herbert RA, Codd GA (eds) Microbes in extreme environments. Academic, London, pp 25–54

Grant WD, Gemmell RT, McGenity TJ (1998a) Halophiles. In: Horikoshi K, Grant WD (eds) Extremophiles. Microbial life in extreme environments. Wiley-Liss, New York, pp 93–132

Grant WD, Gemmell RT, McGenity TJ (1998b) Halobacteria: the evidence for longevity. Extremophiles 2:279–287

Graur D, Pupko T (2001) The Permian bacterium that isn't. Mol Biol Evol 18:1143–1146

Hagemann M (2011) Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev 35:87–123

Hagemann M, Schoor A, Mikkat S, Effmert U, Zuther E, Marin K, Fulda S, Vinnemeyer J, Kunert A, Milkowski C, Probst C, Erdmann N (1999) The biochemistry and genetics of the synthesis of osmoprotective compounds in cyanobacteria. In: Oren A (ed) Microbiology and biogeochemistry of hypersaline environments. CRC Press, Boca Raton, pp 177–186

Hallsworth JE, Yakimov MM, Golyshin PN, Gillion JLM, D'Auria G, de Lima Alves F, La Cono V, Genovese M, Kew BA, Hayes SL, Harris G, Giuliano L, Timmis KN, McGenity TJ (2007) Limits of life in $MgCl_2$-containing environments: chaotropicity defines the window. Environ Microbiol 9:801–813

Hamaide F, Kushner DJ, Sprott GD (1983) Proton motive force and Na^+/H^+ antiport in a moderate halophile. J Bacteriol 156:537–544

Hartman AL, Norais C, Badger JH, Delmas S, Haldenby S, Madupu R, Robinson J, Khouri H, Ren Q, Lowe TM, Maupin-Furlow J, Pohlschröder M, Daniels C, Pfeiffer F, Allers T, Eisen JA (2010) The complete genome sequence of *Haloferax volcanii* DS2, a model archaeon. PLoS One 5:e9605

Hartmann R, Sickinger H-D, Oesterhelt D (1980) Anaerobic growth of halobacteria. Proc Natl Acad Sci USA 77:3821–3825

Heyer J, Berger U, Hardt M, Dunfield PF (2005) *Methylohalobius crimeensis* gen. nov., sp. nov., a moderately halophilic, methanotrophic bacterium isolated from hypersaline lakes of Crimea. Int J Syst Evol Microbiol 55:1817–1826

Imhoff JF (1993) Osmotic adaptation in halophilic and halotolerant microorganisms. In: Vreeland RH, Hochstein LI (eds) The biology of halophilic bacteria. CRC Press, Boca Raton, pp 211–253

Imhoff JF, Hashwa F, Trüper HG (1978) Isolation of extremely halophilic phototrophic bacteria from the alkaline Wadi Natrun, Egypt. Arch Hydrobiol 84:381–388

Imhoff JF, Sahl HG, Soliman GSH, Trüper HG (1979) The Wadi Natrun: chemical composition and microbial mass development in alkaline brines of eutrophic desert lakes. Geomicrobiol J 1:219–234

Jannasch HW (1957) Die bakterielle Rotfärbung der Salzseen des Wadi Natrun (Ägypten). Arch Hydrobiol 53:425–433

Javor BJ (1989) Hypersaline environments. Microbiology and biogeochemistry. Springer, Berlin

Javor BJ (2002) Industrial microbiology of solar salt production. J Indust Microbiol Biotechnol 28:42–47

Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Arch Microbiol 170:319–330

Kevbrin VV, Zhilina TN, Zavarzin GA (1995) Physiology of homoacetic bacteria *Acetohalobium arabaticum*. Microbiologiya 64:165–170 (in Russian)

Khmelenina VN, Kalyuzhneya MG, Starostina NG, Suzina NE, Trotsenko YA (1997) Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. Curr Microbiol 35:257–261

Koops H-P, Böttcher B, Möller U, Pommerening-Röser A, Stehr G (1990) Description of a new species of *Nitrosococcus*. Arch Microbiol 154:244–248

Krekeler D, Sigalevich P, Teske A, Cypionka H, Cohen Y (1997) A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai) *Desulfovibrio oxyclinae* sp. nov. Arch Microbiol 167:369–375

Kunin V, Raes J, Harris JK, Spear JR, Walker JJ, Ivanova N, von Mering C, Bebout BM, Pace NR, Bork P, Hugenholtz P (2008) Millimeter scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol Systems Biol 4:198

Kushner DJ (1978) Life in high salt and solute concentrations: halophilic bacteria. In: Kushner DJ (ed) Microbial life in extreme environments. Academic, London, pp 317–368

Kushner DJ (1985) The *Halobacteriaceae*. In: Woese CR, Wolfe RS (eds) The bacteria. A treatise on structure and function, vol VIII, Archaebacteria. Academic, Orlando, pp 171–214

Lai M-C, Gunsalus RP (1992) Glycine betaine and potassium ions are the major compatible solutes in the extremely halophilic methanogen *Methanohalophilus* strain Z7302. J Bacteriol 174:7474–7477

Lanyi JK (1974) Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriol Rev 38:272–290

Lanyi JK (1986) Halorhodopsin: a light-driven chloride ion pump. Ann Rev Biophys Biophys Chem 15:11–28

Lanyi JK (2003) Bacteriorhodopsin. Ann Rev Physiol 66:665–688

Lanyi JK, MacDonald RE (1976) Existence of electrogenic hydrogen/sodium transport in *Halobacterium* cell envelope vesicles. Biochemistry 15:4608–4614

Lanyi JK, Silverman MP (1972) The state of binding of intracellular K^+ in *Halobacterium cutirubrum*. Can J Microbiol 18:993–995

Lanyi JK, Silverman MP (1979) Gating effects in *Halobacterium halobium* membrane transport. J Biol Chem 254:4750–4755

Lanyi JK, Stevenson J (1970) Studies of the electron transport chain of extremely halophilic bacteria IV. Role of hydrophobic forces in the structure of menadione reductase. J Biol Chem 245:4074–4080

Larsen H (1973) The halobacteria's confusion to biology. Antonie Van Leeuwenhoek 39:383–396

Lentzen G, Schwarz T (2006) Extremolytes: natural compounds from extremophiles for versatile applications. Appl Microbiol Biotechnol 72:623–634

Lowe SE, Jain MK, Zeikus JG (1993) Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. Microbiol Rev 57:451–509

Luisi BF, Lanyi JK, Weber HJ (1980) Na^+ transport via Na^+/H^+ antiport in *Halobacterium halobium* envelope vesicles. FEBS Lett 117:354–358

Ma Y, Galinski EA, Grant WD, Oren A, Ventosa A (2010) Meeting review. Halophiles 2010: life in saline environments. Appl Environ Microbiol 76:6971–6981

Madern D, Ebel C, Zaccai G (2000) Halophilic adaptation of enzymes. Extremophiles 4:91–98

Mancinelli RL, Hochstein LI (1986) The occurrence of denitrification in extremely halophilic bacteria. FEMS Microbiol Lett 35:55–58

Margesin R, Schinner F (2001) Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5:73–83

Martin DD, Ciulla RA, Roberts MF (1999) Osmoadaptation in Archaea. Appl Environ Microbiol 65:1815–1825

Masui M, Wada S (1973) Intracellular concentrations of Na^+, K^+ and Cl^- of a moderately halophilic bacterium. Can J Microbiol 19:1181–1186

Matheson AT, Sprott GD, McDonald IJ, Tessier H (1976) Some properties of an unidentified halophile: growth characteristics, internal salt concentrations, and morphology. Can J Microbiol 22:780–786

Mavromatis K, Ivanova N, Anderson I, Lykidis A, Hooper SD, Sun H, Kunin V, Lapidus A, Hugenholtz P, Patel B, Kyrpides NC (2009) Genome analysis of the anaerobic thermohalophilic bacterium *Halothermothrix orenii*. PLoS One 4:e4192

McGenity TJ (2010) Halophilic hydrocarbon degraders. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 1939–1951

McMeekin TA, Nichols PD, Nichols SD, Jugasz A, Franzmann PD (1993) Biology and biotechnological potential of halotolerant bacteria from Antarctic saline lakes. Experientia 49:1042–1046

Mermelstein LD, Zeikus JG (1998) Anaerobic nonmethanogenic extremophiles. In: Horikoshi K, Grant WD (eds) Extremophiles. Microbial life in extreme environments. Wiley-Liss, New York, pp 255–284

Mesbah NM, Wiegel J (2008) Life at extreme limits. The anaerobic halophilic alkalithermophiles. Ann NY Acad Sci 1125:44–57

Mesbah NM, Wiegel J (2009) *Natronovirga wadinatrunensis* gen. nov., sp. nov. and *Natranaerobius trueperi* sp. nov., halophilic alkalithermophilic microorganisms from soda lakes of the Wadi An Natrun, Egypt. Int J Syst Evol Microbiol 59:2042–2048

Meury J, Kohiyama M (1989) ATP is required for K^+ active transport in the archaebacterium *Haloferax volcanii*. Arch Microbiol 151:530–536

Mevarech M, Neumann E (1977) Malate dehydrogenase isolated from extremely halophilic bacteria of the Dead Sea. 2. Effect of salt on the catalytic activity and structure. Biochemistry 16:3786–3792

Mevarech M, Frolow F, Gloss LM (2000) Halophilic enzymes: proteins with a grain of salt. Biophys Chem 86:155–164

Mongodin MEF, Nelson KE, Duagherty S, DeBoy RT, Wister J, Khouri H, Weidman J, Balsh DA, Papke RT, Sanchez Perez G, Sharma AK, Nesbø CL, MacLeod D, Bapteste E, Doolittle WF, Charlebois RL, Legault B, Rodríguez-Valera F (2005) The genome of *Salinibacter ruber*: convergence and gene exchange among hyperhalophilic bacteria and archaea. Proc Natl Acad Sci USA 102:18147–18152

Mullakhanbhai MF, Larsen H (1975) *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. Arch Microbiol 104:207–214

Müller V, Oren A (2003) Metabolism of chloride in halophilic prokaryotes. Extremophiles 7:261–266

Ng WV, Kennedy SP, Mahairas GG, Berquist B, Pan M, Shukla HD, Lasky SR, Baliga NS, Thorsson V, Sbrogna J, Swartzell S, Weir D, Hall J, Dahl TA, Welti R, Goo YA, Leithausen B, Keller K, Cruz R, Danson MJ, Hough DW, Maddocks DG, Jablonski PE, Krebs MP, Angevine GM, Dale H, Isenbarger TA, Peck RF, Pohlschröder M, Spudich JL, Jung K-H, Alam M, Freitas T, Hou S, Daniels CJ, Dennis PP, Omer AD, Ebhardt H, Lowe TM, Liang P, Riley M, Hood L, DasSarma S (2000) Genome sequence of *Halobacterium* species NRC-1. Proc Natl Acad Sci USA 97:12176–12181

Nickle DC, Learn GH, Rain MW, Mullins JI, Miller JE (2002) Curiously modern DNA for a "250 million-year-old" bacterium. J Mol Evol 54:134–137

Norton CF, McGenity TJ, Grant WD (1993) Archaeal halophiles (halobacteria) from two British salt mines. J Gen Microbiol 139:1077–1081

Novitsky TJ, Kushner DJ (1975) Influence of temperature and salt concentration on the growth of a facultatively halophilic "Micrococcus" sp. Can J Microbiol 21:107–110

Ollivier B, Fardeau M-L, Cayol J-L, Magot M, Patel BKC, Prensier G, Garcia J-L (1998) *Methanocalculus halotolerans* gen. nov., sp. nov., isolated from an oil-producing well. Int J Syst Bacteriol 48:821–828

Ollivier B, Hatchikian CE, Prensier G, Guezennec J, Garcia J-L (1991) *Desulfohalobium retbaense* gen. nov. sp. nov., a halophilic sulfate-reducing bacterium from sediments of a hypersaline lake in Senegal. Int J Syst Bacteriol 41:74–81

Ollivier B, Caumette P, Garcia J-L, Mah RA (1994) Anaerobic bacteria from hypersaline environments. Microbiol Rev 58:27–38

Oremland RS, King GM (1989) Methanogenesis in hypersaline environments. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 180–190

Oren A (1986) Intracellular salt concentration of the anaerobic halophilic eubacteria *Haloanaerobium praevalens* and *Halobacteroides halobius*. Can J Microbiol 32:4–9

Oren A (1988a) The microbial ecology of the Dead Sea. In: Marshall KC (ed) Advances in microbial ecology, vol 10. Plenum, New York, pp 193–229

Oren A (1988b) Anaerobic degradation of organic compounds at high salt concentrations. Antonie van Leeuwenhoek 54:267–277

Oren A (1993) Ecology of extremely halophilic microorganisms. In: Vreeland RH, Hochstein LI (eds) The biology of halophilic bacteria. CRC Press, Boca Raton, pp 25–53

Oren A (1994) The ecology of the extremely halophilic archaea. FEMS Microbiol Rev 13:415–440

Oren A (1999) Bioenergetic aspects of halophilism. Microbiol Mol Biol Rev 63:334–348

Oren A (2000) Salts and brines. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer, Dordrecht, pp 281–306

Oren A (2001) The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. Hydrobiologia 466:61–72

Oren A (2002a) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Indust Microbiol Biotechnol 28:56–63

Oren A (2002b) Halophilic microorganisms and their environments. Kluwer Scientific, Dordrecht

Oren A (2006a) The order halobacteriales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, vol 3. Springer, New York, pp 113–164

Oren A (2006b) The order haloanaerobiales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, vol 4. Springer, New York, pp 804–817

Oren A (2006c) The genera *Rhodothermus, Thermonema, Hymenobacter and Salinibacter*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, vol 4. Springer, New York, pp 712–740

Oren A (2007) Biodiversity in highly saline environments. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 223–231

Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Sal Syst 4:2

Oren A (2009) Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions. Aquat Microb Ecol 56:193–204

Oren A (2010a) Diversity of halophiles. In: Horikoshi K, Bull A, Robb F, Stetter K, Antranikian G (eds) Extremophiles handbook. Springer, Tokyo

Oren A (2010b) Ecology of halophiles. In: Horikoshi K, Bull A, Robb F, Stetter K, Antranikian G (eds) Extremophiles handbook. Springer, Tokyo

Oren A (2010c) Industrial and environmental applications of halophilic microorganisms. Environ Technol 31:825–834

Oren A (2011) Thermodynamic limits to microbial life at high salt concentrations. Environ Microbiol 13:1908–1923

Oren A, Gurevich P (1993) The fatty acid synthetase of *Haloanaerobium praevalens* is not inhibited by salt. FEMS Microbiol Lett 108:287–290

Oren A, Rodríguez-Valera F (2001) The contribution of *Salinibacter* species to the red coloration of saltern crystallizer ponds. FEMS Microbiol Ecol 36:123–130

Oren A, Gurevich P, Azachi M, Henis Y (1992) Microbial degradation of pollutants at high salt concentrations. Biodegradation 3:387–398

Oren A, Heldal M, Norland S (1997) X-ray microanalysis of intracellular ions in the anaerobic halophilic eubacterium *Haloanaerobium praevalens*. Can J Microbiol 43:588–592

Oren A, Rodríguez-Valera F, Antón J, Benlloch S, Rosselló-Mora R, Amann R, Coleman J, Russell NJ (2004) Red, extremely halophilic, but not archaeal: the physiology and ecology of *Salinibacter ruber*, a bacterium isolated from saltern crystallizer ponds. A. Ventosa. Halophilic microorganisms. Springer, Berlin, pp 63–76

Oren A, Larimer F, Richardson P, Lapidus A, Csonka LN (2005) How to be moderately halophilic with a broad salt tolerance: clues from the genome of *Chromohalobacter salexigens*. Extremophiles 9:275–279

Oxley APA, Lanfranconi MP, Wurdemann D, Ott S, Schreiber S, McGenity TJ, Timmis KN, Nogales B (2010) Halophilic archaea in the human intestinal mucosa. Environ Microbiol 12:2398–2410

Parnell JJ, Rompato G, Latta LC IV, Pfender ME, Van Nostrand JD, He Z, Zhou J, Andersen G, Champine P, Ganesan B, Weimer BC (2010) Functional biogeography as evidence of gene transfer in hypersaline microbial communities. PLoS One 5:e12919

Pieper U, Kapadia G, Mevarech M, Herzberg O (1998) Structural features of halophilicity derived from the crystal structure of dihydrofolate reductase from the Dead Sea archaeon, *Haloferax volcanii*. Structure 6:75–88

Post FJ (1977) The microbial ecology of the Great Salt Lake. Microb Ecol 3:143–165

Pundak S, Eisenberg H (1981) Structure and activity of malate dehydrogenase of the extreme halophilic bacteria of the Dead Sea. 1. Conformation and interaction with water and salt between 5 M and 1 M NaCl concentration. Eur J Biochem 118:463–470

Pundak S, Aloni S, Eisenberg H (1981) Structure and activity of malate dehydrogenase of the extreme halophilic bacteria of the Dead Sea. 2. Inactivation, dissociation and unfolding at NaCl concentrations below 2 M. Salt, salt concentration and temperature dependence of enzyme stability. Eur J Biochem 118:471–477

Purdy KJ, Cresswell-Maynard TD, Nedwell DB, McGenity TJ, Grant WD, Timmis KN, Embley TM (2004) Isolation of haloarchaea that grow at low salinities. Environ Microbiol 6:591–595

Rainey FA, Zhilina TN, Boulygina ES, Stackebrandt E, Tourova TP, Zavarzin GA (1995) The taxonomic status of the fermentative anaerobic bacteria: description of Haloanaerobiales ord. nov., Halobacteroidaceae fam. nov., *Orenia* gen. nov. and further taxonomic rearrangements at the genus and species level. Anaerobe 1:185–199

Reed RH (1986) Halotolerant and halophilic microbes. In: Herbert RA, Codd GA (eds) Microbes in extreme environments. Academic, London, pp 55–81

Reistad R (1970) On the composition and nature of the bulk protein of extremely halophilic bacteria. Arch Microbiol 71:353–360

Rengpipat S, Lowe SE, Zeikus JG (1988) Effect of extreme salt concentrations on the physiology and biochemistry of *Halobacteroides acetoethylicus*. J Bacteriol 170:3065–3071

Roberts MF (2005) Organic compatible solutes of halotolerant and halophilic microorganisms. Sal Syst 1:5

Roberts MF (2006) Characterization of organic compatible solutes of halotolerant and halophilic microorganisms. In: Rainey FA, Oren A (eds) Extremophiles, vol 35, Methods in microbiolog. lsevier/Academic, Amsterdam, pp 615–647

Rodriguez-Valera F (1988) Characteristics and microbial ecology of hypersaline environments. In: Rodriguez-Valera F (ed) Halophilic bacteria, vol 1. CRC Press, Boca Raton, pp 3–30

Rodriguez-Valera F (1993) Introduction to saline environments. In: Vreeland RH, Hochstein LI (eds) The biology of halophilic bacteria. CRC Press, Boca Raton, pp 1–23

Roeßler M, Müller V (2002) Chloride, a new environmental signal molecule involved in gene regulation in a moderately halophilic bacterium, *Halobacillus halophilus*. J Bacteriol 184:6207–6215

Rubentschik L (1929) Zur Nitrifikation bei hohen Salzkonzentrationen. Zentralbl Bakteriol Abt II 77:1–18

Saiz-Jimenez C, Laiz L (2000) Occurrence of halotolerant/halophilic bacterial communities in deteriorated monuments. Int Biodeter Biodegr 46:319–326

Savage KN, Krumholz LR, Oren A, Elshahed MS (2007) *Haladaptatus paucihalophilus* gen. nov., sp. nov., a halophilic archaeon isolated from a low-salt, high-sulfide spring. Int J Syst Evol Microbiol 57:19–24

Savage KN, Krumholz LR, Oren A, Elshahed MS (2008) *Halosarcina pallida* gen. nov., sp. nov., a halophilic archaeon isolated from a low-salt, sulfide-rich spring. Int J Syst Evol Microbiol 58:856–860

Schäfer G, Engelhard M, Müller V (1999) Bioenergetics of the Archaea. Microbiol Mol Biol Rev 63:570–620

Schwibbert K, Marin-Sanguino M, Bagyan I, Heidrich G, Seitz H, Rampp M, Schuster SC, Klenk H-P, Pfeiffer F, Oesterhelt D, Kunte HJ (2010) A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581^{T}. Environ Microbiol. DOI:10.1111/j.1462-2920.2010.02336.x

Shindler DB, Wydro RM, Kushner DJ (1977) Cell-bound cations of the moderately halophilic bacterium *Vibrio costicola*. J Bacteriol 130:698–703

Sokolov AP, Trotsenko YA (1995) Methane consumption in (hyper)saline habitats of Crimea. FEMS Microbiol Ecol 18:299–304

Soliman GSH, Trüper HG (1982) *Halobacterium pharaonis* sp. nov., a new, extremely haloalkaliphilic archaebacterium with low magnesium requirement. Zbl Bakt Hyg, I Abt Orig C 3:318–329

Strahl H, Greie JC (2008) The extremely halophilic archaeon *Halobacterium salinarum* R1 responds to potassium limitation by expression of the K^+--transporting KdpFABC P-type ATPase and by a decrease in intracellular K^+. Extremophiles 12:741–752

Tadeo X, López-Méndez B, Trigueros T, Laín A, Castaño D, Millet O (2009) Structural basis for the amino acid composition of proteins from halophilic Archaea. PLoS Biol 7:e1000257

Tapilatu YH, Grossi V, Acquaviva M, Militon C, Bertrand J-C, Cuny P (2010) Isolation of hydrocarbon-degrading extremely halophilic archaea from an uncontaminated hypersaline pond (Camargue, France). Extremophiles 14:225–231

Tindall BJ, Trüper HG (1986) Ecophysiology of the aerobic halophilic archaebacteria. Syst Appl Microbiol 7:202–212

Tindall BJ, Mills AA, Grant WD (1980) An alkaliphilic red halophilic bacterium with a low magnesium requirement from a Kenyan soda lake. J Gen Microbiol 116:257–260

Tindall BJ, Ross HNM, Grant WD (1984) *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaebacteria. Syst Appl Microbiol 5:41–57

Trüper HG, Galinski EA (1990) Biosynthesis and fate of compatible solutes in extremely halophilic phototrophic eubacteria. FEMS Microbiol Rev 75:247–254

Trüper HG, Severin J, Wohlfarth A, Müller E, Galinski EA (1991) Halophily, taxonomy, phylogeny and nomenclature. In: Rodriguez-Valera F (ed) General and applied aspects of halophilic microorganisms. Plenum, New York, pp 3–7

van der Wielen PWJJ, Bolhuis H, Borin S, Daffonchio D, Corselli C, Giuliano L, D'Auria G, de Lange GJ, Huebner A, Varnavas SP, Thomson J, Tamburini C, Marty D, McGenity TJ, Timmis KN, BioDeep Scientific Party (2005) The enigma of prokaryotic life in deep hypersaline anoxic basins. Science 307:121–123

Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Vreeland RH (1987) Mechanisms of halotolerance in microorganisms. CRC Crit Rev Microbiol 14:311–356

Vreeland RH, Litchfield CD, Martin EL, Elliot E (1980) *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. Int J Syst Bacteriol 30:485–495

Vreeland RH, Mierau BD, Litchfield CD, Martin EL (1983) Relationship of the internal solute composition to the salt tolerance of *Halomonas elongata*. Can J Microbiol 29:407–414

Vreeland RH, Rosenzweig WD, Powers DW (2000) Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. Nature 407:897–900

Wagner G, Hartmann R, Oesterhelt D (1978) Potassium uniport and ATP synthesis in *Halobacterium halobium*. Eur J Biochem 89:169–179

Walsby AE (1980) A square bacterium. Nature 283:69–71

Walsby AE (2005) Archaea with square cells. Trends Microbiol 13:193–195

Ward DM, Brock TD (1978) Hydrocarbon degradation in hypersaline environments. Appl Environ Microbiol 35:353–359

Welsh DT, Lindsay YE, Caumette P, Herbert RA, Hannan J (1996) Identification of trehalose and glycine betaine as compatible solutes in the moderately halophilic sulfate reducing bacterium *Desulfovibrio halophilus*. FEMS Microbiol Lett 140:203–207

Wohlfarth A, Severin J, Galinski EA (1990) The spectrum of compatible solutes in heterotrophic halophilic eubacteria of the family *Halomonadaceae*. J Gen Microbiol 136:705–712

Wolfe-Simon F, Switzer Blum J, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, Webb PK, Davies PCW, Anbar AD, Oremland RS (2011) A bacterium that can grow by using arsenic instead of phosphorus. Science 332:1163–1166

Wood AP, Kelly DP (1991) Isolation and characterisation *of Thiobacillus halophilus* sp. nov., a sulphur-oxidising autotrophic eubacterium from a Western Australian hypersaline lake. Arch Microbiol 156:277–280

Zaccai G, Eisenberg H (1991) A model for the stabilization of a halophilic protein. G. di Prisco. Life under extreme conditions. Springer, Berlin, pp 125–137

Zavarzin GA, Zhilina TN, Pusheva MA (1994) Halophilic acetogenic bacteria. In: Drake HL (ed) Acetogenesis. Chapman and Hall, New York, pp 432–444

Zhilina TN, Zavarzin GA (1987) *Methanohalobium evestigatum* gen. nov., sp. nov., extremely halophilic methane-producing archaebacteria. Dokl Akad Nauk SSSR 293:464–468, in Russian

Zhilina TN, Zavarzin GA (1990) Extremely halophilic, methylotrophic, anaerobic bacteria. FEMS Microbiol Rev 87:315–322

Zhilina TN, Zavarzin GA (1994) Alkaliphilic anaerobic community at pH 10. Curr Microbiol 29:109–112

Zhilina TN, Zavarzin GA, Detkova EN, Rainey FA (1996) *Natroniella acetigena* gen. nov. sp. nov., an extremely haloalkaliphilic, homoacetic bacterium: a new member of *Haloanaerobiales*. Curr Microbiol 32:320–326

20 Alkaliphilic Prokaryotes

Terry Ann Krulwich[1] · *Masahiro Ito*[2,3]
[1]Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY, USA
[2]Graduate School of Life Sciences, Toyo University, Gunma, Japan
[3]Japan and Bio-Nano Electronics Research Centre, Toyo University, Kawagoe, Saitama, Japan

Introduction and Definitions

Introduction

Alkaliphilic prokaryotes, in their rich phylogenetic diversity and metabolic versatility, are central participants in useful bioprocessing settings, such as sulfide-removing bioreactors (Sorokin et al. 2008; Sarethy et al. 2011). They have potential bioremediation capacity and are a major resource for enzymes that have many different applications to biotechnology (Horikoshi 1999; Fujinami and Fujisawa 2010; Sarethy et al. 2011). Often, such enzymes come from "polyextremophiles" whose exoenzymes, like the growth of their bacterial hosts, are also thermoresistant or cold resistant and/or salt resistant, i.e., from thermoalkaliphiles, psychrophilic alkaliphiles, or haloalkaliphiles (Yumoto et al. 2002, 2004; Wiegel and Kevbrin 2004; Mesbah and Wiegel 2008). With increasing numbers of complete alkaliphile genome sequences, identification of genes encoding such enzymes is greatly facilitated. Further, protein engineering of alkaliphile enzymes is increasingly used to enhance the useful properties of alkaliphile enzymes, based on studies of structure-function comparisons of the alkaliphile enzymes and their neutralophile counterparts (Shirai et al. 1997a, 2001, 2007; Dubnovitsky et al. 2005).

The insights gained from these "applied" structure-function studies have significant crossover impact on overlapping and distinct efforts to gain insight into the design and mechanistic principles involved in alkaliphily. Research on the cell biological, physiological, and bioenergetic adaptations that underpin alkaliphily is the other major focus of studies on this group of extremophiles. Studies of how alkaliphiles meet the challenge of retaining function of enzymes and other molecules that are exposed to high external pH, the challenge of maintaining a much lower cytoplasmic pH than very high external pH values (i.e., pH homeostasis), and the challenge of meeting the increased energy costs of life at high pH have led to discovery of novel molecules and mechanisms that turn out to be relevant way beyond alkaliphiles (Janto et al. 2011; Krulwich et al. 2011a, b). For example, studies of alkaliphile bioenergetics have led to discovery of the following: two new types of Mot protein pairs that form the stator-channel elements for flagellar rotors, the Na^+-coupled MotPS type and a MotAB type that uses both Na^+ and H^+ coupling depending on external pH (Ito et al. 2004a; Terahara et al. 2008); the first prokaryotic voltage-gated Na^+ channel whose structure was recently reported (Ren et al. 2001; Payandeh et al. 2011); and the novel Mrp-type hetero-oligomeric cation/proton antiporter comprising its own cation/proton antiporter-3 (CPA3) family in the transporter classification database (Saier et al. 2006) and which shares homology with a domain of respiratory complex I while having important physiological roles among non-alkaliphilic bacteria (Hamamoto et al. 1994; Mathiesen and Hägerhäll 2003; Swartz et al. 2005).

This essay begins with definitions and a brief historical perspective on studies of alkaliphily. These sections will be followed by a review of work on the ecology, the physiology and bioenergetics, and applications of alkaliphiles and their component enzymes and transporters. Advantage will be taken

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_58,

of the growing number of sequenced alkaliphile genomes, structures of alkaliphile proteins, and the beginnings of proteomic work which provide an update of rapidly expanding areas of study of these fascinating and useful bacteria.

Definitions

A range of pH values are used by different investigators to define extreme alkaliphiles, alkaliphiles, and alkaline-tolerant bacteria. Extremely alkaliphilic bacteria are generally defined as those that grow at an external pH $\geq$ 10.0 (the more extreme strains growing at pH $>$ 12), moderate alkaliphiles as those that can grow in the pH 9.0–10.0 range, and alkaline-tolerant bacteria as those that can survive and grow suboptimally at ~pH 9.0. Alkaliphiles can be obligate alkaliphiles, exhibiting poor or no growth below pH 9.0, or facultative alkaliphiles, exhibiting significant growth at pH values near neutral. In general, even facultative alkaliphiles grow less well at neutral pH than at high pH, which is thought to reflect the disadvantage, at neutral pH, of many of the adaptations that make growth at very alkaline pH possible (Krulwich et al. 2011a, b), as will be noted in connection with specific adaptations that are expected to have that effect. Extreme alkaliphiles are among a larger group of bacteria, including acidophiles, thermophiles, psychrophiles, and barophiles, which grow under particular extreme conditions and are referred to as "extremophiles" (Horikoshi and Bull 2011). Polyextremophiles exhibit significant growth under conditions of multiple extreme conditions, but the alkaliphilic capacity of haloalkaliphiles, thermoalkaliphiles, or halothermoalkaliphiles is usually somewhat lower than that of the most extreme alkaliphiles alone (Bowers et al. 2009; Hicks et al. 2010). Thermodynamic limits probably constrain the extent to which energy-dependent responses to multiple extreme conditions can each be managed optimally (Bowers et al. 2009; Oren 2010). Yet, robust responses to particular stresses are required for all alkaliphiles, relative to neutralophiles, since some stresses are exacerbated by high pH. For example, the cytotoxicity of Na^+ is higher at high pH than at neutral pH (Padan et al. 2005; Wei et al. 2007), generation of toxic reactive oxygen species is elevated at high pH (Selivanov et al. 2008), and major environments of alkaliphiles present them with challenges of elevated osmolarity (Banciu et al. 2004, 2008).

Historical Notes

Koki Horikoshi, a leading investigator of alkaliphiles, was the first to initiate broad-based studies of these bacteria, starting in the late 1960s. He noted that when he began his alkaliphile work, he found only 16 prior literature references to alkaliphilic bacteria (Horikoshi 2006). The earliest reports of bona fide alkaliphiles were those of *Bacillus pasteurii* by Gibson (1934) and of *Bacillus alcalophilus* by Vedder (1934). Interestingly, while *B. alcalophilus* strains have since been isolated from soil, the Vedder strain, ATCC276547, was isolated from human feces. During the early 1960s, indigo dye reduction was shown to depend upon maintaining sufficient alkalinity, and the fermentation process was improved by adding alkaliphilic *Bacillus* sp. strain S-8, which had been isolated from an indigo ball undergoing fermentation at high pH (Takahara et al. 1961; Takahara and Tanabe 1962). Although other methods took over for indigo blue dye production, a recent return to traditional methods has been accompanied by isolation of psychrotolerant alkaliphiles and other alkaliphilic strains from fermentation liquor and indigo balls (Yumoto et al. 2004; Aino et al. 2008). Over the years, Horikoshi's laboratory and others' advanced this tradition of optimizing alkaliphile fermentation processes or their products while also contributing to alkaliphile taxonomy and characterization (Horikoshi 1996; Ito et al. 1998). The diversity of alkaliphiles was subsequently extended by Grant and colleagues (Grant et al. 1986; Jones et al. 1998) and then others (Sorokin et al. 1999; Rees et al. 2004; Zhilina et al. 2004, 2005; Mesbah et al. 2007; Joshi et al. 2008) in extending the identification of bacteria (as well as archaea) that thrive in natural, selective environments such as the highly alkaline soda lakes in Africa, Asia, and the West Coast of the United States.

Horikoshi's group first reported that the alkaliphile isolates studied in their laboratory require and thrive in added Na^+ (Kurono and Horikoshi 1973). Aono and Horikoshi subsequently reported on the composition of the peptidoglycan of alkaliphilic *Bacillus* species and presented data on the cell wall-associated acid teichuronic acid and teichuronopeptide in *Bacillus halodurans* C-125 (formerly called *B. lentus* str. C-125) (Aono and Horikoshi 1983; Aono et al. 1984), initiating a theme of negatively charged surface components that is important in these organisms. Physiological studies of alkaliphiles were extended by the work of Nosoh's group (Koyama et al. 1976; Koyama 1996) and by the emerging physiological characterizations of alkaliphiles from soda lakes. During the 1970s, the Krulwich laboratory undertook physiological studies on the involvement of antiporters in resolving the problem of cytoplasmic pH regulation by extreme alkaliphiles that indicated a central role for them and identified the first alkaliphile antiporter, NhaC, from *Bacillus pseudofirmus* OF4 (Mandel et al. 1980; Ivey et al. 1991; Ito et al. 1997). Studies by Horikoshi, Kudo, and colleagues subsequently led to the identification of the critically important Mrp antiporter (Kudo et al. 1990; Hamamoto et al. 1994). Several laboratories also initiated work on the bioenergetics of both anaerobic and aerobic alkaliphiles, which identified elements of the sodium cycle that supports much of the bioenergetic work and cell surface elements that make contributions (Hirota et al. 1981; Hirota and Imae 1983; Krulwich et al. 1985; Suigyama et al. 1986; Koyama 1989a, b; Krulwich and Guffanti 1989; Aono et al. 1999; Gilmour et al. 2000; Ito et al. 2004a, b). The Krulwich laboratory also focused on the problem of how aerobic alkaliphiles that grow non-fermentatively achieve H^+-coupled oxidative phosphorylation (Krulwich 1995). Under highly alkaline growth conditions in which the cytoplasmic pH is maintained well below the external pH, the bulk proton motive force (PMF, alkaline, and positive inside relative to outside) that was described by Peter Mitchell

(Mitchell 1961) is too low to support the observed level of respiration-dependent ATP synthesis (Krulwich 1995; Krulwich et al. 2007; Hicks et al. 2010). Research in this area by multiple laboratories ultimately impacted general concepts of energy coupling to bioenergetic work (Mulkidjanian et al. 2005, 2006; Hicks et al. 2010; Krulwich et al. 2011), while the larger interacting H^+ and Na^+ cycles promoted discovery of specific transporters and channels that have already been noted.

At the same time, studies of the stability and pH optimum for alkaliphile enzymes were initiated that drew upon three-dimensional crystal structures and properties that could be deduced from molecular characterizations (Sobek et al. 1992; van der Laan et al. 1992; Shirai et al. 1997a, b, 2001; Kobayashi et al. 1999; Dubnovitsky et al. 2005). Most recently, studies of the physiology and bioenergetic properties of alkaliphiles were enhanced by the growth of applicable molecular biological and imaging techniques as well as proteome and genomic insights. The current burgeoning number of alkaliphile genomes, for which the *Bacillus halodurans* C-125 genome sequence was the first one (Takami et al. 2000), and all the "omics" that have begun to follow have created enormous new opportunities for research and discovery of both a basic and applied nature.

The older literature on bacteria that grow at high pH referred to these organisms as "alkalophiles," but this was replaced by "alkaliphiles" during the 1990s because the latter terminology preserves the "alkali" root as noted by Hans Trüper (Krulwich and Ivey 1990).

Ecology and Diversity

Ecology

A comprehensive set of lists of over 73 Gram-positive alkaliphiles was published in a review of alkaliphile ecology and diversity in 2007 (Yumoto 2007). The 18 alkaliphilic bacteria that are listed in *Table 20.1* are a much smaller subset that exemplifies at least some of the diversity of the much larger, and ever growing, total group of alkaliphiles. The 18 bacteria in *Table 20.1* all have sequenced genomes. The Gram-positive strains among them illustrate the rapid current pace of identification of new alkaliphile species since a significant proportion of them was described after compilation of Yumoto's 2007 list. The group of 18 is a mix of 12 Gram-positive and six Gram-negative, 10 aerobic/microaerobic and eight anaerobe alkaliphiles. Most of them are from natural environmental settings, with the largest number coming from soda lakes and the second largest number from soils. Two members of the group are from industrial or man-made settings.

Natural Environments

Soda lakes have been the outstanding source of alkaliphilic bacterial isolates. Soda lakes are stable, alkaline, and often extremely alkaline (e.g., pH >11.5) environments that are widely distributed and typically found in dry inland places where high evaporation rates lead to high salt concentrations. Their NaCl concentrations range from about 5 % w/v to >15 % w/v. The soda lakes have a paucity of calcium and magnesium ions because they are depressions formed from non-sedimentary rocks; sodium, chloride, and bicarbonate/carbonate and sulfate are dominant ions. The soda lakes often exhibit the pronounced color of organisms (e.g., cyanobacteria) that are the primary photosynthetic actors in the nutrient cycle, and the hypersaline lakes often are the color of haloalkaliphiles (Grant et al. 1986, 2003; Jones et al. 1998; Sorokin and Kuenen 2005a). Evidence from fossil soda lakes, which are similar to soda lakes found today, suggests that these environments are of great antiquity. These observations have led to the suggestion that substantial evolution of many prokaryotes found in this type of environment occurred in the soda lakes, i.e., these communities are very ancient sources of new species of bacteria (Zavarzin 1993). However, divergence over time is apparent since the alkaliphiles found in soda lakes of a particular region tend to be quite different from the alkaliphiles from soda lakes in different regions, such that there are reports of a majority of genotypes (>85 %) being unique to a region (Foti et al. 2006). This undoubtedly reflects the divergence in the properties of the soda lakes from different regions.

Other natural enrichments, such as alkaline hot springs, are the source of interesting, generally alkaline-tolerant organisms (Gorlenko et al. 2004) but are insufficiently buffered to support the extraordinarily high pH values that are consistently maintained in some soda lakes (Jones et al. 1998). The alkaliphiles isolated from soils from different regions, deep sea bottoms, and river sediments are frequently obligate alkaliphiles (Guffanti et al. 1980, 1986; Horikoshi and Akiba 1982; Takami et al. 1999), suggesting that such alkaliphiles find high pH domains within the larger environment and have the capacity to survive when the pH dips even in their niche. Similarly, two strains of alkaliphilic *Bacillus* and *Paenibacillus* inhabit another natural niche for alkaliphilic strains, the alkaline region of the termite hind gut (Thongaram et al. 2005). They were found to require alkaline pH even though they presumably survived passage through regions of lower pH (Thongaram et al. 2003).

Industrial and Other Environments of Man-Made Origin

In addition to the indigo dye process that has already been noted, sodium hydroxide has been used extensively in paper and pulp processing, and calcium hydroxide has been used in cement manufacture. Mining operations and certain food-processing activities also are settings for alkaliphile enrichment (Jones et al. 1998). Alkaliphilic bacteria are isolated from bioreactors and bioremediation processes that benefit from their ability to function at high pH (Sorokin et al. 2008) and are also enriched during various leaching processes that produce an alkaline leachate, e.g., borax leachate and carbonate leaching (Ye et al. 2004; Ghauri et al. 2006). *Thioalkalivibrio sulfidophilus*

Table 20.1
Alkaliphilic eubacteria whose genomes have been or are in the process of being sequenced

	GenBank	Genome sequencing status	Gram + or −	Source	Motility	Mot type	NaChBac	Mrp antiporter (subunitorder in the operon)	F-type ATP synthase-coupling ion[a]
Aerobes									
Arthrospira platensis NIES-39	AP011615.1	Complete	−	Saline soda lake	Yes (gliding)	−	No	Yes (DCDEFGBB)	H^+
Bacillus alcalophilus Vedder1934	None	In progress	+	Human feces	Yes	MotPS	Unknown	Unknown	H^+
Bacillus clausii KSM-K16	AP006627.1	Complete	+	Soil	Yes	MotAB	Yes	Yes (ABCDEFG), (ABCDEFG)	H^+
Bacillus halodurans C-125	BA000004.3	Complete	+	Soil	Yes	MotPS	Yes	Yes (ABCDEFG)	H^+
Bacillus pseudofirmus OF4	CP001878.1	Complete	+	Soil in New York State	Yes	MotPS	Yes	Yes (ABCDEFG)	H^+
Bacillus cellulosilyticus DSM2522	CP002394.1	Complete	+	Soil	Yes	MotPS	Yes	Yes (ABCDEFG)	H^+
Bacillus selenitireducens MLS10	CP001791.1	Complete	+	Alkaline, hypersaline, arsenic-rich mud from Mono Lake, California	No	MotPS	Yes	Yes (ABCDEFG)	H^+
Oceanobacillus iheyensis HTE831	BA000028.3	Complete	+	Deep-sea soil	Yes	MotAB, MotPS	Yes	Yes (ABCDEFG), (ABCDEFG)	H^+
*Caldalkalibacillus thermarum*TA2.A1	AFCE00000000	Draft assembly	+	Alkaline thermal spring	Yes	MotAB	Yes	Yes (ABCDEFG)	H^+
Thioalkalivibrio sulfidophilus HL-EbGr7	CP001339.1	Complete	−	Thiopaq bioreactor	Yes	MotAB, PomAB	Yes	Yes (ABCDEFG)	H^+

Anaerobes									
Alkalilimnicola ehrlichii MLHE-1	CP000453.1	Complete	–	Mono Lake anoxic bottom water	Yes	MotAB, PomAB	Yes	Yes (EFGBCDD)	H^+
Alkaliphilus metalliredigens QYMF	CP000724.1	Complete	+	Borax leachate ponds	Yes	MotAB?, MotPS?	No	Yes (EFGBBBCD)	Na^{+b}
Alkaliphilus oremlandii OhILAs	CP000853.1	Complete	+	Sediments from Ohio River	Yes	MotAB?	No	No	Na^+
Desulfonatronospira thiodismutans ASO3-1	ACJN00000000	Draft assembly	–	Sediments from highly alkaline saline soda lake on the Kulunda Steppe, Altai, Russia	No	MotAB, MotABB?	No	Yes (EFGBCDD), (FGBCDD)	H^+
Desulfurivibrio alkaliphilus AHT2	CP001940.1	Complete	–	Sediments from a highly alkaline saline soda lake in Egypt	No	PomAB	Yes	Yes (EFGBCDDD), (EFGBCDD)	H^+
Dethiobacter alkaliphilus AHT 1	ACJM00000000	Draft assembly	+	Sediments from a highly alkaline saline soda lake in northeastern Mongolia	Yes	MotPS?	No	Yes (BCDEFGBBDDDDDA), (EFGBBBCDDA)	(V-type)
Halanaerobium hydrogeniformans	CP002304	Complete	–	Soap Lake, WA	No	–	No	Yes (EFGBBCDD)	Na^+
Natranaerobius thermophilus JW/NM-WN-LF [c]	CP001034.1	Complete	+	Saline lakes in the Wadi El Natrun depression, Egypt	No	MotPS?	No	Yes (EFGBBBCDA)	Na^{+b}

[a]The coupling ion hypothesis is based on the absence (for H^+) or presence (for Na^+) of the Na^+-binding motif in *c*-subunits known to bind Na^+ (Pogoryelov et al. 2009)

[b]The *c*-subunits of *Alkaliphilus metalliredigens* QYMF and *Natranaerobius thermophilus* JW/NM-WN-LF are 184 and 185 amino acids, respectively, more than twice the normal length of an F-type *c*-subunit. Alignment of these *c*-subunits with the *Ilyobacter tartaricus c*-subunit is indicative of Na^+binding

HL-EbGr7, listed in Table 20.1, is a microaerophilic γ-proteobacterium that was enriched from a mix of soda lake *Thioalkalivibrio* strains in a Thiopaq bioreactor used to remove H_2S from biogas; this strain emerged as the dominant sulfur-oxidizing bacterium under conditions in which sulfide was the substrate (Sorokin et al. 2008; Muyzer et al. 2011). The other bacterium listed in Table 20.1 that originated from an industry-related setting, anaerobic *Alkaliphilus metalliredigens* QYMF, was isolated from alkaline borax leachate ponds in California and was found to reduce Fe(III), Co(III), or Cr(VI) under alkaline conditions (Ye et al. 2004). Waste disposal also can generate settings that support alkaliphile enrichment. One example is sludge from a sewage plant in Atlanta, Georgia, which was the source of anaerobic, thermoalkaliphilic *Clostridium thermoalcaliphilum* and moderately thermoalkaliphilic *Clostridium paradoxum* (Li et al. 1993, 1994). A second example is the extremely alkaline (pH > 12) complexes in the Lake Calumet area of southeast Chicago that were filled with slag from industrial dumping leading to alkalinization of water and selection for bacteria in the sediment and ground water itself, including an impressive variety of alkaliphilic β-proteobacterium, *Bacillus*, and *Clostridium* species (Roadcap et al. 2006).

Diversity

Alkaliphiles found in the soda lakes are amazingly diverse, encompassing cyanobacteria, haloalkaliphiles anaerobic and aerobic *Bacillus* species including strains that respire oxyanions of selenium and arsenic and others that reduce metals, diverse *Clostridium* species, phototrophic purple bacteria, nitrogen-fixing bacteria, a myriad of chemolithotrophic bacteria that are the basis for a complete sulfur cycle, nitrifying bacteria, methanotrophic strains, thiocyanate-oxidizing species, and hydrogen-producing haloanaerobes (Blum et al. 1998; Sorokin and Kuenen 2005a, b; Asao et al. 2011; Brown et al. 2011; Sorokin et al. 2011). Some of the anaerobic sulfate-reducing bacteria have been shown to be magnetotactic (Lefevre et al. 2011). Alkaliphilic cyanobacteria are among the primary photosynthetic organisms that produce oxygen; such organisms include *Spirulina*, *Cyanospira*, *Synechococcus*, and *Chroococcus*. Anoxygenic phototrophic bacteria, *Chromatiaceae* and *Ectothiorhodospira*, that use reduced sulfur compounds participate in the primary production via photosynthesis and also are part of the sulfur-oxidizing limb of the sulfur cycle of the soda lakes (Bryantseva et al. 1999; Asao et al. 2011; Sorokin et al. 2011). The sulfur cycle also includes aerobic sulfur-oxidizing organisms such as γ-Proteobacteria *Thioalkalivibrio, Thioalkalimicrobium*, and *Thioalkalispira* species (Sorokin et al. 2011) as well as anaerobic sulfate-reducing organisms such as *Desulfonatonum, Desulfonatronospira*, and *Desulfonatronovibrio* (Zhilina et al. 1997; Sorokin et al. 2011).

Among the earliest described alkaliphilic anaerobes that were intensively studied is *Amphibacillus xylanus* (Niimura et al. 1987, 1989, 1995). This interesting alkaliphile depends upon high concentrations of ammonium for optimal growth and possesses several transporters that are stimulated by NH_4^+ (Koyama 1989; Koyama 1993). More recently, Clostridium strains as well as other anaerobe genera such as *Alkaliphilus*, *Desulfonatronospira, Natranaerobius, Halanaerobium*, and many others from soda lakes have been studied in detail and developed for various applications.

Because thermoalkaliphiles were not described in the earliest work on alkaliphiles, this combination of extreme adaptations was considered to be incompatible with life. However, this supposition has been negated strongly by the identification of diverse thermoalkaliphiles as well as thermohaloalkaliphiles (Kevbrin et al. 2003; Zhao et al. 2011). These include among others: novel, obligately alkaliphilic *Clostridium* species isolated from sewage that have been noted (Li et al. 1993, 1994; Wiegel 1998); an asporogenous, Gram-positive ammonifying anaerobe from soda lake deposits, *Tindallia magadii* (Kevbrin et al. 1998); a xylan-degrading, anaerobic alkalithermophile, strain LB3A (Prowe et al. 1996; Sunna et al. 1997); and an actinomycete, *Thermoactinomyces* sp. strain HS682 (Tsuchiya et al. 1992).

Global Adaptations in Alkaliphiles

Alkaliphiles Have Generally Lower Protein Isoelectric Points Than In Neutralophiles

In 1991, data comparing charged amino acid distribution were presented for prosequences (~84 amino acids) of subtilisin enzymes from *Bacillus* species. These data indicated that there was little overall homology between them and functionally comparable extracellular protease enzymes from two alkaliphilic *Bacillus* sp., one strain designated *Bacillus* sp. strain YaB and the other *Bacillus alcalophilus* PB92 (ATCC31408), a strain of that alkaliphilic species of soil origin. The lack of homology resulted from dramatically different contents of basic and acidic amino acids, with the alkaliphile domains having two or three basic residues and 26 acidic residues whereas the neutralophile examples had 15 basic amino acids and 10–13 acidic ones (van der Laan et al. 1991). Subsequently, externally exposed domains of numerous membrane proteins from alkaliphiles were found to be much more acidic than the homologous proteins for neutralophiles; this is shown in Table 20.2 for external segments of several proteins, and more have been noted as part of a recent genome report for alkaliphilic *B. pseudofirmus* OF4 (Janto et al. 2011). Moreover, the average isoelectric point (pI) for all predicted proteins of the extracellular, cell-wall-, and membrane-associated, compartments of several alkaliphilic *Bacillus* species is significantly lower than those of neutralophilic *Bacillus* species; even the cytoplasmic proteins exhibit a small significant trend in that direction (Janto et al. 2011). What might be the utility of the acidic protein segments on the external surface? Since acidic residues would retain charge at very high pH, it is possible that this supports function of some segments that require charge for function, e.g., the domain of CtaC which must pick up electrons from Qcr and pass them into

Table 20.2

Charge distributions of segments of alkaliphile membrane proteins just outside the membrane surface relative to homologous regions from non-alkaliphiles: membrane associate protease FtsH, functionally important segments of cytochrome oxidase (CtaC), menaquinol-cytochrome c reductase (QcrC or B/C), and the externally exposed segments of motility channel proteins, MotB and MotPS

Strain		GenBank	FtsH			CtaC			QcrC			MotB			MotS		
			# of acidic and basic amino acids		Overall charge	# of acidic and basic amino acids		Overall charge	# of acidic and basic amino acids		Overall charge	# of acidic and basic amino acids		Overall charge	# of acidic and basic amino acids		Overall charge
			D + E	H, K, +R		D + E	H, K, +R		D + E	H, K, +R		D + E	H, K, +R		D + E	H, K, +R	
Alkaliphile	*B. pseudofirmus* OF4	CP001878.1	20	5	−15	42	20	−22	23	3	−20	Absent			45	18	−27
	B. halodurans C-125	BA000004.3	17	6	−11	48	18	−30	24	5	−19	Absent			46	24	−22
	B. selenitireducens MLS10	CP001791	19	2	−17	52	16	−36	31	2	−29	Absent			48	16	−32
	B. cellulosilyticus DSM2522	CP002394.1	18	4	−14	47	17	−30	25	4	−21	Absent			44	19	−25
	O. iheyensis HTE831	BA000028.3	18	3	−15	44	15	−29	23	5	−18	46	17	−29	43	19	−24
	B. clausii KSM-K16	AP006627.1	19	4	−15	46	19	−27	23	4	−19	37	18	−19	Absent		
	*C. thermarum*TA2.A1	AFCE00000000	14	9	−5	39	18	−21	Absent			49	24	−25	Absent		
Neutralophile	*B. subtilis sub sp. subtilis* str. 168	AL009126	12	12	0	38	41	+3	17	16	−1	37	39	+2	26	36	+10
	B. licheniformis ATCC 14580	CP000002	12	10	−2	45	41	−4	19	13	−6	32	36	+4	28	37	+9
	B. amyloliquefaciens FZB42	CP000560.1	13	13	0	36	40	+4	15	16	+1	32	38	+6	22	32	+10
	B. atrophaeus 1942	CP002207.1	11	11	0	42	38	−4	19	13	−6	38	31	−7	24	30	+6
	B. megaterium DSM 319	CP001982.1	15	13	−2	37	40	+3	16	15	−1	30	28	−2	21	22	+1
	B. cereus E33L	CP000001	11	11	0	33	38	+5	13	19	+6	32	33	+1	30	30	0
	G. thermodenitrificans NG80-2	CP000557.1	13	11	−2	36	33	−3	Absent			29	30	+1	Absent		
	G. thermoglucosidasius C56-YS93	CP002835.1	13	10	−3	38	37	−1	Absent			29	29	0	Absent		
	G. kaustophilus HTA426	BA000043.1	13	11	−2	32	39	+7	Absent			29	30	+1	Absent		

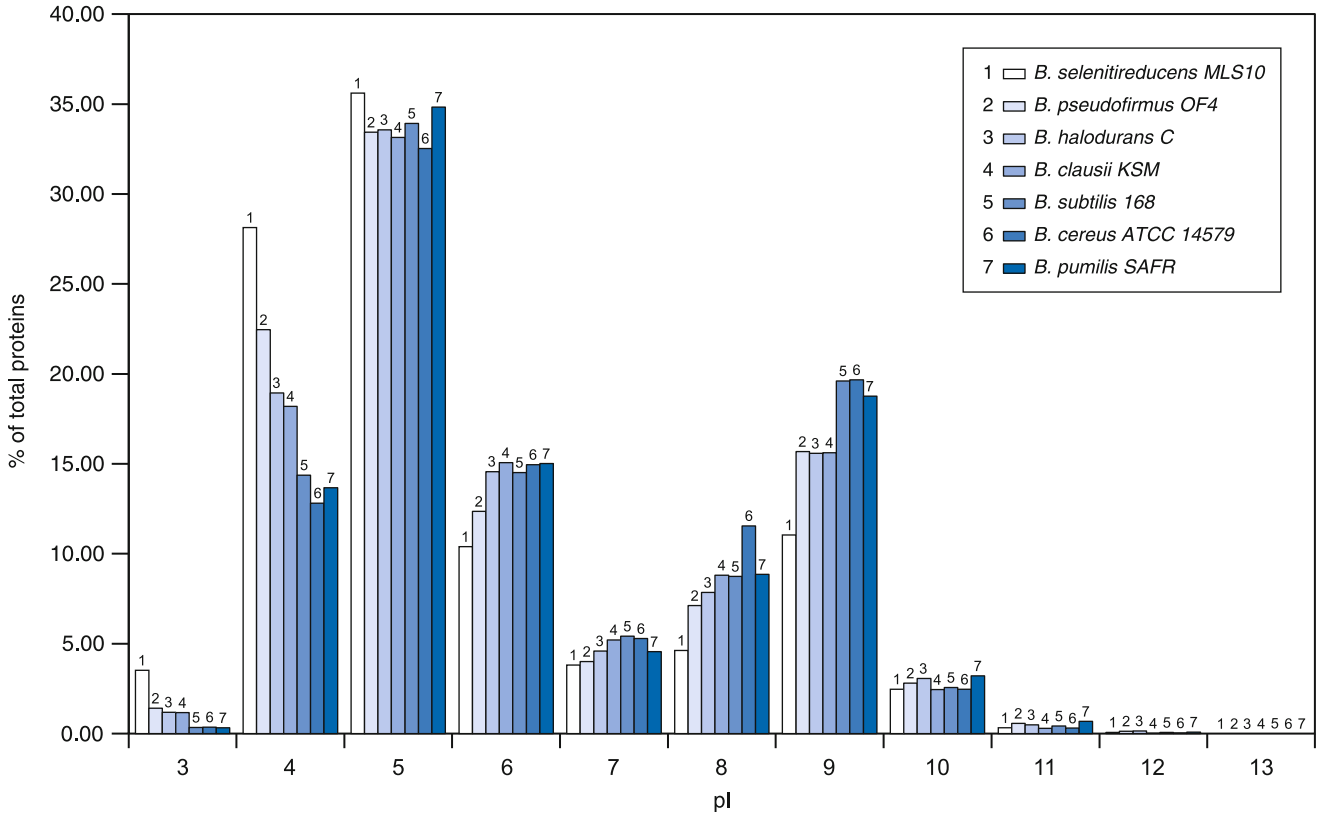


Fig. 20.1

Average isoelectric point, pI values, for the overall proteome predicted from genomes of selected alkaliphilic and neutralophilic *Bacillus* species. Predicted amino acid sequences from seven complete *Bacillus* genomes (four alkaliphiles and three neutralophiles, listed in order in the key that identified the different shading and symbols that identify the species) were submitted to the Compute pI/Mw tool at the Swiss Institute of Bioinformatics ExPASy Proteomics Server (http://ca.expasy.org/tools/pi_tool.html). The data were then binned according to the pI (3 = pI values greater than 3 and less than 4) and expressed as a percentage of the total number of proteins

the major catalytic subunit of Cta. Another possibility is that negative charges on the surface bind protons to enhance localized presence of protons for cytoplasmic pH homeostasis and, in alkaliphiles that carry out oxidative phosphorylation, for ATP synthesis. The negatively charged amino acids may also bind sodium ions, whose circulation is important for bioenergetic work cycles as described below.

It has been reported that the distribution of pIs among all the predicted proteins of individual bacterial genomes varies between halophiles such as *Halobacterium* sp. NRC-1, where a lower protein pI range is found than neutralophiles, and bacteria with acidophilic capacity such as *Helicobacter pylori*, where a higher pI range is found than for neutralophile protein pIs (Tomb et al. 1997; Kennedy et al. 2001; Schwartz et al. 2001; Knight et al. 2004). As shown in *Fig. 20.1*, the protein pI distribution for four alkaliphiles (numbered 1–4 in the figure) showed a greater % total proteins with pI values below 5 and a lower % total proteins with pI values of ~9 than the three neutralophiles chosen for comparison (numbered 5–7 in the figure). *B. selenitireducens* MLS10 followed by *B. pseudofirmus* OF4 exhibited the most pronounced trend in this direction and are both extreme alkaliphiles. It is possible that high halotolerance (Blum et al. 1998) contributes to the *B. selenitireducens* pattern along with adaptation to alkaliphily.

It is important to note, however, that not all adaptations that are suggested for alkaliphile extracellular enzymes involve substitution of a more acidic residue for more basic one. Shirai et al. (1997, 2001, 2007) have applied analyses of "molecular phylogeny" such as ASET, ancestor sequence evolutionary trace, to compare alkaliphile versions of enzymes with homologues that represent putative ancestral forms that predate evolutionary adaptation to alkaliphily. Further, they have drawn upon the increasing number of sequences for amylases, proteases, and cellulases as well as enzymes for which there are structural data. The results show a consistent general trend during alkaline adaptation of these enzymes: decreases in Lys and Asp residues and increases in Arg, His, and Glu. Much, but not all, of the Lys decrease and the Arg increase correlates with remodeling of ion pairs, i.e., Arg-Glu/Asp pairs, predominantly Arg-Glu, replace Lys-Asp pairs (Shirai et al. 2007). The use of Arg instead of Lys is likely to be adaptive to high external pH at which the pI of Arg will make it more reliably charged than Lys. Although the

substitutions resulted in a modest increase in the overall pI of an M-protease from alkaliphilic *Bacillus* sp. KSM-K16 (Shirai et al. 1997), this was not a general finding (Shirai et al. 2001). In addition to the changes in charged amino acids, there were also presumably adaptive changes in usage of different hydrophobic amino acids (Shirai et al. 2007). As will be further noted in connection with applications, the correlation of various changes in amino acid usage with structure/function changes that underpin alkaline adaptation of useful extracellular enzymes is of great interest. Papageorgiou and colleagues (Dubnovitsky et al. 2005; Kapetaniou et al. 2006) have studied alkaline adaptation of a cytoplasmic enzyme, phosphoserine aminotransferase, from the extreme alkaliphile *Bacillus alcalophilus* and facultative alkaliphile *Bacillus circulans* ssp. *alkalophilus*. These two enzymes and the *E. coli* homologue to which they were compared are homodimers, each of which has two domains. The alkaliphile enzymes were found to have distinctive structural features in the vicinity of the active site. In addition, the alkaliphile enzymes, relative to the neutralophile example, were reported to have increased hydrophobic interactions between the two monomer domains, increased number of negative charges exposed on the surface that is exposed to solvent, as well as a reduction in total ion pairs but an increase in hydrogen bonds. These trends will need to be further tested with direct comparisons between a greater number of cytoplasmic enzyme homologues from both neutralophiles and alkaliphiles, but the findings so far are very intriguing. The cytoplasmic pH of alkaliphiles is higher, when they are grown at pH values above about 9.5, than is tolerated by neutralophiles (Padan et al. 2005; Slonczewski et al. 2009; Krulwich et al. 2011). As will be discussed further under bioenergetics, it is possible that cytoplasmic enzymes of alkaliphiles have general adaptations that contribute to tolerance of a higher pH that is higher than found in neutralophiles. Those adaptations may be somewhat distinct from the adaptations of extracellular enzymes since the rise in cytoplasmic pH is dramatic as pH_{out} rises, but the cytoplasmic pH still remains lower than that of the external environment.

Buffering Capacity

If the cytoplasm and/or outer surface of alkaliphilic bacteria were to have unusually high buffering capacity in the alkaline pH range, this would potentially be a defense against sudden alkalinization of the external pH. However, while any delay would be helpful during short-term adaptation to an alkaline challenge, buffering would be limited and would thus be unable to function as a sustainable barrier against cytoplasmic proton loss to suddenly increased alkalinity outside. Data from several assessments of buffering capacity of alkaliphilic *Bacillus* species, in comparison with neutralophilic Firmicutes, indicate that a high pH-grown alkaliphile, e.g., pH 10.5-grown *B. alcalophilus*, had a high cytoplasmic buffering capacity relative to the buffering power of neutralophiles or the alkaliphile grown at a lower pH. The cell surface was consistently shown to account for a significant proportion of the buffering capacity measured (Ruis and Loren 1998; Slonczewski et al. 2009).

Last Resort: Mobile Elements to Introduce Further Potentially Adaptive Change?

Another strategy that might be considered as a global strategy to support alkaliphily, or any "extremophily," is perhaps a strategy of last resort in which genomic rearrangements are fostered, thus increasing the generation of variants among which some could survive an alkaline challenge that is not manageable for the wild-type strain. Numerous and novel insertion sequences were noted in the genome of extremely alkaliphilic *B. halodurans* C-125, and there were indications of past rearrangements (Takami et al. 2001). In alkaliphilic *B. pseudofirmus* OF4, three distinct interspersed, noncoding repeated DNA elements, two of which were novel, were found between genes in large numbers; in addition, mobile elements represented approximately 10 % of the gene content of two resident plasmids and are proposed to be a reservoir of "change agents" that could foster survival under a challenge that the wild type cannot meet (Janto et al. 2011). It will also be of interest to see whether such patterns are a common feature of the genomes of extreme alkaliphiles.

Cells of *B. pseudofirmus* OF4 that were recovered from pH-controlled continuous cultures maintained at pH 11.2, yielded variants that have increased activity of antiport, sodium/proton exchange across the membrane. This activity, as described below, is critical to alkaline pH homeostasis, and the variants initiated growth at pH 11 more rapidly than wild-type cells (Sturr et al. 1994). A detailed analysis was not conducted of the properties of such variants, but properties such as their genomic, transcriptome and proteome changes, and their surface properties could reveal new information about global adaptation strategies as well as specific ones.

Cell Surface

Secondary Cell Wall Polymers (SCWPs)

The peptidoglycan of alkaliphilic *Bacillus* species has the same general composition as that of other *Bacillus* species (Aono et al. 1984). Alkaliphilic *Bacillus* species also possess secondary cell wall polymers (SCWPs) as part of the cell wall layer that are highly acidic and significantly add to the negative charge of the alkaliphile cell surface. Two of the most intensively studied alkaliphilic *Bacillus* species, *B. halodurans* C-125 and *B. pseudofirmus* OF4, have different anionic SCWPs associated with their peptidoglycan layers, both of which are present during growth at near-neutral pH and present in increased amounts during growth at highly alkaline pH (Aono and Horikoshi 1983; Aono and Uramoto 1986; Aono 1987; Aono et al. 1993b; Gilmour et al. 2000). The SCWPs of *B. halodurans* C-125 consist of a teichuronic acid composed of galacturonic acid, glucuronic acid, and *N*-acetylfucosamine (Aono and Uramoto 1986), and the teichuronopeptide (TUP) is composed of a polyglutamate and polyglucuronic complex (Aono et al. 1993a, b). A mutant of *B. halodurans* C-125 with defective teichuronopeptide synthesis exhibits defects in growth at the upper end of the wild-type pH

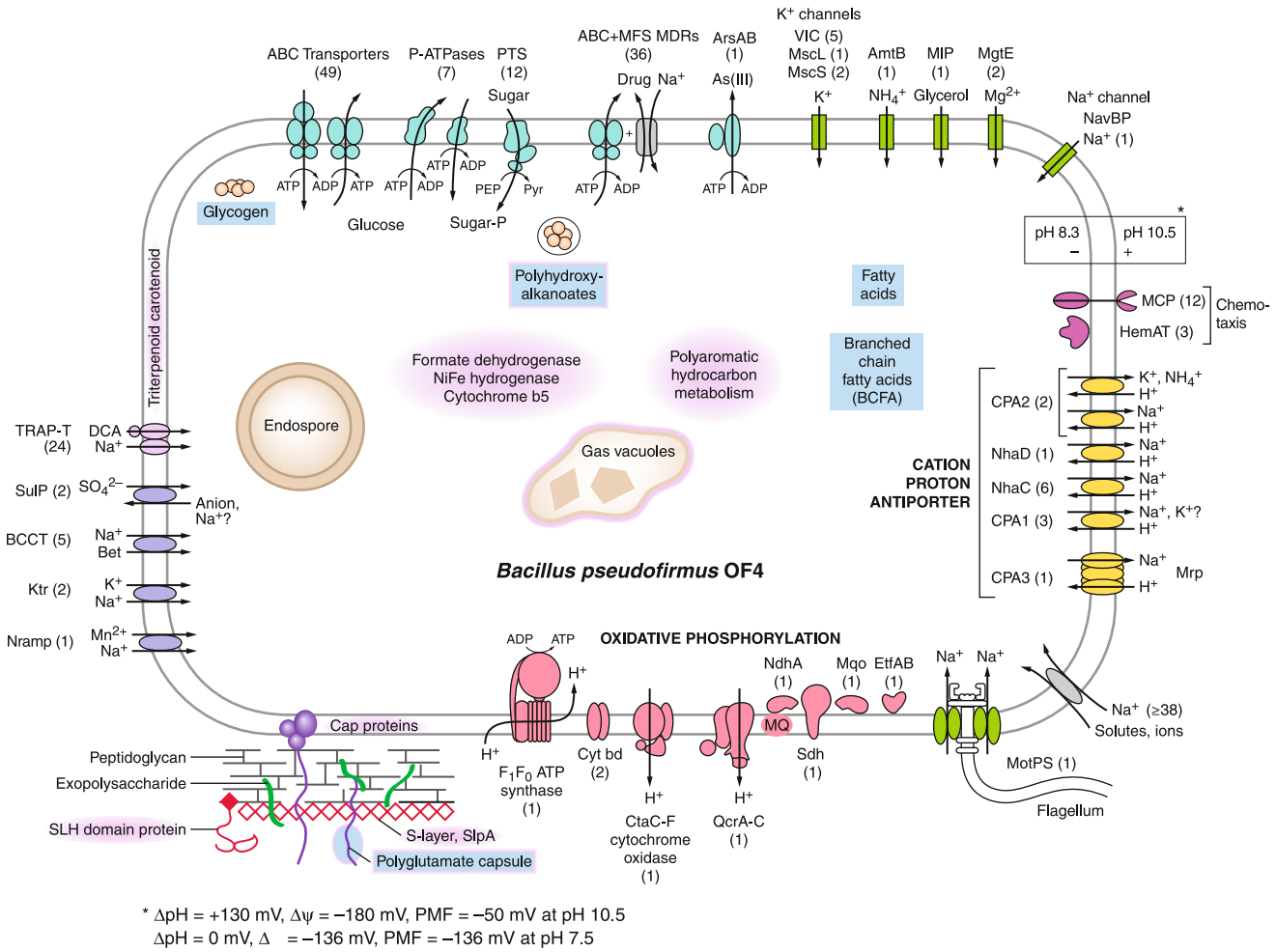
range and in cytoplasmic pH homeostasis (Aono and Ohtani 1990; Aono et al. 1999; Ito and Aono 2002). The acidic SCWPs of *B. pseudofirmus* OF4 do not include comparable compounds, and genes for teichuronopeptide synthesis are absent from the genome (Janto et al. 2011). Rather, this extreme alkaliphile has SCWP patterns similar to those found in the *Bacillus cereus* group of *Bacillus* species, composed of an acidic S-layer protein and also a cell wall-attached γ-polyglutamate polymer that would add to surface acidity and probably is also one of several forms of stored nutrients (Gilmour et al. 2000; Janto et al. 2011). Interestingly, deletion of the S-layer-encoding *slpA* gene enhances the growth rate at of *B. pseudofirmus* OF4 at pH 7.5 but increases the lag upon initiation of growth at ∼pH 11 (Gilmour et al. 2000). Therefore, the expensive synthesis of the S-layer at near-neutral pH is adverse to growth at that pH but optimizes the alkaliphile's ability to adapt to a sudden alkaline shift, an indication of "hardwired" readiness for the extreme condition (Gilmour et al. 2000; Krulwich et al. 2011). Genomic data suggest that alkaliphilic *B. pseudofirmus* OF4 may synthesize additional extracellular polysaccharides that have not yet been described, e.g., products of *espX*, BY kinase genes, and *cpsD* and *cpsC* genes (Janto et al. 2011). A representation of a cell wall and SCWP layer from this alkaliphile is part of the schematic model of a *B. pseudofirmus* OF4 cell shown in ❯ *Fig. 20.2*.

Membranes

Alkaliphile cell membranes need to resist proton leakage to the outside, but evidence of their adaptations to fulfill that role is mostly inferential. With a growing number of alkaliphile genomes and genetically tractable strains, this is an area in which experimental data would be valuable. A comparative study has been conducted of membrane lipids from obligately alkaliphilic *B. alcalophilus* and *B. pseudofirmus* RAB; two facultative alkaliphiles that are closely related to *B. pseudofirmus* RAB, i.e., *B. pseudofirmus* OF1 and OF4; and non-alkaliphilic *B. subtilis*. The alkaliphile membranes all contained higher concentrations of anionic phospholipids, especially cardiolipin, than membranes from *B. subtilis*, and all had appreciable amounts of squalene (Clejan et al. 1986). There were also differences in the contents of unsaturated fatty acids and branched chain fatty acids that differed between obligate and facultative alkaliphiles and that are consistent with observations on membranes from alkaliphilic *Bacillus* strain YN-2000 (Yumoto et al. 2000). Studies of *Bacillus* YN-2000 revealed significant increases in the anionic phospholipid content of the membranes at high pH compared to near-neutral pH (Enomoto and Koyama 1999; Yumoto et al. 2000). Two other types of molecules are notable in connection with alkaliphile membranes. First, a flotillin-like protein, which could be a marker of particular lipid domains, was identified in *B. halodurans* C-125, and both its transcription and translation were found to be alkali inducible (Zhang et al. 2005). While this protein has not found in all extreme alkaliphiles, its role in this well-studied example will be of interest. A second group of molecules are the yellow membrane-associated triterpenoid carotenoid pigments that are found in some groups of alkaliphilic *Bacillus* strains (Aono and Horikoshi 1991), which are probably similar to pigments found in other non-alkaliphilic *Bacillus* strains and have been intensively studied recently (Khaneja et al. 2009; Perez-Fons et al. 2011). They are likely to be involved in resistance to photodamage or reactive oxygen species but might also confer other properties on the membrane that impact alkaliphily.

Bioenergetics of Alkaliphilic Bacteria

Central problems of extremely alkaliphilic *B. pseudofirmus* OF4 are illustrated by the data shown in ❯ *Fig. 20.3*, which show the doubling time (tg) of cells in continuous aerobic cultures of malate-yeast extract medium at the pH values shown on the x-axis. The alkaliphile maintains a cytoplasmic pH (pH_{in}) much lower than the external pH when growing at very alkaline external pH values (pH_{out}); the internal pH can be as much as 2.3 pH units lower than pH_{out}. Over a range of pH_{out} from 7.5 to 9.5, *B. pseudofirmus* OF4 maintains a pH_{in} close to pH 7.5, and the pH_{in} only goes up to about pH 8.2 as pH_{out} values close to 10.5. This raises two problems. Problem 1 is how is such a large pH gradient (ΔpH), with a more acidic pH inside than outside, generated by cells that are pumping protons out during respiration (see ❯ *Fig. 20.2*)? Further, although the alkaliphile still grows optimally at pH 10.5, there is a large pH gradient that is opposite the productive orientation from the point of view of energizing bioenergetic work. As shown in the footnote in ❯ *Fig. 20.2*, the PMF that energizes proton-coupled transport, motility, and ATP synthesis is much lower at pH 10.5 than at pH 7.5. The PMF is low at high pH because, although the transmembrane electrical potential (ΔΨ) component rises with rising pH_{out} (❯ *Fig. 20.3*), the increase in the ΔΨ is not large enough to compensate for the large "reversed" ΔpH that results from successful pH homeostasis. Problem 2 is how does ion-coupled bioenergetic work occur robustly at such low values of the PMF? Further, at external pH values above pH 10.5, the capacity for pH homeostasis no longer keeps reasonably strong pace with the increasing external pH. Rather, the cytoplasmic pH goes up significantly, and, in turn, the doubling time increases markedly (❯ *Fig. 20.3*). This shows the importance of pH homeostasis to retaining an optimal growth rate. However, although growth is slower, the alkaliphile still grows at an external pH > 11, with a cytoplasmic pH above 9.5 (❯ *Fig. 20.3*). By contrast, neutralophilic bacteria such as *E. coli* or *B. subtilis* maintain a steady cytoplasmic pH over an external pH range from ∼5.5 to 8.5 but go into growth arrest when challenged sufficiently, i.e., by shifts to external pH values ≥9, that cause the cytoplasmic pH to rise above 8 (❯ *Fig. 20.4*) (Zilberstein et al. 1979; Wiegert et al. 2001). The growth of *B. pseudofirmus* OF4 and other alkaliphiles at external pH values at which the capacity for pH homeostasis is exceeded raises a third problem. Problem 3 is how do alkaliphilic *Bacillus* species tolerate a much higher cytoplasmic pH value than neutralophilic bacteria and retain the ability to grow?



* ΔpH = +130 mV, Δψ = –180 mV, PMF = –50 mV at pH 10.5
ΔpH = 0 mV, Δ = –136 mV, PMF = –136 mV at pH 7.5

Fig. 20.2

Features of *Bacillus pseudofirmus* OF4 revealed by the genome that are proposed adaptations to the energy demands and overlapping stresses related to alkaliphily. The primary transporter groups (ABC, PTS) are predicted to have roles in antibiotic resistance/cell envelope stress, nutrient uptake, and ion homeostasis. The ABC-type MDRs (multidrug transporters) included in the 36 total MDRs are also counted in the ABC transporter total of 49. The selected groups of secondary transporters that are shown have potential or established roles in cytoplasmic pH homeostasis (cation proton antiporters, shown on the *right pole*) and sodium-dependent efflux of toxins and uptake of nutrients, ions, or compatible solutes. The representative channels that are shown have roles in motility, pH homeostasis, osmoregulation, and cation and nutrient uptake. The flagellum, usually one per cell, is found on the long side of the rod-shaped cell and is energized via energetically downhill movement of Na^+ through MotPS channels in the stator. The voltage-gated sodium channel and chemotaxis receptors are colocalized at a cell pole, and unreleased spores are also observed at a cell pole. The other localizations are not known. Outside bottom left corner: peptidoglycan and additional cell wall-associated polymers and proteins are shown. Potential energy sources that are shaded include glycogen, polyhydroxyalkanoates, fatty acids and branched chain fatty acids, and a polyglutamate capsule. The gas vacuoles and hydrogenase may extend the aerobic metabolic capacity of the organism under otherwise oxygen-limiting conditions. Examples of elements that are distinct from those found in closely related *Bacillus halodurans* C-125 are two of the putative energy storage forms, polyhydroxyalkanoates and polyglutamate capsule; cell wall S-layer and SLH-domain proteins; cell membrane triterpenoid carotenoid; gas vacuoles; a NiFe hydrogenase/cytochrome b5 locus; enzymes and regulators of poly-aromatic hydrocarbon (PAH) metabolism; and a malonate utilization locus. PAH degradation is only one example chosen for this figure of a great catabolic versatility, and it is also an example of potential remediation capacity (This figure was redrawn and modified from Fig. 4 in Janto et al. (2011))

A fourth problem should also be raised here and is implicit in the data shown in *Fig. 20.4*, in which the pH range for significant growth of many facultative alkaliphiles does not extend down to pH 7 and the low end of the range for obligate alkaliphiles is higher, at ~pH 9. Problem 4 is why do extreme alkaliphiles fail to grow or grow suboptimally near-neutral pH?

In addition to the four problems raised by the data in *Figs. 20.3* and *20.4*, which will be considered further

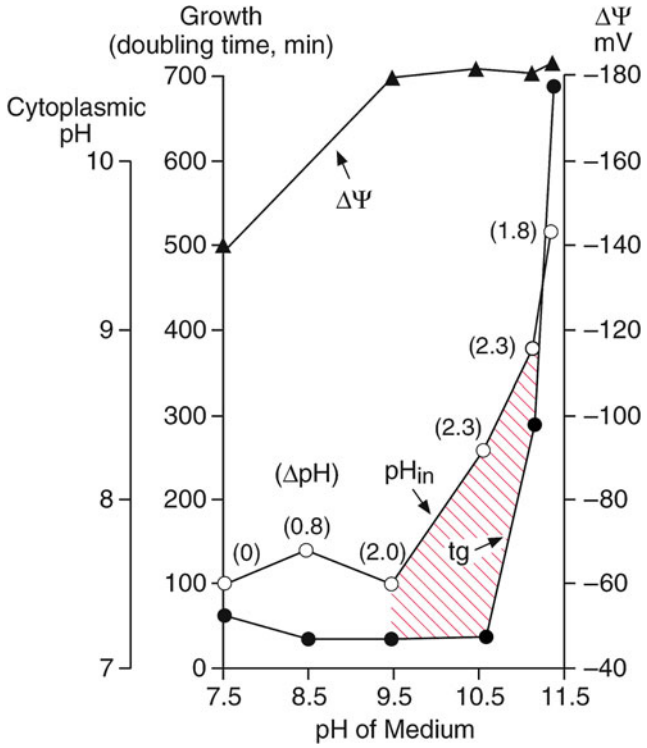


Fig. 20.3

Cytoplasmic pH, proton motive force (PMF) parameters, and doubling time (tg) of *Bacillus pseudofirmus* OF4 in pH-controlled continuous cultures maintained at the indicated external pH values. The pH_{in} (cytoplasmic pH) is shown by the *open circles*, with the ΔpH (external pH – cytoplasmic pH, in pH units) shown in *parentheses*. The doubling time (tg), in minutes, is shown by the *filled circles* and the transmembrane electrical potential, ΔΨ, in millivolts, is shown by the *filled triangles*. The *red-line* highlighting draws attention to the rise in cytoplasmic pH that accompanies increases in external pH beyond pH 9.5 (This figure is a modified version of Fig. 1 from Krulwich (1995) based on work by Sturr et al. (1994))

below, there is one other general problem related to alkaliphile bioenergetics, i.e., an increased energy requirement for growth at high pH. This arises from the high energy cost of pH homeostasis and the added energy needed for the ATP synthesis at high pH since a proton is involved in the synthetic reaction $ADP^{3-} + HPO_4^{2-} + H^+ \rightarrow ATP^{4-} + H_2O$ (Hicks et al. 2010). There is thus an expectation that alkaliphiles will exhibit some combination of metabolic robustness, metabolic versatility, and perhaps specific strategies to support their energy needs. For example, the genome of *B. pseudofirmus* OF4 suggests that it draws upon an impressive array of metabolic energy sources as well as multiple forms of energy reserves and has adaptive strategies to support metabolism under conditions of reduced oxygen (Janto et al. 2011); several of these features are highlighted in ❯ *Fig. 20.2*. Proteomic studies of both *B. pseudofirmus* OF4 and the Gram-negative alkaliphile *Alkalimonas amylolytica* N10 indicate upregulation of central energy-yielding metabolic pathways at highly alkaline pH relative to a lower pH that also supported growth (Gilmour et al. 2000; Wang et al. 2009). In the schematic diagram of the aerobic polyextremophile, *C. thermarum* TA2.A1 in ❯ *Fig. 20.5*, EtfAB (Electron transfer flavoprotein A/B) is shown as a possible mediator of electron transfer to the respiratory chain quinone, e.g., from dehydrogenase reactions. This is speculative but reflects the interesting presence of four candidates pairs of EtfAB (plus one orphan EtfB) in the *C. thermarum* TA2.A1 genome (Kalamorz et al. 2011). Moreover, one of those pairs (CathTa2_1992/CathTA2_1991) is contiguous with a FAD-dependent oxidoreductase (CathTA2_1993) that is homologous to known electron transfer protein-ubiquinone reductases that could mediate the electron transfer from dehydrogenases via EtfAB to respiratory ubiquinone (Watmough and Frerman 2010). Both *B. pseudofirmus* OF4 and *B. halodurans* C-125 are predicted to have only one EtfAB pair, and although ❯ *Fig. 20.3* shows the *B. pseudofirmus* OF4 EtfAB with respiratory-associated proteins, it is less likely than the *C. thermarum* TA2.A1 EtfAB to mediate electron transport to ubiquinone or menaquinone since no electron transfer protein-ubiquinone reductase homologue is evident in its genome. The possible metabolic contributions of single and multiple EtfAB pairs to energy conservation will be of interest in the context of special strategies of alkaliphiles in meeting their energy needs.

A different set of adaptations to provide "extra" energy to alkaliphiles appear to enhance the energy made available through electron transport. For respiratory components of alkaliphilic *Bacillus* strains, low midpoint potentials of the Qcr Rieske Fe-S protein as well as cytochrome *c* and *b* species have been reported, including cytochrome *c* species that are part of a terminal oxidase complex (Lewis et al. 1981; Hicks and Krulwich 1995; Goto et al. 2005; Muntyan and Bloch 2008), whereas the *a*-type cytochrome components of cytochrome oxidase that finally transfer the electrons to oxygen have midpoint potentials comparable to those found in neutralophiles (❯ *Fig. 20.6*) (Muntyan and Bloch 2008). These observations indicate the possibility that respiratory alkaliphiles take advantage of a larger span of redox potentials and hence greater opportunity for energy conservation than neutralophiles. The span of redox potentials has similarly been cited as an advantage of proton coupling to ATP synthesis as opposed to use of Na^+ coupling (Mulkidjanian et al. 2008). Na^+ coupling to F-type ATP synthases has turned out not to be used by respiratory alkaliphiles for ATP synthesis (❯ *Fig. 20.5*) contrary to the predictions of Skulachev (1989a, b, 1992). Instead, such coupling has been found in synthases only in bacteria in specialized metabolic niches and in F-type ATPases of fermentative and anaerobic alkaliphiles that pump Na^+ outward, not in the inward synthetic direction, e.g., *C. paradoxum* and *N. thermophilus* JW/NM-WN-LF (❯ *Fig. 20.5*) (Dimroth and Cook 2004; Ferguson et al. 2006; Meier et al. 2006; Mesbah and Wiegel 2011). Another potential strategy to expand available energy is the use of reduced ferredoxin as an a electron donor to the Na^+-translocating Rnf complex, since this makes available energy from a region of redox potentials that is not accessed by many organisms or cells, as discussed by Biegel et al. (2011). Two Rnf

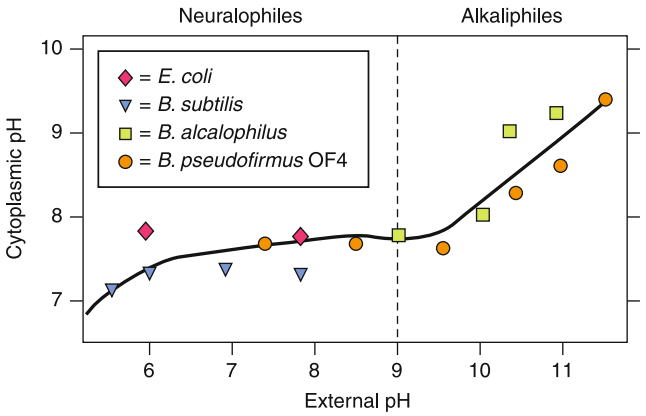


Fig. 20.4

The cytoplasmic pH of two neutralophiles and two extremely alkaliphiles, one of which is facultative, over a range of pH values. Values for the cytoplasmic pH are shown for two neutralophiles, *B. subtilis* growing over a pH range from ~5.5 to 7.9 (Shioi et al. 1978) and *E. coli* growing over a range of ~6–7.9 (Zilberstein et al. 1979), and two alkaliphiles, facultative *B. pseudofirmus* OF4 growing in a range of ~7.5–11.4 (Sturr et al. 1994) and obligately alkaliphilic *B. alcalophilus* ATCC27647 growing in a pH range from ~9.0 to 11 (Guffanti et al. 1978)

loci are predicted by the genome sequence of anaerobic polyextremophile *N. thermophilus* JW/NM-WN/LF, at least one of which could play a role in the energetics of the organism as shown as a hypothesis in ❯ *Fig. 20.5*.

Problem 1: How is such a large pH gradient, more acidic inside than outside, generated in cells that are actively pumping protons out during respiration (see ❯ *Fig. 20.2*)?

Extremely alkaliphilic aerobes, such as *B. pseudofirmus* OF4 (❯ *Fig. 20.2*) and *B. halodurans* C-125 (❯ *Fig. 20.5*, bottom right panel), require the activity of the Mrp-type (CPA3) antiporter that was first revealed by work on a mutant of *B. halodurans* C-125. A partial fragment of the *mrp* operon was identified as the locus of a mutation that resulted in loss of the normal capacity for pH homeostasis such that the mutant was non-alkaliphilic in its pH range for growth (Hamamoto et al. 1994). While alkaliphiles, like other bacteria, have multiple cation/proton antiporters, which catalyze efflux of $Na^+(Li^+)$ and/or K^+ or Ca^{2+} in exchange for external H^+, the Mrp antiporters appear to have particularly important roles in alkaliphile pH homeostasis. They also have unique structural complexity relative to other cation/proton antiporters (Padan et al. 2005; Swartz et al. 2005; Krulwich et al. 2009; Slonczewski et al. 2009). While most cation/proton antiporters are composed of a single hydrophobic gene product, sometimes as a homo-oligomer and/or sometimes with an additional peripheral or integral membrane subunit, Mrp-type antiporters require a larger number of distinct hydrophobic proteins (Hiramatsu et al. 1998; Ito et al. 2000; Morino et al. 2008). For all but one of the aerobes shown in ❯ *Table 20.1*, 7 hydrophobic Mrp proteins that comprise the hetero-oligomeric antiporter are products of *mrpABCDEFG* operons, as are the Mrp antiporters of many neutralophiles, including *B. subtilis* (Ito et al. 1999; Swartz et al. 2005). Other aerobic bacteria have a 6-protein Mrp version in which MrpA and MrpB are fused, but even bigger differences are seen in Mrp systems from anaerobes (also archaea), in which MrpA is often missing; there is more than one copy of other Mrp proteins, especially MrpB and MrpD; and the gene order of the operons differs from those of most common aerobe Mrp types (❯ *Table 20.1*) (Swartz et al. 2005). Some aerobes and some anaerobes have more than one Mrp antiporter locus, sometimes with different cation specificities (Swartz et al. 2005). Both the alkaliphilic *B. pseudofirmus* OF4 and *B. subtilis* Mrp antiporters have been shown to form 7-protein hetero-oligomeric complexes, and in the alkaliphile, both dimeric and monomeric forms are found (Kajiyama et al. 2007; Morino et al. 2008, 2010). In the alkaliphile, mutagenesis data suggest that the larger dimeric form may be the most active form (Morino et al. 2010). The MrpA, MrpD, and MrpC proteins are homologues and presumed to share an ancestor with NuoL, NuoM/N, and NuoK proteins of the mitochondrial Complex I, a proton-pumping NADH:ubiquinone oxidoreductase. They are both, in turn, homologous with subunits of ion-pumping bacterial hydrogenases (Friedrich and Scheide 2000; Mathiesen and Hägerhäll 2002, 2003; Swartz et al. 2005; Efremov and Sazanov 2011a, b). The Mrp-Nuo homologues share conserved residues that are essential for function (Kajiyama et al. 2009; Efremov et al. 2010; Morino et al. 2010; Efremov and Sazanov 2011a, b; Krulwich et al. 2011). Other Mrp residues have been shown by mutagenesis studies to affect formation of one or both of the hetero-oligomeric complexes; to be important for either chaperone or assembly functions, since their mutation leads to reduced amounts of membrane-associated Mrp proteins; or to be candidates for involvement in putative catalytic functions of Mrp that are distinct from cation/proton antiport (❯ *Fig. 20.7*) (Morino et al. 2008, 2010; Kajiyama et al. 2009).

Mrp antiporters catalyze electrogenic antiport, i.e., the ratio of H^+ entering/Na^+ exiting >1, so that net positive charge moves inward during an antiport reaction turnover; this makes it possible for the antiporter to use the transmembrane potential, $\Delta\Psi$, and achieve the net accumulation of cytoplasmic protons relative to the outside, which is crucial for alkaline pH homeostasis (Macnab and Castle 1987; Padan et al. 2005). It has been hypothesized that the large surface of a dimeric Mrp hetero-oligomeric complex that is exposed on the outside surface of bacteria may be engineered to bind and funnel protons into the antiporter; this would support the kinetic competency required to sustain pH homeostasis at high external pH that would otherwise be impossible in the proton-poor environment (Morino et al. 2008, 2010). The large number of additional cation/proton antiporters that are found in each alkaliphile (❯ *Fig. 20.5*) is likely to be important under specific conditions, e.g., one of the six NhaC-type sodium:proton antiporters of *B. pseudofirmus* OF4 plays a role primarily near-neutral pH in this facultative alkaliphile (Ito et al. 1997; Janto et al. 2011). Effects, under different growth conditions, of a comprehensive set of deletions in the antiporter complement have not yet been

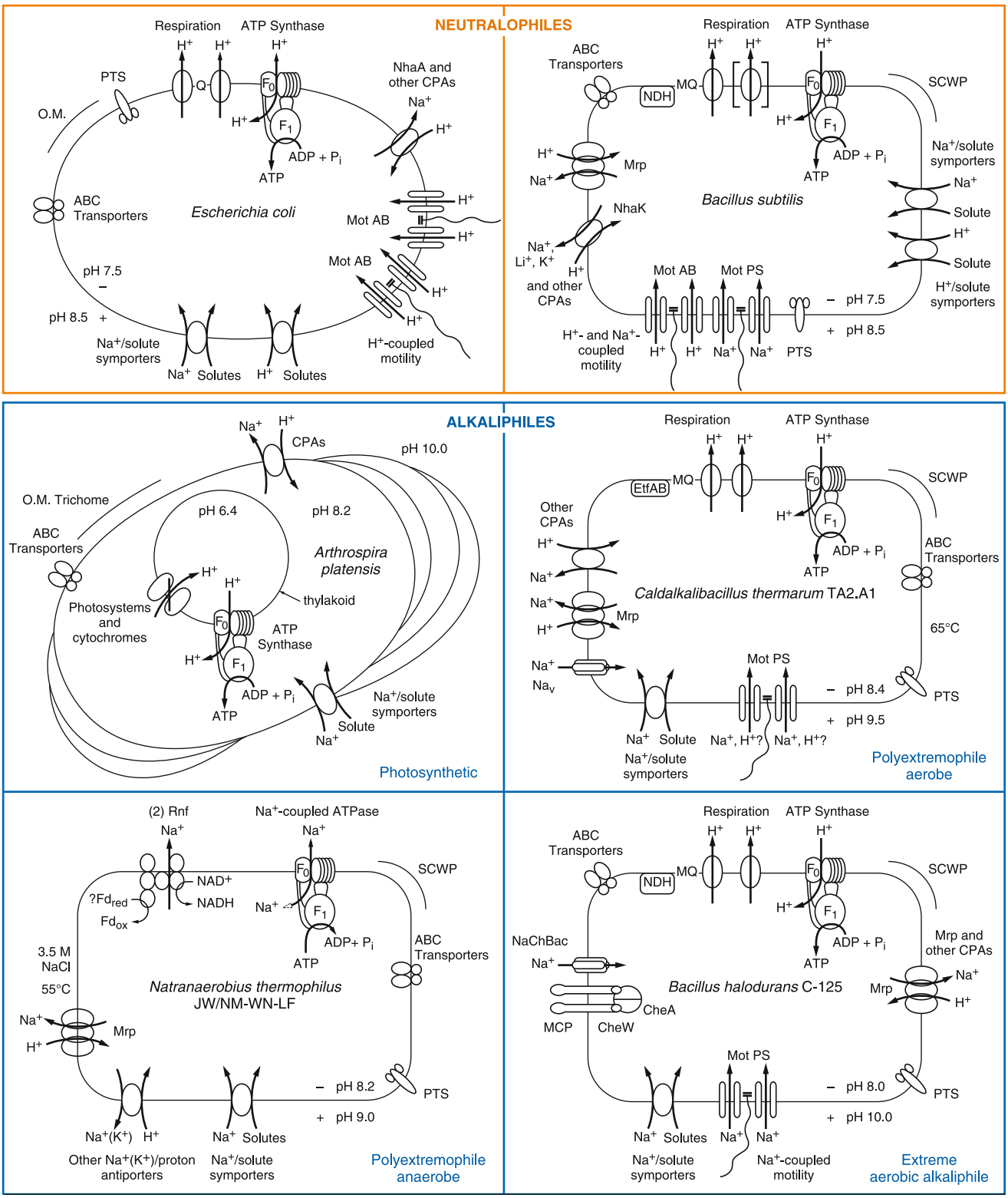


Fig. 20.5

Bioenergetic features of two model neutralophiles and four different alkaliphiles. A list follows of the organisms in the diagrams with each genus/species name followed first by the GenBank accession # for genomic information, in parentheses, and then by a reference for data shown on pH homeostasis, i.e., the cytoplasmic pH at a particular external pH: neutralophiles *Escherichia coli* (NC010473) (Padan and Schuldiner 1986) and *Bacillus subtilis* (NC000964) (Shioi et al. 1978) and alkaliphiles cyanobacterium *Arthrospira* (formally *Spirulina*) *platensis* (ACSK00000000) (Belkin and Boussiba 1991; Pogoryelov et al. 2003), the thermoalkaliphilic aerobe *Caldalkalibacillus thermarum* TA2.A1 (AFCE00000000) (Olsson et al. 2003), the thermohaloalkaliphilic anaerobe *Natranaerobius thermophilus* JW/NM-WN-LF

reported in an alkaliphile, nor has a set of expression data under multiple conditions been reported for a full antiporter complement.

Since cation/proton antiporters are secondary transporters that depend upon a PMF for energization and especially the transmembrane electrical potential component, the $\Delta\Psi$, alkaliphile pH homeostasis depends on generation of this potential by respiration in respiratory alkaliphiles or other primary electrogenic ion pumps in anaerobes. In addition, for the majority of alkaliphiles that depend upon sodium:proton antiporters for pH homeostasis, there must be multiple sodium ion uptake systems to promote reentry of sodium ions to guarantee sustained cation/proton antiport. A major part of that need is generally met by use, in most alkaliphiles, of Na^+-coupled transporters and channels for much of their transport and flagellar motility but not for other crucial bioenergetic work.

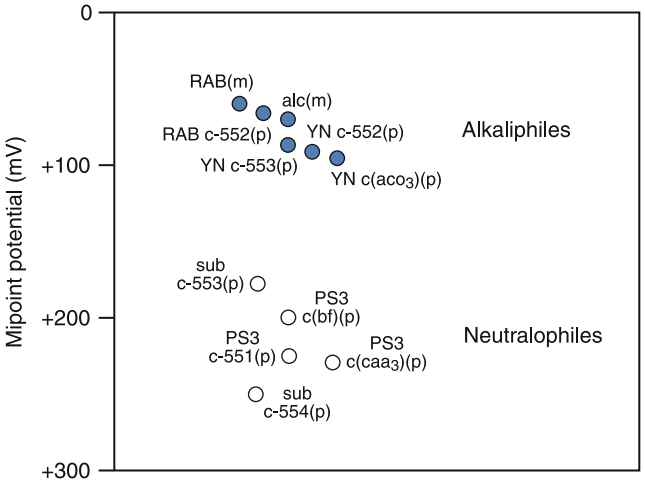


Fig. 20.6

Midpoint potentials of various alkaliphile *c*-cytochromes. Data from alkaliphiles, RAB, *B. pseudofirmus* RAB; alc, *B. alcalophilus*; and YN, *Bacillus* YN-2000, are shown in comparison to those from non-alkaliphilic *Bacillus* species, sub, *B. subtilis* and PS3, *Bacillus* PS3. The designation m represents values calculated from composite membrane data, and p represents values calculated from measurements with purified protein (This figure was reproduced from Hicks and Krulwich (1995) with permission from the publisher)

Problem 2: How does ion-coupled bioenergetic work occur robustly at such low values of the PMF?

Transport: The genome sequence of *B. halodurans* C-125 (Takami et al. 2000) confirmed an already established strategy of using of Na^+-coupled solute transporters (Na^+/solute symporters), which can use a sodium-motive force, SMF, rather than depend upon the low PMF for solute uptake. The SMF of most alkaliphiles is much larger than the PMF since ongoing extrusion of Na^+ is carried out by the large Na^+/H^+ antiport complement as well as additional primary Na^+ pumps in different species, e.g., a Na^+-efflux ABC transporter in *B. pseudofirmus* OF4 and a Na^+-coupled Rnf transporter in *T. sulfidophilus* HL-ebGr7 (Wei et al. 1999; Muyzer et al. 2011). The presence of a larger number of ABC-type transporters, which are energized by ATP hydrolysis rather than requiring either an SMF or PMF, was also noted in the *B. halodurans* C-25, with 111 ABC transporters as opposed to the 82 found in neutralophile *B. subtilis* (Takami et al. 2000). However, this latter strategy is not universal, e.g., *B. pseudofirmus* OF4 has only 49 ABC transporter systems (Janto et al. 2011). By contrast, *B. pseudofirmus* OF4 has a particularly large complement of Na^+-coupled systems as exemplified by predicted contents of Na^+-coupled tripartite ATP-independent periplasmic transporter (TRAP-T) systems (Rabus et al. 1999), 24 in *B. pseudofirmus* OF4 versus 16 in *B. halodurans* C-125, while neutralophilic species have low single-digit numbers of such transporters. Na^+/solute symporters have been shown to have a major role in the Na^+ reentry that supports pH homeostasis in *B. pseudofirmus* OF4. The capacity of this alkaliphile for maintaining a steady cytoplasmic pH during a sudden alkaline shift in the pH_{out} is strongly dependent upon the presence of solutes that are taken up together with Na^+ (Krulwich et al. 1985; Ito et al. 2004).

Motility: Na^+-coupled flagellar motility, powered by PomAB, was established in non-alkaliphilic, alkaline-tolerant *Vibrio* species (Atsumi et al. 1992; Asai et al. 1997) and it had been shown by Imae and others that alkaliphilic *Bacillus* species utilized a SMF to energize flagellar motility (Hirota et al. 1981; Hirota and Imae 1983). The later *B. halodurans* C-125 gene sequence made it possible for the alkaliphile stator channel to be identified and verified. The single MotAB-like pair in *B. halodurans* C-125

(ACJM00000000) (Mesbah et al. 2009), and the extremely alkaliphilic aerobe *Bacillus halodurans* C-125 (BA00004.3) (Ito and Aono 2002). Sources for the data on ion coupling for the F-type ATP synthases/ATPases and for the Mot stator/channels for flagellar motility are described in footnotes to Table 1.34.1; "?" indicates uncertainty about the ion specificity. Respiratory chain complexes and carriers, sodium-coupled Rnf systems, were predicted by genome searches; hypothesized functions are indicated by "?". SCWP = secondary cell wall polymers; OM trichome is an outer layer of *Arthrospira* (Ciferri 1983); OM = outer membrane of *E. coli*; ABC = ATP-coupled transport systems; PTS = phosphotransferase transport systems. The voltage-gated sodium channels, NaChBac (Ren et al. 2001) and its homologue Na_V, are shown near cell poles (together with chemotaxis machinery MCP, CheAW in *B. halodurans* C125) based on experimental work in *B. pseudofirmus* OF4 (Fujinami et al. 2007). The *dotted arrowhead* indicating a possible inward Na^+ flux for the *N. thermophilus* ATPase is based on data indicating a modest synthetic capacity although outward pumping is presumed to be the physiological activity (Mesbah and Wiegel 2011). The hypothesized role for one of the two predicted Rnf systems in Ferredoxin:NAD^+ oxidation:reduction is based on analogy with other systems (Biegel and Müler 2010; Biegel et al. 2011)

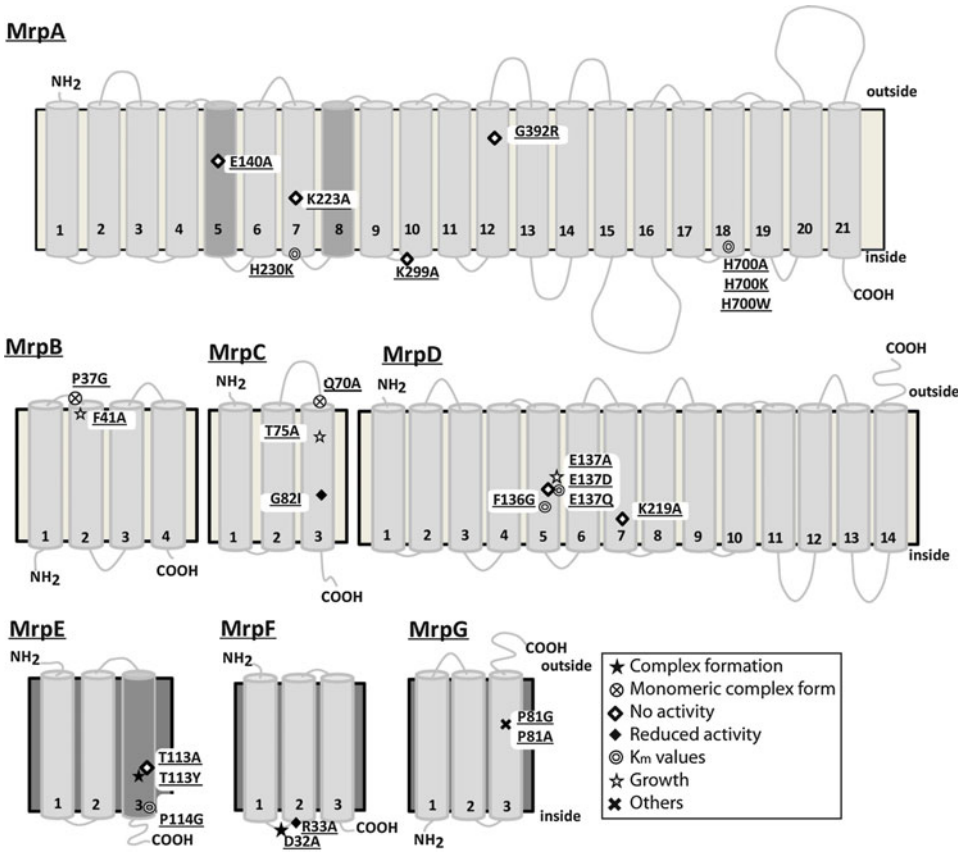


Fig. 20.7

Topological models for the seven Mrp proteins with locations of mutations shown to affect structure and functional properties of the hetero-oligomeric sodium/proton antiporter. The transmembrane segments shown were predicted by analyses using ConPred II, HMMTOP, and TMHMM (available on the World Wide Web). *Light-gray* transmembrane segments were predicted by all of the algorithms. *Dark-gray* shading in MrpA indicates transmembrane segments predicted only by two algorithms, HMTOP and TMHMM, and *dark-gray* shading in MrpE indicates transmembrane segments only predicted by ConPred II. The types of changes caused by indicated mutations are shown in the key (This figure is a modified version of Fig. 1 in Morino et al. (2010))

had a stronger resemblance to a *B. subtilis* MotAB-like pair of unknown function and to a MotAB-like pair that was then identified in *B. pseudofirmus* OF4 than to established H^+-coupled MotAB stator channels of *B. subtilis* and other neutralophiles. Follow-up experiments verified that the new stator-channel group, named MotPS (for pH and salt), is indeed a Na^+-coupled stator channel that supports motility of *B. pseudofirmus* OF4 and functions as a second unanticipated Na^+-coupled stator channel in *B. subtilis* in which it contributes to motility at elevated Na^+, pH, and viscosity; MotPS could also be identified in other neutralophilic *Bacillus* species such as *B. licheniformis* and *B. megaterium* (Ito et al. 2004, 2005; Fujinami et al. 2009). In alkaliphiles, the use of MotPS also makes a small contribution as a reentry pathway for Na^+ in support of pH homeostasis (Ito et al. 2004).

Subsequently, the genome of more moderately alkaliphilic *Bacillus clausii* KSM-K16 revealed a single MotAB-like channel whose sequence was closer to H^+-coupled MotAB stator channels than to Na^+-coupled stator channel, and yet the organism exhibits motility in the broad range of pH from 7 to 11. Further study revealed that this MotAB variant is a distinct category of a "hybrid" stator channel that uses H^+ coupling in a near-neutral range in which the PMF is significant but uses Na^+ coupling at high pH in the presence of a high SMF. Trios of mutations were identified that could change the bifunctional stator channel to greater use of either H^+ or Na^+ (Terahara et al. 2008). For the motile alkaliphiles listed in *Table 20.1*, assessments of their predicted ion-coupling profiles for motility have been noted. *Figure 20.8* lists alkaliphilic *Bacillus* species whose genomes reveal MotPS, the moderately alkaliphilic *Oceanobacillus iheyensis* and neutralophilic *B. subtilis* that are examples with both MotAB (H^+-type) and MotPS (Na^+-coupled) stator channels and *B. clausii* as the first example of bacterium with a bifunctional MotAB with hybrid ion coupling. It is notable that extremely alkaliphilic and facultative *B. halodurans* C-125 and *B. pseudofirmus* OF4, both of which have MotPS genes only, are nonmotile when growing near-neutral pH. This can be overcome by raising the Na^+ concentration with *B. pseudofirmus*

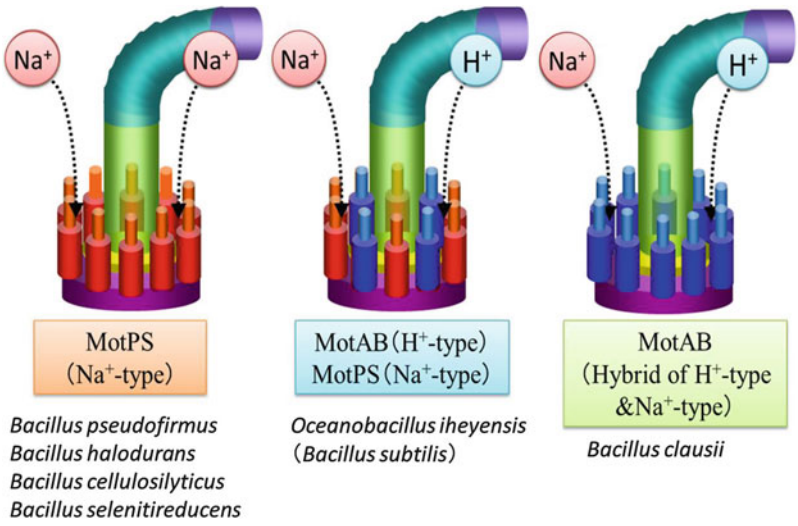


Fig. 20.8

Examples of alkaliphilic *Bacillus* species with flagellar stator rotors with three different ion-coupling patterns. Na^+-coupled MotPS flagellar stator channels are the only type found in *B. pseudofirmus* OF4, *B. halodurans* C-125, *B. cellulosilyticus*, and *B. selenitireducens*. *B. clausii* is an alkaliphile example with a hybrid stator that used both Na^+ and H^+ in a pH-dependent pattern, whereas alkaliphile *Oceanobacillus iheyensis* and neutralophile *B. subtilis* are both examples of rotors with a mixture of distinct H^+ (MotAB) and Na^+-coupled (MotPS) stator channels. Predictions are based on alignments as shown for *B. clausii* in Terahara et al. (2008)

OF4 since flagella are present throughout the pH range but are apparently competitively inhibited by H^+, whereas *B. halodurans* C-125 requires elevated pH for induction of flagellar synthesis (Aono et al. 1992; Fujinami et al. 2007b).

A voltage-gated sodium channel of the NaChBac family: Voltage-gated Na^+ channels of the NaChBac family were discovered in *B. halodurans* C-125 (Ren et al. 2001) and are predicted to be present in all almost all of the aerobic alkaliphiles listed in *Table 20.1*, but of the eight anaerobes, only two of the strains with respiratory capacity appear positive. NaChBac family channels are also found in diverse non-alkaliphilic bacteria that have aspects of saline and/or alkali conditions as part of their ecology (Koishi et al. 2004). These channels are of broad interest beyond alkaliphiles because of their structural similarity to subunits of physiologically important eukaryotic channels (Charalambous and Wallace 2011). In the context of their native alkaliphile settings, NaChBac-type channels bridge transport and motility. In *B. pseudofirmus* OF4, the Na_vBP member of the family is part of the Na^+ reentry complement of the Na^+ cycles that is alkali activated and contributes to pH homeostasis. Na_vBP is also essential for normal chemotaxis in this alkaliphile (Ito et al. 2004). The mechanistic basis for the striking effects of Na_vBP status on chemotaxis has not yet been unraveled, but the possibility of direct interaction with methylated chemotaxis proteins (Mcps) is raised by the localization of Na_vBP at the poles as are Mcps (*Fig. 20.9*).

Oxidative phosphorylation: All the other bioenergetic work of alkaliphiles described thus far makes use of a substantial SMF to avoid the problem of the low PMF at high pH, except for cation/proton antiporter-dependent proton accumulation itself. Thus far, only the cation/proton antiporters bring H^+ inward, as they must, to support the pH homeostasis that results in the low PMF. Extensive experimental evidence ultimately established that respiration-dependent ATP synthesis, oxidative phosphorylation (OXPHOS), is the other H^+-coupled process in alkaliphilic *Bacillus* species, and this is now broadly accepted (Dimroth and Cook 2004; von Ballmoos et al. 2008). Earlier, it was anticipated that OXPHOS would be Na^+ coupled, i.e., that respiratory chains of alkaliphilic bacteria would pump Na^+ in instead of H^+ to produce an SMF, which would power Na^+-coupled ATP synthases (Skulachev 1989a, b; Sobek et al. 1992). This was a reasonable hypothesis since, as with Na^+-coupled solute symporters and flagellar motility, Na^+-coupled OXPHOS would bypass the problem of the necessarily low PMF. However, this expectation has not at all been fulfilled. It is probable that the energy needs of alkaliphiles make the use of oxidation-reduction potential of a H^+-coupled respiratory chain of critical importance and ATP synthesis also appears to support alkaline pH homeostasis (Krulwich et al. 2007, 2011; Hicks et al. 2010). To date, no example of Na^+-coupled OXPHOS has been found in an alkaliphile. There is a well-established family of Na^+-translocating complexes, Nqr-Rnf (Häse and Barquera 2001; Kerscher et al. 2008; Juarez et al. 2010; Biegel et al. 2011), whose members can couple electron transport to Na^+ extrusion in a variety of marine and alkaliphilic bacteria, but many respiratory alkaliphiles lack Nqr-Rnf family members or any other putative respiratory Na^+ pumps and have exclusively H^+-pumping respiratory chains, e.g., *B. pseudofirmus* OF4 and *B. halodurans* C-125 (*Figs. 20.2* and *20.5*). Although none of the bacteria shown schematically in *Fig. 20.5* has

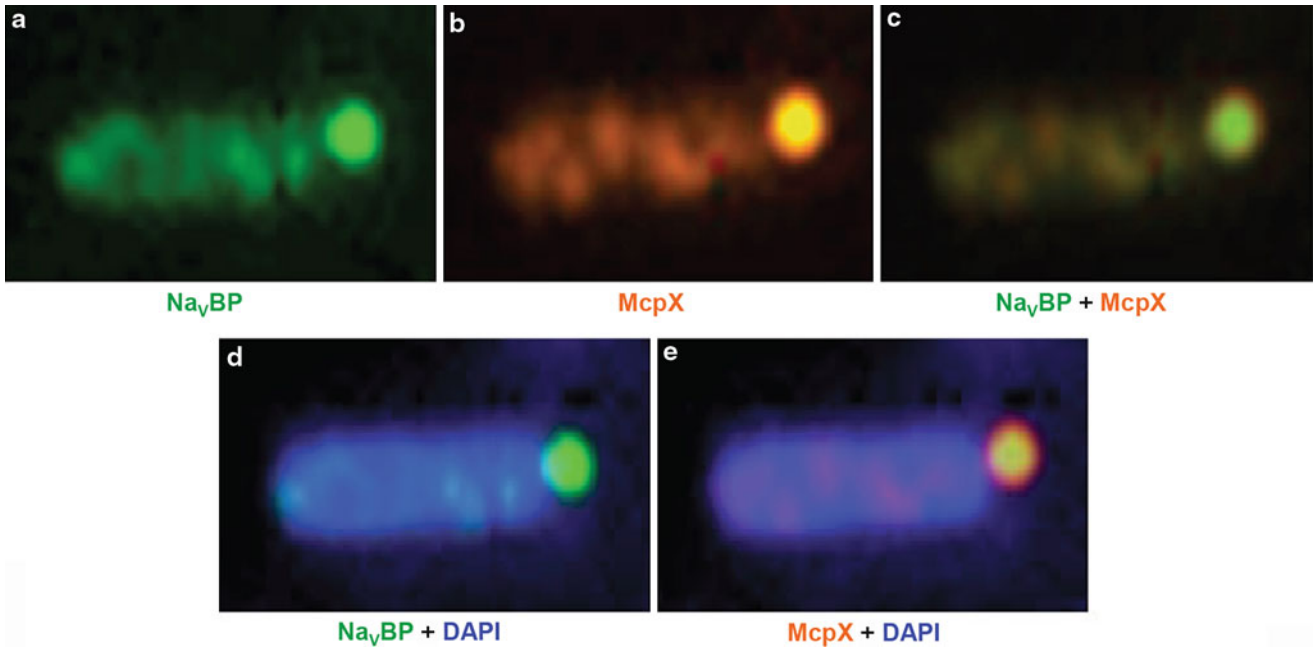


Fig. 20.9

Immunofluorescence microscopy analyses of the cellular localization of the voltage-gated sodium channel Na_VBP and the methylated chemotaxis receptor protein McpX in *B. pseudofirmus* OF4. (a) Na_VBP, immunofluorescence microscopy for Na_VBP (Alexa Fluor 488 rabbit anti-mouse IgG is *green*). (b) McpX, immunofluorescence microscopy for McpX (Alexa Fluor 546 goat anti-rabbit IgG is *red*). (c) Overlay images of the images in (a) (Na_VBP) and (b) (McpX). (d) Overlay images in (a) (Na_VBP) and DIC, differential-interference contrast microscopy. (e) Overlay images in (b) (McpX) and DIC, differential-interference contrast microscopy. DAPI, 4′,6-diamidino-2-phenylindole, is a DNA stain (The figure is an unpublished figure from a project described in Fujinami et al. (2007a))

a H^+-pumping NADH:ubiquinone oxidoreductase (the structural counterpart of mitochondrial respiratory complex I), there are alkaliphiles whose genomes indicate the presence of such a complex, e.g., in *Bacillus cellulosilyticus* DSM2522 (*Table 20.1*). Some of the alkaliphiles that have an Rnf-Nqr complex do not carry out OXPHOS. For example, *N. thermophilus* JW/NM-WN-LF has a Na^+-coupled ATPase that is proposed to function physiologically in the Na^+ efflux direction (Mesbah and Wiegel 2011) (*Fig. 20.5*) as also reported for the alkaliphilic anaerobe *C. paradoxum* (Ferguson et al. 2006). Respiratory marine bacteria as well as alkaliphilic bacteria that have Rnf-Nqo family complexes also have H^+-pumping respiratory chain components and use a H^+-coupled ATP synthase, so the Rnf-Nqo may contribute to the membrane potential, but its major role is support of the transporters, flagella, etc., that require an SMF for energization as predicted for alkaliphilic *T. sulfidophilus* (Muyzer et al. 2011) as well as in neutralophile *Vibrio cholerae* (Häse and Barquera 2001; Padan et al. 2005). As indicated in *Fig. 20.5*, photosynthetic energization of ATP by alkaliphilic *Arthrospira* (*Spirulina*) *platensis* is also H^+ coupled and occurs under sequestered conditions in thylakoid compartments of the cyanobacterium (Bakels et al. 1993). The ATP synthase of the cyanobacterial alkaliphile *A. platensis* is sequestered in an organelle that has a large pH gradient in the direction favorable to H^+-coupled ATP synthesis; however, the use of a PMF that is entirely in the form of a transmembrane pH gradient may bring its own challenges as evidenced by the high stoichiometry of the rotor of its ATP synthase, as discussed in comparison with other alkaliphiles below.

How, then, do alkaliphilic bacteria such as *B. pseudofirmus* OF4, whose H^+-coupled respiratory chain complexes and ATP synthase are in the cytoplasmic membrane, successfully carry out OXPHOS? The conundrum is that if energization involves the bulk transmembrane PMF as posited by Mitchell (Mitchell 1961), it should be impossible to synthesize ATP robustly when the cytoplasmic pH is 2 units lower than the external pH, since that energetically counterproductive pH gradient is not offset by an unusually large transmembrane potential (Guffanti and Hicks 1991; Sturr et al. 1994). Yet *B. pseudofirmus* OF4 grows non-fermentatively on malate and synthesizes ATP both at pH 7.5, with a PMF of −136 mV, and also at pH 10.5, with a PMF of −50 mV (*Fig. 20.2*). As proposed by Williams (Williams 1978) and supported by the alkaliphile results (Krulwich 1995; Krulwich et al. 2007), it is most likely that H^+ movements from respiratory pumps to the ATP synthase are sequestered in some manner at or near the membrane surface, allowing their arrival at the synthase to be faster than their equilibration with the highly alkaline bulk phase. This general suggestion has received wide attention and support recently as models have predicted interfacial barriers to rapid proton equilibration from the near membrane surface to the bulk (Georgievskii et al. 2002;

Cherepanov et al. 2004; Mulkidjanian et al. 2006) and experiments that take advantage of new probes and biophysical techniques support rapid proton movements on a membrane surface (Heberle and Dencher 1990, 1992; Brändén et al. 2006). The possibility that the membrane components, e.g., anionic lipids such as cardiolipin, play a role in proton transfer is a related issue of interest (Haines and Dencher 2002). The new models and experiments that have fueled a strong new look at the idea of rapid surface-associated proton movements usually cite the alkaliphile OXPHOS conundrum as a biological paradigm but propose that their models apply more broadly than alkaliphiles to H^+. Thus, the alkaliphile work has impacted general thinking about proton-coupled bioenergetic work. Meanwhile, studies of the OXPHOS in extremely alkaliphilic *Bacillus* strains show that beyond rapid, sequestered proton movement between pumps and the synthase, there are requirements for alkaliphile-specific adaptations of the OXPHOS machinery.

Although less work has been conducted on the respiratory chain proteins and protein complexes of alkaliphiles than on the ATP synthases, there are two types of observations in work on *B. pseudofirmus* OF4 that indicate special adaptations related to the respiratory chain. First, the *B. pseudofirmus* OF4 respiratory chain has three terminal oxidases that catalyze the final transfers of electrons and reduce molecular oxygen to H_2O, a proton-pumping cytochrome caa_3-type CtaC-F cytochrome oxidase, as well as two PMF-generating but non-proton-pumping cytochrome *bd*-type oxidases (Fig. 20.2). In neutralophiles, most of which also have multiple terminal oxidases, non-fermentative growth and the OXPHOS it requires can occur as long as there is a single terminal oxidase. By contrast, in *B. pseudofirmus* OF4, deletion of the Cta terminal oxidase eliminates growth on malate even though one of the *bd*-type oxidases is greatly upregulated (Gilmour and Krulwich 1997). Possibly, active proton pumping at the terminal oxidase site is essential in the alkaliphile, but the possibility that Cta and ATP synthase are closer in proximity to each other in the membrane or dynamically interact has also been raised (Liu et al. 2007). Second, as noted in connection with global adaptations, both the Qcr and Cta complexes, the two H^+-pumping complexes of the respiratory chain, have externally exposed segments that are far more acidic than those found in neutralophile homologues, whose importance for function is yet to be directly tested.

F-type ATP synthases of extremely alkaliphilic *Bacillus* species have specific motifs in the two subunits of the F_o sector of the ATP synthase, the *a*-subunit and the *c*-subunit. The *a*-subunit is thought to be involved in passage of external protons to an interface in which they can be passed to the *c*-subunit rotor ring, and after a protonated subunit has completed rotation and returns to the *a*-*c* interface, the *a*-subunit is involved in proton release to the cytoplasm (Fig. 20.10a) (von Ballmoos et al. 2009; Hicks et al. 2010; Okuno et al. 2011). Several of the alkaliphile-specific motifs in these two subunits are required for ATP synthesis at high pH but not at near-neutral pH (Wang et al. 2004; Liu et al. 2009, 2011; Fujisawa et al. 2010). The implication of this important finding is that for these alkaliphiles, the interfacial barriers to proton equilibration with the bulk and rapid movement of protons at the membrane surface could be necessary but not sufficient to achieve OXPHOS at very high external pH. An alkaliphile-specific motif that is likely to be in the path of protons into the ATP synthase is a pair of residues in helices four and five, Lys180 and Gly212 (*B. pseudofirmus* OF4 numbering) that are found in three extreme alkaliphiles, with Lys180 found in a larger range of alkaliphiles (Fig. 20.10b); the two residues can be switched in position with retention of function although the double mutant is leaky, which is adverse for function at alkaline pH. Studies of mutants with substitution in the Lys180 position indicate that it has a critical functional role for non-fermentative growth at high pH and also plays a role at low pH when the PMF is reduced by a protonophore (Fujisawa et al. 2010). The results of mutagenesis of the *B. pseudofirmus* OF4 *a*-subunit Lys180 differ from results with the same substitutions in the comparable position of thermoalkaliphile *C. thermarum* TA2.A1 (McMillan et al. 2007). Further study of sequence differences between the two subunits (outlined with boxes in Fig. 20.10b), and of effects of their replacement with the neutralophile consensus, suggests that the thermoalkaliphile has a distinct variant that presumably meets its specific set of challenges more optimally than the alkaliphile pattern and vice versa (Fujisawa et al. 2010).

A crystal structure of the homo-oligomeric ring that is the rotor of the *B. pseudofirmus* OF4 ATP synthase has been solved at 2.5 Å. A view perpendicular to the membrane is shown in Fig. 20.11a, with the *B. pseudofirmus* OF4 *c*-ring in between *c*-ring structures of the 11-subunit ring from the Na^+-coupled *Ilyobacter tartaricus* synthase and the 15-subunit ring of the H^+-coupled *A. platensis* enzyme (Preiss et al. 2010). Although *B. pseudofirmus* OF4 ring has 13-subunits (Fig. 20.11b), the width of its ring is closer to that of the 15-subunit *A. platensis* ring than to that of the 11-subunit *I. tartaricus* ring (Fig. 20.11a). The greater width is probably needed to accommodate the AxAxAxA motif in the N-terminal, inner helix, that replaces the GxGxGxG motif found in neutralophiles as well as in the photosynthetic alkaliphile *A. platensis* (Fig. 20.10c). The motifs of the *B. pseudofirmus* OF4 *c*-ring are probably also related to its having somewhat less of an "hourglass"-like shape than the others. The second major motif of the *c*-subunit is the PxxExxP motif in the outer, C-terminal helices, in which the first proline, Pro51, is the alkaliphile-specific residue (Fig. 20.10c). This residue completes a pair of prolines that flank the proton-binding carboxylate Glu54 and have a major impact on the binding site. The binding site was captured in an "ion-locked" state with a bound water molecule in the site and has a significantly different pattern of ion binding compared to the *I. tartaricus* and *A. platensis* examples (Preiss et al. 2010). The *B. pseudofirmus* OF4 *c*-ring has 13 subunits (Fig. 20.11b) as does the ring from *C. thermarum* TA2.A1 (Meier et al. 2007). The number of *c*-subunits/rotor, i.e., the *c*-subunit stoichiometry, is the same as the number of H^+ translocated during the full rotation of the *c*-ring, which is accompanied by synthesis of three ATP. At high stoichiometries of *c*-subunits/rotor, the rotor better adapted to synthesize ATP at a low PMF although it is less efficient than rotors with fewer subunits under normal

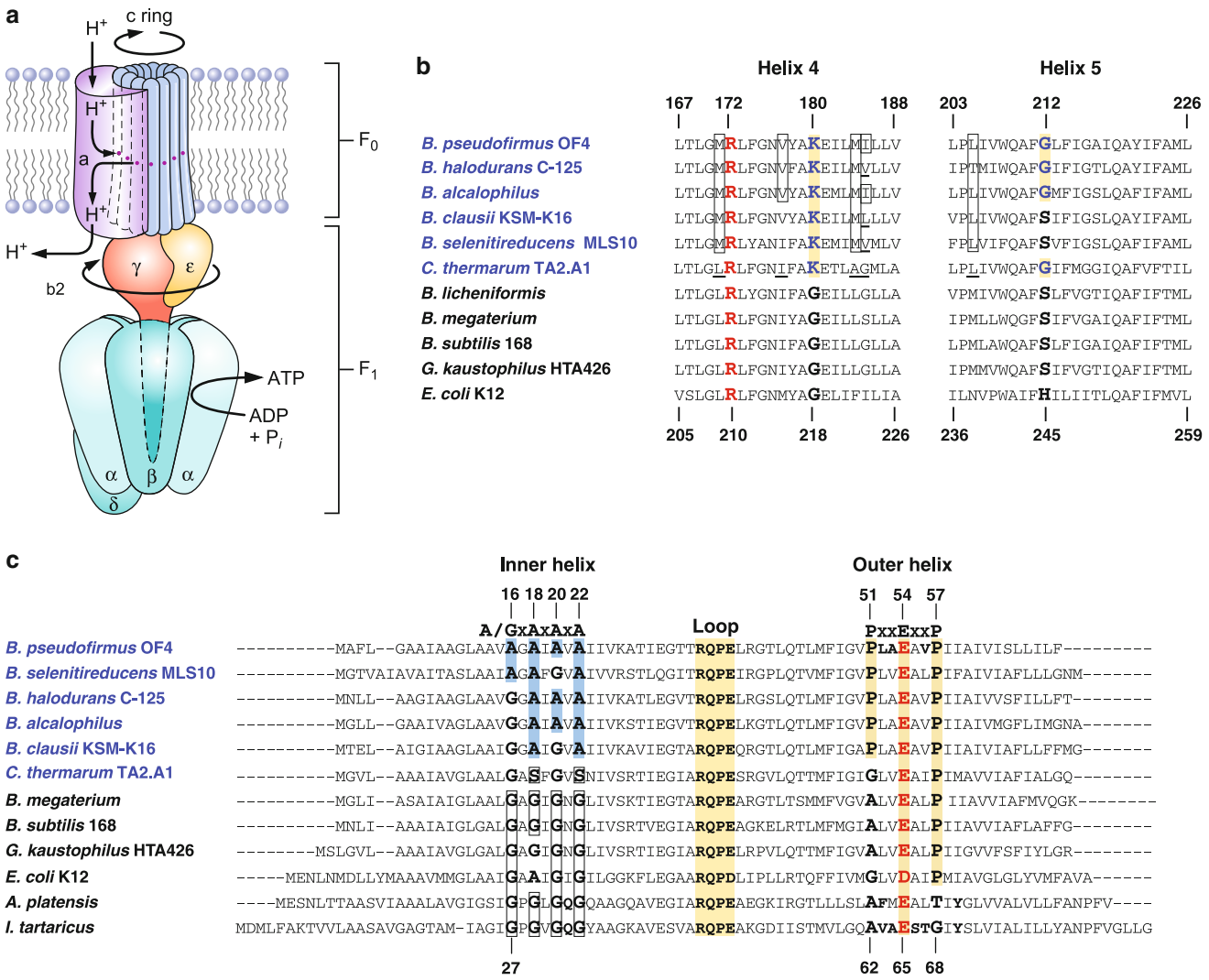


Fig. 20.10

A schematic model of a H^+-coupled bacterial ATP synthase and alignments showing alkaliphile-specific motifs of the proton-translocating *a*- and *c*-subunits. (a) A model of the proton-coupled ATP synthase showing inward, energetically favorable H^+ translocation powering ATP synthesis (Hicks et al. 2010); (b) An alignment of *a*-subunit helices 4 and 5 is shown as these regions are thought to have a major role in proton translocation, especially Arg172. Alkaliphilic-specific residues Lys180 and Gly212 are highlighted by shading. Boxed regions highlight residues in extreme aerobic alkaliphiles that are not found in the polyextremophile, thermoalkaliphile *C. thermarum* TA2.A1 (Fujisawa et al. 2010). (c) Alignment of the *c*-subunits of six alkaliphilic Firmicutes (including *C. thermarum*) and three neutralophilic Firmicutes, *E. coli, A. platensis*, and *Ilyobacter tartaricus*, which have a sodium-coupled synthase (Liu et al. 2011). The alkaliphile A/GxAxAxA motif of the N-terminal helix, which is the inner helix of the *c*-ring rotor, is *shaded*, while the GxGxGxG of neutralophiles is *boxed*. In the outer C-terminal helix of the hairpin-like subunit, the alkaliphile-specific Pro51 is highlighted as part of a PxxExxP motif of alkaliphiles (Arechaga and Jones 2001), and the carboxylate in the middle is *shaded*; note its essential role in proton translocation

PMF conditions (Pogoryelov et al. 2005; Hicks et al. 2010). The c_{13} rotors of the two non-photosynthetic alkaliphile enzymes, which function under conditions of low PMF, are near the high end of reported rotor stoichiometries, 8–15 *c*-subunits/rotor (Pogoryelov et al. 2005, 2009; Watt et al. 2010). However, the stoichiometry is not nearly high enough to fully account for the observed synthesis by these two alkaliphiles at alkaline pH values (Hicks et al. 2010). Moreover, it is the photosynthetic alkaliphilic cyanobacterium *A. platensis* and the spinach chloroplast ATP synthase *c*-ring rotors, with respective stoichiometries of 15 and 14, that have the highest reported stoichiometries (Seelert et al. 2000, 2003; Pogoryelov et al. 2005, 2009), suggesting that an adequate PMF that is entirely in the form of a transmembrane chemical gradient (ΔpH), albeit large, creates a constraint that fosters use of a high stoichiometry even more forcefully than alkaliphily.

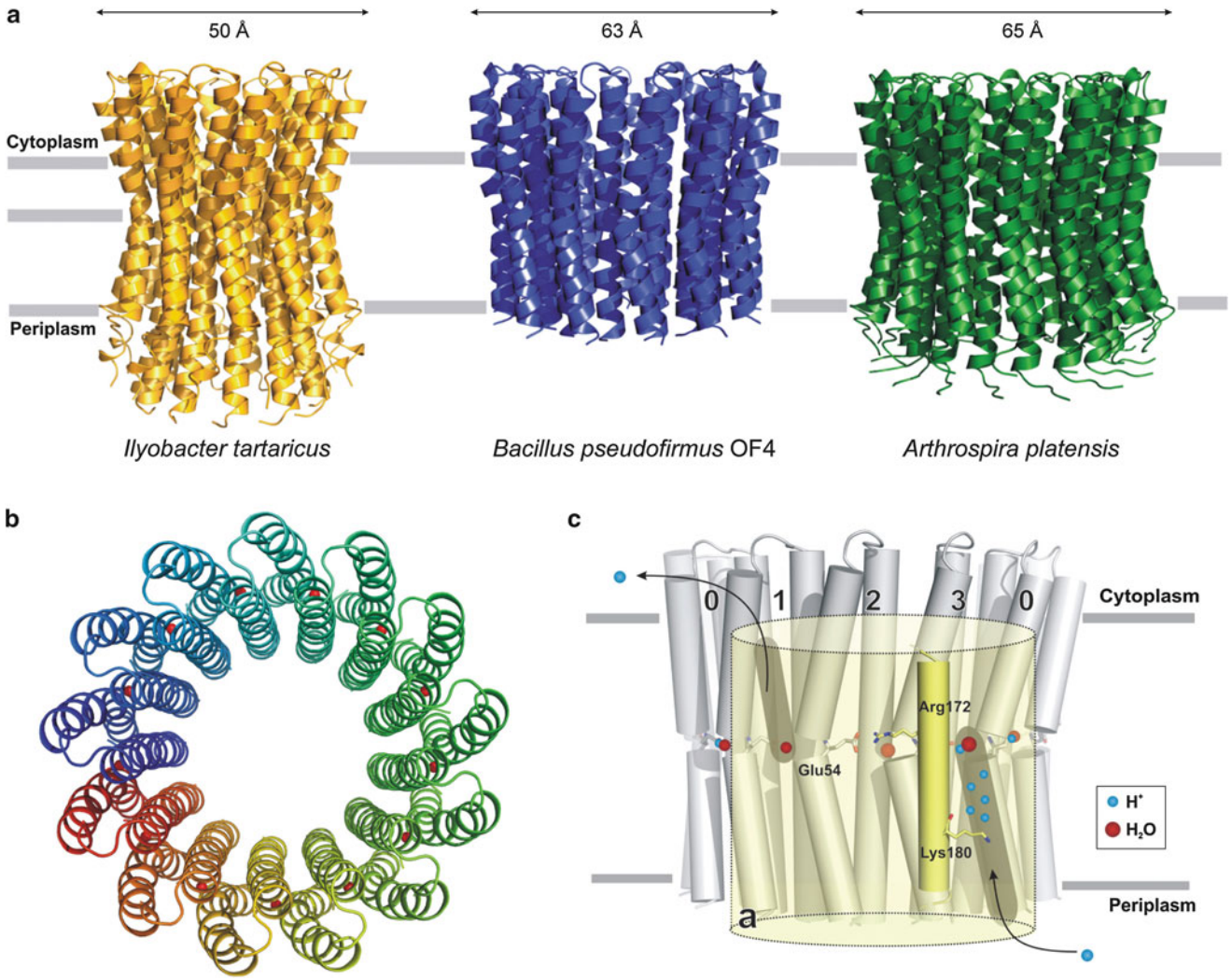


Fig. 20.11

Alkaliphile-specific motifs of the proton-conducting ATP synthase *a*- and *c*-subunits, the 13-subunit *c*-ring structure, and a hypothetical model of proton movement through the membrane segment of the alkaliphile synthase. (a) Views of structures of the *c*-ring rotors of the Na^+-coupled ATP synthase of *Ilyobacter tartaricus* (11 *c*-subunits/ring) (Meier et al. 2005) and two H^+-coupled rotors from *Bacillus pseudofirmus* OF4 (13 *c*-subunits/ring) (Preiss et al. 2010) and *Arthrospira (Spirulina) platensis* (15 *c*-subunits/ring) (Pogoryelov et al. 2005, 2009). The widths of the rings are shown. (b) A view of the *c*-rotor ring perpendicular to the membrane from the cytoplasmic side. The *red circles* are bound water molecules in the proton-binding sites on the outer C-terminal helices. (c) A model showing the c_{13} ring rotor (*gray*) with the neighboring *a*-subunit (stator, *yellow*), and the view is slanted to the membrane plane. A selection of *c*-subunits is shown with the ion-coordinating glutamate residue (Glu54) and helix 4 of the *a*-subunit with the conserved and functionally important Arg172 and alkaliphile-specific Lys180 (Valiyaveetil and Fillingame 1998; Wang et al. 2004; Fujisawa et al. 2010). During ATP synthesis by the enzyme, the rotor moves from left to right. Two access pathways, to and from the binding sites in the membrane, are indicated in *gray* (Steed and Fillingame 2009; Lau and Rubinstein 2010) (The figure is a modified combination of images from Hicks et al. (2010) (a) and Preiss et al. (2010) (b, c))

Mutagenesis studies of two major alkaliphile-specific motifs in the *c*-subunit have been enhanced by availability of a crystal structure for the *c*-ring. As noted above, one motif is near the middle of each of the two helices of this hairpin-like subunit. The two motifs are highlighted in the alignment in ❯ *Fig. 20.10c*. Mutagenesis studies have shown that both the AxAxAxA motif in the N-terminal helix and the alkaliphile-specific Pro51, near the proton-binding carboxylate Glu54 of the C-terminal helix, have important roles in alkaliphily (Liu et al. 2009, 2011). Further partnered biochemical and structural studies should clarify details of how the motifs impact the *c*-rotor structure and contribute to ATP synthesis at high pH. A diagrammatic model of ion translocation by the *B. pseudofirmus* OF4 ATP synthase is shown in ❯ *Fig. 20.11c*, which shows the *a*-subunit Lys180 and interactions of the ion-binding site as it moves through the *a*- and *c*-subunit interfaces (Preiss et al. 2010).

Molecular dynamics simulations predict that the proton is present in the ion-binding site as a protonated glutamate (Glu54) since protonation of the bound water would be transient and would be quickly followed by irreversible transfer to the carboxylate (Leone et al. 2010).

It is notable that the ATP synthase motifs described for alkaliphilic *Bacillus* strains are not found in Gram-negative alkaliphiles such as alkaliphilic *T. sulfidophilus* HL-EbGr7, raising the possibility that their outer membrane has beneficent properties with respect to proton retention or that the challenges of Gram-negative and Gram-positive alkaliphiles select for different adaptations as appears to be the explanation for differently adapted ATP synthase subunits in thermoalkaliphile *C. thermarum* TA2.A1 and *B. pseudofirmus* OF4.

Problem 3: How do alkaliphilic *Bacillus* species tolerate a much higher cytoplasmic pH value than neutralophilic bacteria and retain the ability to grow?

This is an area that will require much more data on cytoplasmic components. The structural studies that indicate differences between cytoplasmic phosphoserine aminotransferases from alkaliphilic *Bacillus* strains and one neutralophilic example are tantalizing (Dubnovitsky et al. 2005; Kapetaniou et al. 2006). It may also turn out when more data are available that any adaptive trends for cytoplasmic enzymes might be different, at least in the details, from the adaptations noted already for extracellular enzymes from alkaliphiles (Shirai et al. 1997, 2001, 2007). Possibly, there will turn out to be specific "weak spots" that are particularly vulnerable to malfunction at elevated cytoplasmic pH and that are specifically adapted in alkaliphiles to avoid such malfunction. Candidates might include divisome-associated proteins, since septation phenotypes were observed in *B. pseudofirmus* OF4 cells grown in continuous culture at pH 11.2 (Sturr et al. 1994). Further, there may be alkaliphile-specific proteins that play roles as chaperones or some sort of novel adaptors since two small proteins were identified in the *B. pseudofirmus* OF4 genome that only have homologues in a small group of alkaliphilic *Bacillus* species (BpOF4_10505 and BpOF4_10510). Another small *B. pseudofirmus* OF4 protein of potential interest in this context has a broader range of homologues, but the top match is BH2819 from *B. halodurans* C-125, which was cloned as a gene that plays a required role in alkaliphily (Aono et al. 1993).

Problem 4: Why do alkaliphiles fail to grow or grow poorly at pH ~ 7?

The most likely explanation for obligate alkaliphily or poor growth of extreme alkaliphiles at near-neutral pH is that their multiple adaptations for growth at very high pH are in most cases adverse for growth in a lower pH range (Krulwich et al. 2011). In *B. pseudofirmus* OF4, the following can be cited as potential problems: The 13 *c*-subunit stoichiometry would make the ATP synthase less efficient at near-neutral pH (Hicks et al. 2010), expression of the S-layer has been shown to be detrimental to growth at pH 7.5 except that it enhances adaptation if a sudden transition to alkali is imposed (Gilmour et al. 2000), and higher concentrations of Na^+ are required to support flagellar motility and Na^+-coupled transporters at near-neutral pH because of the competitive inhibition of these processes by the H^+ concentration at near-neutral pH (Gilmour et al. 2000; Fujinami et al. 2007). It also seems likely that global adaptation of the pI values of proteins exposed on or outside the external membrane will be a disadvantage at near-neutral pH. Membrane lipids, too, may be adapted to minimize proton leakage if a shift to high pH occurs but carry a cost because of some property associated with this constitutively adapted lipid complement that makes them less optimal at near-neutral pH.

Applications of Alkaliphiles to Biotechnology

Applications of alkaliphiles to biotechnology continue to expand as the databases on alkaliphile genomes and the place of alkaliphiles in metagenomes expand, and the number of well-characterized alkaliphiles grows. Major areas of application remain: the use of alkaline enzymes, bioprocesses including bioremediation, and use of alkaliphile products. The largest industrial application of alkaliphile enzymes has been in laundry and dishwasher detergents (Ito et al. 1998; Horikoshi 1999; Fujinami and Fujisawa 2010; Sarethy et al. 2011). Alkaliphile proteases, which also have additional applications (Sarethy et al. 2011), have been the most extensively identified and developed enzyme group (Gupta et al. 2002; Saeki et al. 2007; Takimura et al. 2007). The structural biology on alkaliphile and non-alkaliphile homologues of proteases has been partnered with phylogenetic analyses that assess pathways and evolutionary distances (*Fig. 20.12*). These analyses have provided insights into alkaliphile adaptations of structure-function that will continue to be enhanced as the structure database grows (Shirai et al. 1997). Similar analyses have also been extended to amylases and cellulases (Shirai et al. 2001, 2007). Alkaliphile xylanases can be used to bleach alkali-treated wood pulp and avoid using environmentally problematic chlorine for that task, and various uses have been explored for alkaliphile pectinases and chitinases and catalases (Sarethy et al. 2011).

The two other types of applications, use of products made by alkaliphile enzymes and use of alkaliphiles themselves in bioprocesses, have been explored extensively. Alkaliphile cyclomaltodextrin glucanotransferases (CGTases) have been explored for use in producing cyclodextrins, which have a variety of uses in pharmaceuticals, cosmetics, and food flavoring (Horikoshi 1999; Sarethy et al. 2011). Other products of potential interest are carotenoids, which are found widely in alkaliphiles (Aono and Horikoshi 1991), and siderophores, which are important for alkaliphiles in iron acquisition (McMillan et al. 2010; Janto et al. 2011; Miethke et al. 2011) and have been considered as having biotechnological applications (Sarethy et al. 2011). A variety of bioprocessing uses of alkaliphiles have also been explored, and others have been suggested. Indigo blue dye production has been noted and has returned as a bioprocess to which a growing number of

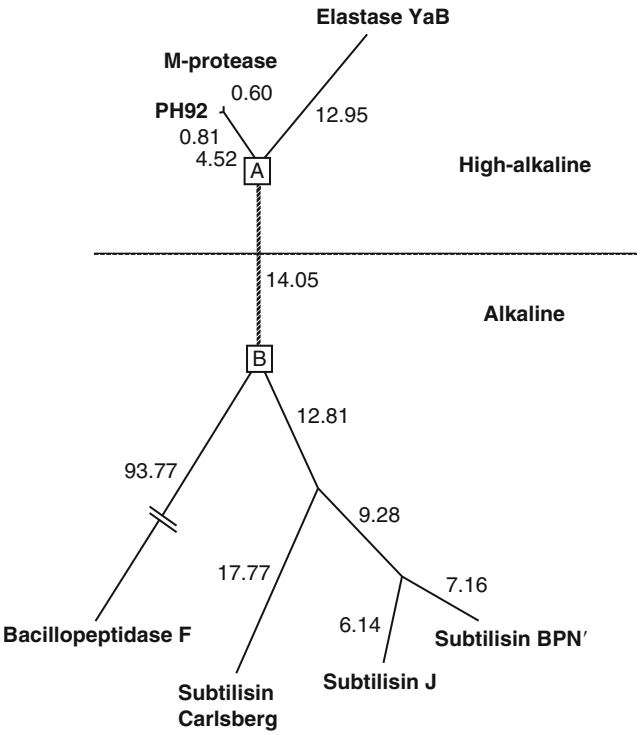


Fig. 20.12

The phylogenetic tree of M-protease and related proteases showing evolutionary distances. The evolutionary distances are shown on the branches in percent between accepted point mutations. Two boxes indicate positions of distinct hypothetical ancestors A and B. All of the branches are more than 95 % probable from 1,000 bootstrap reconstructions (This figure was reproduced from Shirai et al. (1997), with permission from the publisher)

alkaliphile isolates are applied (Sarethy et al. 2011). Other bioprocesses involving alkaliphiles are also receiving increased attention. The psychrophilic alkaliphile *Dietzia psychralcaliphila* was isolated from a drain pool of a fish-egg-processing plant and shown to grow on hydrocarbons, leading to the suggestion that it has potential for bioremediation of oil-contaminated soils or water in cold climate conditions (Yumoto et al. 2002). Haloalkaliphilic sulfur-oxidizing bacteria in mixed cultures have been suggested for treatment of inorganic sulfur compounds in petroleum industry effluents (Olguin-Lora et al. 2011). Thiopaq reactors containing sulfur-oxidizing haloalkaliphiles of the *Thioalkalivibrio* genus have been used for natural gas desulfurization (Sorokin et al. 2008), and biological treatment of alkaline sulfidic refinery waste, sulfidic spent caustic, was conducted in bioreactors under aerobic, haloalkaline conditions. In these latter reactors, *Thioalkalivibrio* species mediated conversion of the sulfide to sulfate, and more than 90 % of influent benzene was removed, possibly by other haloalkaliphilic bacteria of several genera that were found in the reactors (de Graaff et al. 2011). The list can be expected to grow.

Conclusions

Alkaliphilic bacteria continue to raise challenging physiological and bioenergetic questions whose answers are likely to continue to yield insights and lead to identification of new molecules that play roles beyond the extremophiles. The era of genomics, metagenomics, and an array of other new tools should also facilitate the application of findings to ecology and phylogenetics. Application of alkaliphile enzymes to biotechnology has already intersected significantly with bioengineering, providing an intersection between applied and basic mechanistic efforts. There is great potential for expansion of the use of information gained about the adaptations that underpin enzyme function in the alkaline pH range, at a variety of conditions of temperature, salinity, and oxygen, to problems of bioremediation and diverse kinds of bioprocessing. There is also potential for comparable studies of the adaptations of a wide range of alkaliphile products that will further enhance our insights into alkaliphily while suggesting bioengineering opportunities for increasing their usefulness.

Acknowledgments

Work from the authors' laboratories was supported by research grants GM28454 from the National Institutes of Health (to T.A.K.) and by The Strategic Research Foundation Grant-Aided Project for Private Universities and Grant-in-Aid for Scientific Research (B) No. 21370074 of the Ministry of Education, Culture, Sports, Science and Technology of Japan (to M.I.). We thank Benjamin Janto for providing *Fig. 20.1*, Shun Fujinami for *Fig. 20.8*, and David Hicks for *Fig. 20.10*. We are also grateful to many colleagues in our own laboratories and collaborators from other institutions who have contributed to our work and shared ideas, questions, and new findings on alkaliphiles with us.

References

Aino K, Hirota K, Matsuno T, Morita N, Nodasaka Y, Fujiwara T, Matsuyama H, Yoshimune K, Yumoto I (2008) *Bacillus polygoni* sp. nov., a moderately halophilic, non-motile obligate alkaliphile isolated from indigo balls. Int J Syst Evol Microbiol 58:120–124

Aono R (1987) Characterization of structural components of cell walls of alkaliphilic strain of *Bacillus* sp. C-125. Biochem J 245:467–472

Aono R, Horikoshi K (1983) Chemical composition of cell walls of alkalophilic strains of *Bacillus*. J Gen Microbiol 129:1083–1087

Aono A, Horikoshi K (1991) Carotenes produced by alkaliphilic yellow-pigmented strains of *Bacillus*. Agric Biol Chem 55:2643–2645

Aono R, Ohtani M (1990) Loss of alkalophily in cell-wall-component-defective mutants derived from alkalophilic *Bacillus* C-125 Isolation and partial characterization of the mutants. Biochem J 266:933–936

Aono R, Uramoto M (1986) Presence of fucosamine in teichuronic acid of the alkalophilic *Bacillus strain* C-125. Biochem J 233:291–294

Aono R, Horikoshi K, Goto S (1984) Composition of the peptidoglycan of alkalophilic *Bacillus* spp. J Bacteriol 157:688–689

Aono R, Ogino H, Horikoshi K (1992) pH-dependent flagella formation by facultative alkaliphilic *Bacillus* sp. C-125. Biosci Biotechnol Biochem 56:48–53

Aono R, Hayakawa A, Hashimoto M, Kaneko H, Nakamura S, Horikoshi K (1993a) Cloning of a gene required for the alkaliphily of alkaliphilic *Bacillus* sp. strain C-125. Nucleic Acids Symp Ser 29:139–140

Aono R, Ito M, Horikoshi K (1993b) Occurrence of teichuronopeptide in cell walls of group 2 alkaliphilic *Bacillus* spp. J Gen Microbiol 139:2739–2744

Aono R, Ito M, Machida T (1999) Contribution of the cell wall component teichuronopeptide to pH homeostasis and alkaliphily in the alkaliphile *Bacillus lentus* C-125. J Bacteriol 181:6600–6606

Arechaga I, Jones PC (2001) The rotor in the membrane of the ATP synthase and relatives. FEBS Lett 494:1–5

Asai Y, Kojima S, Kato H, Nishioka N, Kawagishi I, Homma M (1997) Putative channel components for the fast-rotating sodium-driven flagellar motor of a marine bacterium. J Bacteriol 179:5104–5110

Asao M, Pinkart HC, Madigan MT (2011) Diversity of extremophilic purple phototrophic bacteria in Soap Lake, a Central Washington (USA) soda lake. Environ Microbiol. doi:10.1111/j.1462-2920.2011.02449x

Atsumi T, McCarter L, Imae Y (1992) Polar and lateral flagellar motors of marine Vibrio are driven by different ion-motive forces. Nature 355:182–184

Bakels RH, van Walraven HS, Krab K, Scholts MH, Kraayenhof R (1993) On the activation mechanism of the H^+-ATP synthase and unusual thermodynamic properties in the alkalophilic cyanobacterium *Spirulina platensis*. Eur J Biochem 213:957–964

Banciu H, Sorokin DY, Kleerebezem R, Muyzer G, Galinski EA, Kuenen JG (2004) Growth kinetics of haloalkaliphilic, sulfur-oxidizing bacterium *Thioalkalivibrio versutus* strain ALJ 15 in continuous culture. Extremophiles 8:185–192

Banciu HL, Sorokin DY, Tourova TP, Galinski EA, Muntyan MS, Kuenen JG, Muyzer G (2008) Influence of salts and pH on growth and activity of a novel facultatively alkaliphilic, extremely salt-tolerant, obligately chemolithoautotrophic sufur-oxidizing Gammaproteobacterium *Thioalkalibacter halophilus* gen. nov., sp. nov. from South-Western Siberian soda lakes. Extremophiles 12:391–404

Belkin S, Boussiba S (1991) Resistance of Spirulina platensis to ammonia at high pH values. Plant Cell Physiol 32:953–958

Biegel E, Müler V (2010) Bacterial Na^+-translocating ferredoxin: NAD^+ oxidoreductase. Proc Natl Acad Sci USA 107:18138–18142

Biegel E, Schmidet S, Gonzalez JM, Müller V (2011) Biochemistry, evolution and physiological function of the Rnf complex, a novel ion-motive electron transport complex in prokaryotes. Cell Mol Life Sci 68:613–634

Blum JS, Bindi AB, Buzzelli J, Stolz JF, Oremland RS (1998) *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30

Bowers KJ, Mesbah NM, Wiegel J (2009) Biodiversity of poly-extremophilic Bacteria: Does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life? Saline Systems 5:9

Brändén M, Sandén T, Brzezinski P, Widengren J (2006) Localized proton microcircuits at the biological membrane-water interface. Proc Natl Acad Sci USA 103:19766–19770

Brown SD, Begemann MB, Mormile MR, Wall JD, Han CS, Goodwin LA, Pitluck S, Land ML, Hauser LJ, Elias DA (2011) Complete genome sequence of the haloalkaliphilic, hydrogen-producing bacterium *Halanaerobium hydrogeniformans*. J Bacteriol 193:3682–3683

Bryantseva I, Gorlenko VM, Kompantseva EI, Imhoff JF, Suling J, Mityushina L (1999) *Thiorhodospira sibirica* gen. Nov., sp. nov., a new alkaliphilic purple sulfur bacterium from a Siberian soda lake. Int J Syst Bacteriol 49:697–703

Charalambous K, Wallace BA (2011) NaChBac: the long lost sodium channel ancestor. Biochemistry 50:6742–6752

Cherepanov DA, Junge W, Mulkdjanian AY (2004) Proton transfer dynamics at the membrane/water interface: dependence on the fixed and mobile pH buffers, on the size and form of membrane particles, and on the interfacial potential barrier. Biophys J 86:665–680

Ciferri O (1983) Spirulina, the edible microorganism. Microbiol Rev 47:551–578

Clejan S, Krulwich TA, Mondrus KR, Seto-Young D (1986) Membrane lipid composition of obligately and facultatively alkalophilic strains of *Bacillus* spp. J Bacteriol 168:334–340

de Graaff M, Bijmans MFM, Abbas B, Euverink G-JW, Muyzer G, Janssen AJH (2011) Biological treatment of refinery spent caustics under halo-alkaline conditions. Bioresource Technol 102:7257–7264

Dimroth P, Cook GM (2004) Bacterial Na^+ - or H^+ -coupled ATP synthases operating at low electrochemical potential. Adv Microb Physiol 49:175–218

Dubnovitsky AP, Kapetaniou EG, Papageorgiou AC (2005) Enzyme adaptation to alkaline pH: atomic resolution (1.08 A) structure of phosphoserine aminotransferase from *Bacillus alcalophilus*. Protein Sci 14:97–110

Efremov RG, Sazanov LA (2011a) Respiratory complex I: 'steam engine' of the cell? Curr Opin Struct Biol 21:532–540

Efremov RG, Sazanov LA (2011b) Structure of the membrane domain of respiratory complex I. Nature 476:414–420

Efremov RG, Bardaran R, Sazanov LA (2010) The architecture of respiratory complex I. Nature 465:441–445

Enomoto K, Koyama N (1999) Effect of growth pH on the phospholipid contents of the membranes from alkaliphilic bacteria. Curr Microbiol 39:270–273

Ferguson SA, Keis S, Cook GM (2006) Biochemical and molecular characterization of a Na^+-translocating F_1F_o-ATPase from the thermoalkaliphilic bacterium *Clostridium paradoxum*. J Bacteriol 188:5045–5054

Foti M, Ma S, Sorokin DY, Rademaker JL, Kuenen JG, Muyzer G (2006) Genetic diversity and biogeography of haloalkaliphilic sulphur-oxidizing bacteria belonging to the genus Thioalkalivibrio. FEMS Microbiol Ecol 56:95–101

Friedrich T, Scheide D (2000) The respiratory complex I of bacteria, archaea and eukarya and its module common with membrane-bound multisubunit hydrogenases. FEBS Lett 479:1–5

Fujinami S, Fujisawa M (2010) Industrial application of alkaliphiles and their enzymes – past, present and future. Environ Technol 31:845–856

Fujinami S, Sato T, Trimmer JS, Spiller BW, Clapham DE, Krulwich TA, Kawagishi I, Ito M (2007a) The voltage-gated Na^+ channel Na_VBP co-localizes with methyl-accepting chemotaxis protein at cell poles of alkaliphilic *Bacillus pseudofirmus* OF4. Microbiology 153:4027–4038

Fujinami S, Terahara N, Lee S, Ito M (2007b) Na^+ and flagella-dependent swimming of alkaliphilic *Bacillus pseudofirmus* OF4: a basis for poor motility at low pH and enhancement in viscous media in an "up-motile" variant. Arch Microbiol 187:239–247

Fujinami S, Terahara N, Krulwich TA, Ito M (2009) Motility and chemotaxis in alkaliphilic *Bacillus* species. Future Microbiol 4:1137–1149

Fujisawa M, Fackelmayer O, Liu J, Krulwich TA, Hicks DB (2010) The ATP synthase a-subunit of extreme alkaliphiles is a distinct variant. J Biol Chem 285:32105–32115

Georgievskii Y, Medvedev ES, Stuchebrukhov AA (2002) Proton transport via the membrane surface. Biophys J 82:2833–2846

Ghauri MA, Khalid AM, Grant S, Grant WD, Heaphy S (2006) Phylogenetic analysis of bacterial isolates from man-made high-pH, high-salt environments and identification of gene-cassette-associated open reading frames. Curr Microbiol 52:487–492

Gibson T (1934) An investigation of the *Bacillus pasteuri* group II. Special physiology of the organisms. J Bacteriol 28:313–322

Gilmour R, Krulwich TA (1997) Construction and characterization of a mutant of alkaliphilic *Bacillus firmus* OF4 with a disrupted *cta* operon and purification of a novel cytochrome *bd*. J Bacteriol 179:863–870

Gilmour R, Messner P, Guffanti AA, Kent R, Scheberl A, Kendrick N, Krulwich TA (2000) Two-dimensional gel electrophoresis analyses of pH-dependent protein expression in facultatively alkaliphilic *Bacillus pseudofirmus* OF4 lead to characterization of an S-layer protein with a role in alkaliphily. J Bacteriol 182:5969–5981

Gorlenko VM, Tsapin S, Namsaraev Z, Teal T, Tourova TP, Engler D, Mielke R, Nealson K (2004) *Anaerobranca californiensis* sp. nov., an anaerobic, alkalithermophilic, fermentative bacterium isolated from a hot spring on Mono Lake. Int J Syst Evol Microbiol 54:739–743

Goto T, Matsuno T, Hishinuma-Narisawa M, Yamazaki K, Matsuyama H, Inoue N, Yumoto I (2005) Cytochrome *c* and bioenergetic hypothetical model for alkaliphilic *Bacillus* spp. J Biosci Bioeng 100:365–379

Grant WD, Tindall BJ, Herbert RA, Codd GA (1986) The alkaline saline environment. In: Microbes in extreme environments. Academic, London

Grant WD, Gerday C, Glansdorff N (2003) Alkaline environments and biodiversity. In: Extremophiles (life under extreme external conditions). Eolss, Oxford, UK, On-line publication http://www.eolss.net

Guffanti AA, Hicks DB (1991) Molar growth yields and bioenergetic parameters of extremely alkaliphilic *Bacillus* species in batch cultures, and growth in a chemostat at pH 10.5. J Gen Microbiol 137:2375–2379

Guffanti AA, Susman P, Blanco R, Krulwich TA (1978) The protonmotive force and a-aminoisobutyric acid transport in an obligately alkalophilic bacterium. J Biol Chem 253:708–715

Guffanti AA, Blanco R, Benenson RA, Krulwich TA (1980) Bioenergetic properties of alkaline-tolerant and alkalophilic strains of *Bacillus firmus*. J Gen Microbiol 119:79–86

Guffanti AA, Finkelthal O, Hicks DB, Falk L, Sidhu A, Garro A, Krulwich TA (1986) Isolation and characterization of new facultatively alkalophilic strains of *Bacillus* species. J Bacteriol 167:766–773

Gupta R, Berg QK, Lorenz P (2002) Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotechnol 59:15–32

Haines TH, Dencher NA (2002) Cardiolipin: a proton trap for oxidative phosphorylation. FEBS Lett 528:35–39

Hamamoto T, Hashimoto M, Hino M, Kitada M, Seto Y, Kudo T, Horikoshi K (1994) Characterization of a gene responsible for the Na^+/H^+ antiporter system of alkalophilic *Bacillus* species strain C-125. Mol Microbiol 14:939–946

Häse CC, Barquera B (2001) Role of sodium bioenergetics in *Vibrio cholerae*. Biochim Biophys Acta 1505:168–178

Heberle J, Dencher NA (1990) Bacteriorhodopsin in ice. Accelerated proton transfer from the purple membrane surface. FEBS Lett 277:277–280

Heberle J, Dencher NA (1992) Surface-bound optical probes monitor proton translocation and surface potential changes during the bacteriorhodopsin photocycle. Proc Natl Acad Sci USA 89:5996–6000

Hicks DB, Krulwich TA (1995) The respiratory chain of alkaliphilic bacteria. Biochim Biophys Acta 1229:303–314

Hicks DB, Liu J, Fujisawa M, Krulwich TA (2010) F_1F_0-ATP synthases of alkaliphilic bacteria: lessons from their adaptations. Biochim Biophys Acta 1797:1362–1377

Hiramatsu T, Kodama K, Kuroda T, Mizushima T, Tsuchiya T (1998) A putative multisubunit Na^+/H^+ antiporter from *Staphylococcus aureus*. J Bacteriol 180:6642–6648

Hirota N, Imae Y (1983) Na^+-driven flagellar motors of an alkalophilic *Bacillus* strain YN-1. J Biol Chem 258:10577–10581

Hirota N, Kitada M, Imae Y (1981) Flagellar motors of alkalophilic *Bacillus* are powered by an electrochemical potential gradient of Na^+. FEBS Lett 132:278–280

Horikoshi K (1996) Alkaliphiles–-from an industrial point of view. FEMS Microbiol Lett 18:259–270

Horikoshi K (1999) Alkaliphiles: some applications of their products for biotechnology. Microbiol Mol Biol Rev 63:735–750

Horikoshi K (2006) Introduction. In: Alkaliphiles, genetic properties and application of enzymes. Springer, Berlin, pp 3–5

Horikoshi K, Akiba T (1982) Alkalophilic Microorganisms. Springer, Heideberg

Horikoshi K, Bull AT (2011) Prologue: definition, categories, distribution, origin and evolution, pioneering studies and emerging fields. In: Horikoshi K, Antranikan G, Bull AT, Robb FT, Stetter KO (eds) Extremophiles handbook. Springer, Berlin, pp 4–15

Ito M, Aono R (2002) Decrease in cytoplasmic pH-homeostatic activity of the alkaliphile *Bacillus lentus* C-125 by a cell wall defect. Biosci Biotechnol Biochem 66:218–220

Ito M, Guffanti AA, Zemsky J, Ivey DM, Krulwich TA (1997) Role of the *nhaC*-encoded Na^+/H^+ antiporter of alkaliphilic *Bacillus firmus* OF4. J Bacteriol 179:3851–3857

Ito S, Kobayashi T, Ara K, Ozaki K, Kawai S, Hatada Y (1998) Alkaline detergent enzymes from alkaliphiles: enzymatic properties, genetics, and structures. Extremophiles 2:185–190

Ito M, Guffanti AA, Oudega B, Krulwich TA (1999) *mrp*, a multigene, multifunctional locus in *Bacillus subtilis* with roles in resistance to cholate and to Na^+ and in pH homeostasis. J Bacteriol 181:2394–2402

Ito M, Guffanti AA, Wang W, Krulwich TA (2000) Effects of nonpolar mutations in each of the seven *Bacillus subtilis* mrp genes suggest complex interactions among the gene products in support of Na^+ and alkali but not cholate resistance. J Bacteriol 182:5663–5670

Ito M, Hicks DB, Henkin TM, Guffanti AA, Powers B, Zvi L, Uematsu K, Krulwich TA (2004a) MotPS is the stator-force generator for motility of alkaliphilic *Bacillus* and its homologue is a second functional Mot in *Bacillus subtilis*. Mol Microbiol 53:1035–1049

Ito M, Xu H, Guffanti AA, Wei Y, Zvi L, Clapham DE, Krulwich TA (2004b) The voltage-gated Na^+ channel Na_vBP has a role in motility, chemotaxis, and pH homeostasis of an alkaliphilic *Bacillus*. Proc Natl Acad Sci USA 101:10566–10571

Ito M, Terahara N, Fujinami S, Krulwich TA (2005) Properties of motility in *Bacillus subtilis* powered by the H^+-coupled MotAB flagellar stator, Na^+-coupled MotPS or hybrid stators MotAS or MotPB J. Mol Biol 352:396–408

Ivey DM, Guffanti AA, Bossewitch JS, Padan E, Krulwich TA (1991) Molecular cloning and sequencing of a gene from alkaliphilic *Bacillus firmus* OF4 that functionally complements an *Escherichia coli* strain carrying a deletion in the *nhaA* Na^+/H^+ antiporter gene. J Biol Chem 266:23483–23489

Janto B, Ahmed A, Liu J, Hicks DB, Pagni S, Fackelmayer OJ, Smith TA, Earl J, Elbourne LDH, Hassan K, Paulsen IT, Kolsto AB, Tourasse NJ, Ehrlich GD, Boissy R, Ivey DM, Li G, Xue Y, Ma Y, Hu F, Krulwich TA (2011) The genome of alkaliphilic *Bacillus pseudofirmus* OF4 reveals adaptations that support the ability to grow in an external pH range from 7.5 to 11.4. Environ Microbiol 13(12):3289–3309

Jones BE, Grant WD, Duckworth AW, Owenson GG (1998) Microbial diversity of soda lakes. Extremophiles 2:191–200

Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Vani AA, Borgave SB, Sarnaik SS (2008) Cultivable bacterial diversity of alkaline Lonar Lake, India. Microb Ecol 55:163–172

Juarez O, Morgan JE, Nilges MJ, Barquera B (2010) Energy transducing redox steps of the Na^+-pumping NADH: quinone oxidoreductase from *Vibrio cholerae*. Proc Natl Acad Sci USA 107:12505–12510

Kajiyama Y, Otagiri M, Sekiguchi J, Kosono S, Kudo T (2007) Complex formation by the *mrpABCDEFG* gene products, which constitute a principal Na^+/H^+ antiporter in *Bacillus subtilis*. J Bacteriol 189:7511–7514

Kajiyama Y, Otagiri M, Sekiguchi J, Kudo T, Kosono S (2009) The MrpA MrpB and MrpD subunits of the Mrp antiporter complex in Bacillus subtilis contain membrane-embedded and essential acidic residues. Microbiology 155:2137–2147

Kalamorz F, Keis S, McMillan DGG, Olsson K, Stanton J-A, Stockwell P, Black MA, Klingeman DM, Land ML, Han CS, Martin SL, Becher SA, Peddie CJ, Morgan HW, Matthies D, Preiss L, Meier T, Brown SD, Cook GM (2011) Draft genome sequence of the thermoalkaliphilic *Caldalkalibacillus thermarum* strain TA2.A1. J Bacteriol 193:4290–4291

Kapetaniou EG, Thanassoulas A, Dubnovitsky AP, Nounesis G, Papageorgiou AC (2006) Effect of pH on the structure and stability of *Bacillus circulans* ssp. *alkalophilus* phosphoserine aminotransferase: thermodynamic and crystallographic studies. Proteins 63:742–753

Kennedy SP, Ng WV, Salzberg SL, Hood L, DasSarma S (2001) Understanding the adaptation of *Halobacterium* species NRC-1 to its extreme environment through computational analysis of its genome sequence. Genome Res 11:1641–1650

Kerscher S, Dröse S, Zickermann V, Brandt U (2008) The three families of respiratory NADH dehydrogenases results. Probl Cell Differ 45:185–222

Kevbrin VV, Zhilina TN, Rainey FA, Zavarzin GA (1998) *Tindallia magadii* gen. nov., sp. nov.: an alkaliphilic anaerobic ammonifier from soda lake deposits. Curr Microbiol 37:94–100

Kevbrin VV, Romanek CS, Wiegel J (2003) Alkalithermophiles: a double challenge from extreme environments. In: Seckbach J (ed) Cellular origins, life in extreme habitats and astrobiology (COLE). Kluwer, Dordrecht

Khaneja R, Perez-Fons L, Fakhry S, Baccigalupi L, Steiger S, To E, Sandmann G, Dong TC, Ricca E, Fraser PD, Cutting SM (2009) Carotenoids found in *Bacillus*. J Appl Microbiol 108:1889–1902

Knight CG, Kassen R, Hebestreit H, Rainey PB (2004) Global analysis of predicted proteomes: functional adaptation of physical properties. Proc Natl Acad Sci USA 101:8390–8395

Kobayashi T, Hatada Y, Higaki N, Lusterio DD, Ozawa T, Koike K, Kawai S, Ito S (1999) Enzymatic properties and deduced amino acid sequence of a high-alkaline pectate layse from an alkaliphilic *Bacillus* isolate. Biochim Biophys Acta 1427:145–154

Koishi R, Xu H, Ren D, Navarro B, Spiller BW, Shi Q, Clapham DE (2004) A superfamily of voltage-gated sodium channels in bacteria. J Biol Chem 279:9532–9538

Koyama N (1989a) Ammonium-dependent transports of amino acids and glucose in a facultatively anaerobic alkalophile. FEBS Lett 253:187–189

Koyama N (1989b) Characterization of the membrane-bound ATPase from a facultatively anaerobic alkalophile. Biochim Biophys Acta 980:255–259

Koyama N (1993) Stimulatory effect of NH_4^+ on the transport of leucine and glucose in an anaerobic alkaliphile. Eur J Biochem 217:435–439

Koyama N (1996) NH_4^+ + Na^+-activated ATPase of a facultatively anaerobic alkaliphile, Amphibacillus xylanus. Anaerobe 2:103–109

Koyama N, Kiyomiya A, Nosoh Y (1976) Na^+-dependent uptake of amino acids by an alkalophilic *Bacillus*. FEBS Lett 72:77–78

Krulwich TA (1995) Alkaliphiles: 'basic' molecular problems of pH tolerance and bioenergetics. Mol Microbiol 15:403–410

Krulwich TA, Guffanti AA (1989) The Na^+ cycle of extreme alkalophiles: a secondary Na^+/H^+ antiporter and Na^+/solute symporters. J Bioenerg Biomembr 21:663–677

Krulwich TA, Ivey DM (1990) Bioenergetics of extreme environments. In: Krulwich TA (ed) Bacterial energetics, vol 12. Academic, New York, pp 417–447

Krulwich TA, Federbush JG, Guffanti AA (1985) Presence of a nonmetabolizable solute that is translocated with Na^+ enhances Na^+-dependent pH homeostasis in an alkalophilic *Bacillus*. J Biol Chem 260:4055–4058

Krulwich TA, Hicks DB, Swartz TH, Ito M (2007) Bioenergetic adaptations that support alkaliphily. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 311–329

Krulwich TA, Hicks DB, Ito M (2009) Cation/proton antiporter complements of bacteria: why so large and diverse? Mol Microbiol 74:257–260

Krulwich TA, Liu J, Morino M, Fujisawa M, Ito M, Hicks D (2011a) Adaptive mechanisms of extreme alkaliphiles. In: Horikoshi K, Antranikan G, Bull A, Robb FT, Stetter K (eds) Extremophiles handbook. Springer, Heidelberg, pp 120–139

Krulwich TA, Sachs G, Padan E (2011b) Molecular aspects of bacterial pH sensing and homeostasis. Nat Rev Microbiol 9:330–343

Kudo T, Hino M, Kitada M, Horikoshi K (1990) DNA sequences required for the alkalophily of *Bacillus* sp. strain C-125 are located close together on its chromosomal DNA. J Bacteriol 172:7282–7283

Kurono Y, Horikoshi K (1973) Alkaline catalase produced by *Bacillus* No. Ku-1. Agric Biol Chem 37:2565–2570

Lau WD, Rubinstein JL (2010) Structure of intact *Thermus thermophilus* V-ATPase by cryo-EM reveals organization of the membrane-bound V_o motor. Proc Natl Acad Sci USA 107:1367–1372

Lefevre CT, Frankel RB, Posfai M, Prozorov T, Bazylinski DA (2011) Isolation of obligately alkaliphilic magnetotactic bacteria from extremely alkaline environments. Environ Microbiol 13(8):2342–2350. doi:10.1111/j.1462-2910.2011.02505.x

Leone V, Krah A, Faraldo-Gómez JD (2010) On the question of hydronium binding to ATP-synthase membrane rotors. Biophys J 99:L53–L55

Lewis RJ, Prince RC, Dutton PL, Knaff DB, Krulwich TA (1981) The respiratory chain of *Bacillus alcalophilus* and its nonalkalophilic mutant derivative. J Biol Chem 256:10543–10549

Li Y, Mandelco L, Wiegel J (1993) Isolation and characterization of a moderately thermophilic anaerobic alkaliphile, *Clostridium paradoxum* sp. nov. Int J Syst Bacteriol 43:450–460

Li Y, Engle M, Weiss N, Mandelco L, Wiegel J (1994) *Clostridium thermoalcaliphilum* sp. nov., an anaerobic and thermotolerant facultative alkaliphile. Int J Syst Bacteriol 44:111–118

Liu X, Gong X, Hicks DB, Krulwich TA, Yu L, Yu CA (2007) Interaction between cytochrome caa_3 and F_1F_0-ATP synthase of alkaliphilic *Bacillus pseudofirmus* OF4 is demonstrated by saturation transfer electron paramagnetic resonance and differential scanning calorimetry assays. Biochemistry 46:306–313

Liu J, Fujisawa M, Hicks DB, Krulwich TA (2009) Characterization of the functionally critical AXAXAXA and PXXEXXP motifs of the ATP synthase *c*-subunit from an alkaliphilic *Bacillus*. J Biol Chem 284:8714–8725

Liu J, Fackelmayer OJ, Hicks DB, Preiss L, Meier T, Sobie EA, Krulwich TA (2011) Mutations in a helix-1 motif of the ATP synthase *c*-subunit of *Bacillus pseudofirmus* OF4 cause functional deficits and changes in *c*-ring stability and mobility on SDS-PAGE. Biochemistry 50:5497–5506

Macnab RM, Castle AM (1987) A variable stoichiometry model for pH homeostasis in bacteria. Biophys J 52:637–647

Mandel KG, Guffanti AA, Krulwich TA (1980) Monovalent cation/proton antiporters in membrane vesicles from *Bacillus alcalophilus*. J Biol Chem 255:7391–7396

Mathiesen C, Hägerhäll C (2002) Transmembrane topology of the NuoL, M and N subunits of NADH: quinone oxidoreductase and their homologues among membrane-bound hydrogenases and bona fide antiporters. Biochim Biophys Acta 1556:121–132

Mathiesen C, Hägerhäll C (2003) The 'antiporter module' of respiratory chain Complex I includes the MrpC/NuoK subunit – a revision of the modular evolution scheme. FEBS Lett 5459:7–13

McMillan DG, Keis S, Dimroth P, Cook GM (2007) A specific adaptation in the a subunit of thermoalkaliphilic F_1F_O-ATP synthase enables ATP synthesis at high pH but not at neutral pH values. J Biol Chem 282:17395–17404

McMillan DG, Vetasquez I, Nunn BL, Goodlett DR, Hunter KA, Lamont I, Sander SG, Cook GM (2010) Acquisition of iron by alkaliphilic bacillus species. Appl Environ Microbiol 76:6955–6961

Meier T, Polzer P, Diederichs K, Welte W, Dimroth P (2005) Structure of the rotor ring of F-Type Na^+-ATPase from *Ilyobacter tartaricus*. Science 308:659–662

Meier T, Ferguson SA, Cook GM, Dimroth P, Vonck J (2006) Structural investigations of the membrane-embedded rotor ring of the F-ATPase from *Clostridium paradoxum*. J Bacteriol 188:7759–7764

Meier T, Morgner N, Matthies D, Pogoryelov D, Keis S, Cook GM, Dimroth P, Brutschy B (2007) A tridecameric c ring of the adenosine triphosphate (ATP) synthase from the thermoalkaliphilic *Bacillus* sp. strain TA2.A1 facilitates ATP synthesis at low electrochemical proton potential. Mol Microbiol 65:1181–1192

Mesbah NM, Wiegel J (2008) Life at extreme limits: the anaerobic halophilic alkalithermophiles. Ann N Y Acad Sci 1125:44–57

Mesbah NM, Wiegel J (2011) The Na^+-translocating F_1F_o-ATPase from the halophilic, alkalithermophile *Natranaerobius thermophilus*. Biochim Biophys Acta. doi:10.1016/j.bbabio.2011.001

Mesbah NM, Abou-El-Ela SH, Wiegel J (2007) Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi An Natrun Egypt. Microb Ecol 54:598–617

Mesbah N, Cook G, Wiegel J (2009) The halophilic alkalithermophile *Natranaerobius thermophilus* adapts to multiple environmental extremes using a large repertoire of $Na^+(K^+)/H^+$ antiporters. Mol Microbiol 74:270–281

Miethke M, Pierik AJ, Peuckert F, Seubert A, Marahiel MA (2011) Identification and characterization of a novel-type ferric siderophore reductase from a gram-positive extremophile. J Biol Chem 286:2245–2260

Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. Nature 191:144–148

Morino M, Natsui S, Swartz TH, Krulwich TA, Ito M (2008) Single gene deletions of *mrpA* to *mrpG* and *mrpE* point mutations affect activity of the Mrp Na^+/H^+ antiporter of alkaliphilic *Bacillus* and formation of hetero-oligomeric Mrp complexes. J Bacteriol 190:4162–4172

Morino M, Natsui S, Ono T, Swartz TH, Krulwich TA, Ito M (2010) Single site mutations in the hetero-oligomeric Mrp antiporter from alkaliphilic *Bacillus pseudofirmus* OF4 that affect Na^+/H^+ antiport activity, sodium exclusion, individual Mrp protein levels or Mrp complex formation. J Biol Chem 285:30942–30950

Mulkidjanian AY, Cherepanov DA, Heberle J, Junge W (2005) Proton transfer dynamics at membrane/water interface and mechanism of biological energy conversion. Biochemistry (Mosc) 70:251–256

Mulkidjanian AY, Heberle J, Cherepanov DA (2006) Protons @ interfaces: implications for biological energy conservation. Biochim Biophys Acta 1757:913–930

Mulkidjanian AY, Dibrov P, Galperin MY (2008) The past and present of sodium energetics: may the sodium-motive force be with you. Biochim Biophys Acta 1777:985–992

Muntyan MS, Bloch DA (2008) Study of redox potential in cytochrome c covalently bound to terminal oxidase of alkaliphilic Bacillus pseudofirmus FTU. Biochemistry (Mosc) 73:107–111

Muyzer G, Sorokin DY, Mavromatis K, Lapidus A, Clum A, Ivanova N, Pati A, d'Haeseleer P, Woyke T, Kyrpides NC (2011) Complete genome sequence of "*Thioalkalivibrio sulfidophilus*" HL-EbGr7 stand. Genomic Sci 4:23–35

Niimura Y, Yanagida T, Uchimura T, Ohara N, Suzuki K, Kozaki M (1987) A new facultative anaerobic xylan-using alkalophile lacking cytochrome, quinone, and catalase. Agric Biol Chem 51:2271–2275

Niimura Y, Koh E, Uchimura T, Ohara N, Kozaki M (1989) Aerobic and anaerobic metabolism in a facultative anaerobe Ep01 lacking cytochrome, quinone and catalase. FEMS Microbiol Lett 61:70–84

Niimura Y, Poole LB, Massey V (1995) *Amphibacillus xylanus* NADH oxidase and *Salmonella typhimurium* alkyl-hydroperoxide reductase flavoprotein components show extremely high scavenging activity for both alkyl hydroperoxide and hydrogen peroxide in the presence of *S. typhimurium* alkyl-hydroperoxide reductase 22-kDa protein component. J Biol Chem 270:25645–25650

Okuno D, Iino R, Noji H (2011) Rotation and structure of F_1F_o-ATP synthase. J Biochem 149:655–664

Olguin-Lora P, Le Borgne S, Castorena-Cortes G, Roldan-Carrillo T, Zapata-Penasco I, Reyes-Avila J, Alcantara-Perez S (2011) Evaluation of haloalkaliphilic sulfur-oxidizing microorganisms with potential application in the effluent treatment of the petroleum industry. Biodegradation 22:83–93

Olsson K, Keis S, Morgan HW, Dimroth P, Cook GM (2003) Bioenergetic properties of the thermoalkaliphilic *Bacillus* sp. strain TA2.A1. J Bacteriol 185:461–465

Oren A (2010) Thermodynamic limits to microbial life at high salt concentrations. Environ Microbiol 13:1908–1923

Padan E, Schuldiner S (1986) Intracellular pH regulation in bacterial cells. Methods Enzymol 125:337–352

Padan E, Bibi E, Ito M, Krulwich TA (2005) Alkaline pH homeostasis in bacteria: new insights. Biochim Biophys Acta 1717:67–88

Payandeh J, Scheuer T, Zheng N, Catterall WA (2011) The crystal structure of a voltage-gated sodium channel. Nature 475:353–358

Perez-Fons L, Steiger S, Khaneja R, Bramley PM, Cutting SM, Sandmann G, Fraser PD (2011) Identification and developmental formation of carotenoid pigments in the yellow/orange *Bacillus* spore-formers. Biochim Biophys Acta 1811:177–185

Pogoryelov D, Sudhir PR, Kovacs L, Gombos Z, Brown I, Garab G (2003) Sodium dependency of the photosynthetic electron transport in the alkaliphilic cyanobacterium *Arthrospira platensis*. J Bioenerg Biomembr 35:427–437

Pogoryelov D, Yu J, Meier T, Vonck J, Dimroth P, Müller DJ (2005) The c_{15} ring of the *Spirulina platensis* F-ATP synthase: F_1/F_0 symmetry mismatch is not obligatory. EMBO Rep 6:1040–1044

Pogoryelov D, Yildiz Ö, Faraldo-Gómez JD, Meier T (2009) High-resolution structure of the rotor ring of a proton-dependent ATP synthase. Nat Struct Mol Biol 16:1068–1073

Preiss L, Yildiz Ö, Hicks D, Krulwich TA, Meier T (2010) A new type of proton coordination in an F_1F_0-ATP synthase rotor ring. PLoS Biol 8:e1000443

Prowe SG, van de Vossenberg JL, Driessen AJ, Antranikian G, Konings WN (1996) Sodium-coupled energy transduction in the newly isolated thermoalkaliphilic strain LBS3. J Bacteriol 178:4099–4104

Rabus R, Jack DL, Kelly DJ, Saier MH Jr (1999) TRAP transporters: an ancient family of extracytoplasmic solute-receptor-dependent secondary active transporters. Microbiology 145:3431–3445

Rees HC, Grant WD, Jones BE, Heaphy S (2004) Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. Extremophiles 8:63–71

Ren D, Navarro B, Xu H, Yue L, Shi Q, Clapham DE (2001) A prokaryotic voltage-gated sodium channel. Science 294:2372–2375

Roadcap GS, Sanford RA, Jin Q, Pardinas JR, Bethke CM (2006) Extremely alkaline (pH > 12) ground water hosts diverse microbial community. Ground Water 44:511–517

Ruis N, Loren JG (1998) Buffering capacity and membrane H^+ conductance of neutrophilic and alkalophilic gram-positive bacteria. Appl Environ Microbiol 64:1344–1349

Saeki K, Ozaki K, Kobayashi T, Ito S (2007) Detergent alkaliphile proteases: enzymatic properties, genes, and crystal structures. J Biosci Bioeng 103:501–508

Saier MH Jr, Tran CV, Barabote RD (2006) TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic Acids Res 34:D181–D186

Sarethy IP, Saxena Y, Kapoor A, Sharma M, Sharma SK, Gupta V, Gupta S (2011) Alkaliphilic bacteria: applications in industrial biotechnology. J Ind Microbiol Biotechnol 38:769–790

Schwartz R, Ting CS, King J (2001) Whole proteome pI values correlate with subcellular localizations of proteins for organisms within the three domains of life. Genome Res 11:703–709

Seelert H, Poetsch A, Dencher NA, Engel A, Stahlberg H, Müller DJ (2000) Proton-powered turbine of a plant motor. Nature 405:418–419

Seelert H, Dencher NA, Müller DJ (2003) Fourteen protomers compose the oligomer III of the proton-rotor in spinach chloroplast ATP synthase. J Mol Biol 333:337–344

Selivanov VA, Zeak JA, Roca J, Cascante M, Trucco M, Votyakova TV (2008) The role of external and matrix pH in mitochondrial reactive oxygen species generation. J Biol Chem 283:29292–29300

Shioi JI, Imae Y, Oosawa F (1978) Protonmotive force and motility of *Bacillus subtilis*. J Bacteriol 133:1083–1088

Shirai T, Suzuki A, Yamane T, Ashida T, Kobayashi T, Hitomi J, Ito S (1997a) High-resolution crystal structure of M-protease: phylogeny aided analysis of the high-alkaline adaptation mechanism. Protein Eng 10:627–634

Shirai T, Yamane T, Hidaka T, Kuyama K, Suzuki A, Ashida T, Ozaki K, Ito S (1997b) Crystallization and preliminary X-ray analysis of a truncated family A alkaline endoglucanase isolated from Bacillus sp. KSM-635. J Biochem 122:683–685

Shirai T, Ishida H, Noda J, Yamane T, Ozaki K, Hakamada Y, Ito S (2001) Crystal structure of alkaline cellulase K: insight into the alkaline adaptation of an industrial enzyme. J Mol Biol 310:1079–1087

Shirai T, Igarashi K, Ozawa T, Hagihara H, Kobayashi T, Ozaki K, Ito S (2007) Ancestral sequence evolutionary trace and crystal structure analyses of alkaline a-amylase from *Bacillus* sp. KSM-1378 to clarify the alkaline adaptation process of proteins. Proteins 66:600–610

Skulachev VP (1989a) Bacterial Na^+ energetics. FEBS Lett 250:106–114

Skulachev VP (1989b) The sodium cycle: a novel type of bacterial energetics. J Bioenerg Biomembr 21:635–647

Skulachev VP (1992) The laws of cell energetics. Eur J Biochem 208:203–209

Slonczewski JL, Fujisawa M, Dopson M, Krulwich TA (2009) Cytoplasmic pH measurement and homeostasis in bacteria and archaea. Adv Microb Physiol 55:1–79

Sobek H, Hecht J-J, Aehle W, Schomburg D (1992) X-ray structure determination and comparison of two crystal forms of a variant (Asn115Arg) of the alkaline protease from *Bacillus alcalophilus* refined at 1.85 A resolution. J Mol Biol 228:108–117

Sorokin DY, Kuenen JG (2005a) Chemolithotrophic haloalkaliphiles from soda lakes. FEMS Microbiol Ecol 52:287–295

Sorokin DY, Kuenen JG (2005b) Haloalkaliphilic sulfur-oxidizing bacteria in soda lakes. FEMS Microbiol Rev 29:685–702

Sorokin DY, Cherepanov A, de Vries S, Kuenen GJ (1999) Identification of cytochrome c oxidase in the alkaliphilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium 'Thioalcalomicrobium aerophilum' strain AL 3. FEMS Microbiol Lett 179:91–99

Sorokin DY, van den Bosch PL, Abbas B, Janssen AJ, Muyzer G (2008) Microbiological analysis of the population of extremely haloalkaliphilic sulfur-oxidizing bacteria dominating in lab-scale sulfide-removing bioreactors. Appl Microbiol Biotechnol 80:965–975

Sorokin DY, Kuenen JG, Muyzer G (2011) The microbial sulfur cycle at extremely haloalkaline conditions of soda lakes. Front Microbiol 2:44

Steed PR, Fillingame RH (2009) Aqueous accessibility to the transmembrane regions of subunit c of the *Escherichia coli* F_1F_0 ATP synthase. J Biol Chem 284:23243–23250

Sturr MG, Guffanti AA, Krulwich TA (1994) Growth and bioenergetics of alkaliphilic *Bacillus firmus* OF4 in continuous culture at high pH. J Bacteriol 176:3111–3116

Suigyama S, Matsukura H, Koyama N, Nosoh Y, Imae Y (1986) Requirement of Na^+ in flagellar rotation and amino acid transport in a facultatively alkalophilic *Bacillus*. Biochim Biophys Acta 852:38–45

Sunna A, Prowe SG, Stoffregen T, Antranikian G (1997) Characterization of the xylanases from the newly isolated thermophilic xylan-degrading *Bacillus thermoleovorans* strain K-3d and *Bacillus flavothermus* strain LB3A. FEMS Microbiol Lett 148:209–216

Swartz TH, Ikewada S, Ishikawa O, Ito M, Krulwich TA (2005) The Mrp system: a giant among monovalent cation/proton antiporters? Extremophiles 9:345–354

Takahara Y, Tanabe O (1962) Studies on the reduction of indigo in industrial fermentation vat (XIX). Taxonomic characteristics of strain No. S-8. J Ferment Technol 40:77–80

Takahara Y, Takahashi Y, Tanabe O (1961) Studies on the reduction of indigo in industrial fermentation vat (XVII). On the growth factor of strain No. S-8. J Ferment Technol 39:183–187

Takami H, Kobata K, Nagahama T, Kobayashi H, Inoue A, Horikoshi K (1999) Biodiversity in deep-sea sites located near the south part of Japan. Extremophiles 3:97–102

Takami H, Nakasone K, Takaki Y, Maeno G, Sasaki R, Masui N, Fuji F, Hirama C, Nakamura Y, Ogasawara N, Kuhara S, Horikoshi K (2000) Complete genome sequence of the alkaliphilic bacterium *Bacillus halodurans* and genomic sequence comparison with *Bacillus subtilis*. Nucleic Acids Res 28:4317–4331

Takami H, Han CG, Takaki Y, Ohtsubo E (2001) Identification and distribution of new insertion sequences in the genome of alkaliphilic *Bacillus halodurans* C-125. J Bacteriol 183:4345–4356

Takimura Y, Saito K, Okuda M, Kageyama Y, Katsuhisa S, Ozaki K, Ito S, Kobayashi T (2007) Alkaliphilic *Bacillus* sp. strain KSM-LD1 contains a record number of subtilisin-like proteases genes. Appl Microbiol Biotechnol 76:395–405

Terahara N, Krulwich TA, Ito M (2008) Mutations alter the sodium versus proton use of a *Bacillus clausii* flagellar motor and confer dual ion use on *Bacillus subtilis* motors. Proc Natl Acad Sci USA 105:14359–14364

Thongaram T, Kosono S, Ohkuma M, Hongoh Y, Kitada M, Yoshinaka T, Trakulnaleamsai S, Noparatnaraporn N, Kudo T (2003) Gut of higher termites as a niche for alkaliphiles as shown by culture-based and culture-independent studies. Microbes Environ 18:152–159

Thongaram T, Hongoh Y, Kosono S, Ohkuma M, Trakulnaleamsai S, Noparatnaraporn N, Kudo T (2005) Comparison of bacterial communities in the alkaline gut segment among various species of higher termites. Extremophiles 9:229–238

Tomb J-F, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty GA, Nelson K, Quackenbush J, Zhou L, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak GG, Glodek A, McKenney K, Fitzegerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Wedman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 388:539–547

Tsuchiya K, Nakamura Y, Sakshita H, Kimura T (1992) Purification and characterization of a thermostable alkaline protease from alkalophilic *Thermoactinomyces* sp. HS682. Biosci Biotechnol Biochem 56:289–303

Valiyaveetil FI, Fillingame RH (1998) Transmembrane topography of subunit *a* in the *Escherichia coli* F_1F_0 ATP synthase. J Biol Chem 273:16241–16247

van der Laan JC, Gerritse G, Mulleners LJ, van der Hoek RA, Quax WJ (1991) Cloning, characterization, and multiple chromosomal integration of a *Bacillus* alkaline protease gene. Appl Environ Microbiol 57:901–909

van der Laan JM, Teplyakov AV, Kelders H, Kalk KH, Misset O, Mulleners JSM, Dijkstra BW (1992) Crystal structure of the high-alkaline serine protease PB92 from *Bacillus alcalophilus*. Protein Eng 5:405–411

Vedder A (1934) *Bacillus alcalophilus* n. sp. benevens enkele ervaringen met sterk alcalische voedingsbodems Ant. v. Leeuwenhoek. J Microbiol Serol 1:141–147

von Ballmoos C, Cook GM, Dimroth P (2008) Unique rotary ATP synthase and its biological diversity. Annu Rev Biophys 37:43–64

von Ballmoos C, Wiedenmann A, Dimroth P (2009) Essentials for ATP synthesis by F_1F_0 ATP synthases. Annu Rev Biochem 78:649–672

Wang Z, Hicks DB, Guffanti AA, Baldwin K, Krulwich TA (2004) Replacement of amino acid sequence features of *a*- and *c*-subunits of ATP synthases of alkaliphilic *Bacillus* with the *Bacillus* consensus sequence results in defective oxidative phosphorylation and non-fermentative growth at pH 10.5. J Biol Chem 279:26546–26554

Wang Q, Han H, Xue Y, Qian Z, Meng B, Peng F, Wnag Z, Tong W, Zha C, Wang Q, Guo Y, Li G, Liu S, Ma Y (2009) Exploring membrane and cytoplasm proteomic response of *Alkalimonas amylolytica* N10 to different external pHs with combination strategy of *de novo* peptide sequencing. Proteomics 9:1254–1273

Watmough NJ, Frerman FE (2010) The electron transfer flavoprotein: ubiquinone oxidoreductases. Biochim Biophys Acta 1797:1910–1916

Watt IN, Montgomery MG, runswick MJ, Leslie AG, Walker JE (2010) Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria. Proc Natl Acad Sci USA 107:16823–16827

Wei Y, Guffanti AA, Krulwich TA (1999) Sequence analysis and functional studies of a chromosomal region of alkaliphilic *Bacillus firmus* OF4 encoding an ABC-type transporter with similarity of sequence and Na^+ exclusion capacity to the *Bacillus subtilis* NatAB transporter. Extremophiles 3:113–120

Wei Y, Liu J, Ma Y, Krulwich TA (2007) Three putative cation/proton antiporters from the soda lake alkaliphile *Alkalimonas amylolytica* N10 complement an alkali-sensitive *Escherichia coli* mutant. Microbiology 153:2168–2179

Wiegel J (1998) Anaerobic alkalithermophiles, a novel group of extremophiles. Extremophiles 2:257–267

Wiegel J, Kevbrin VV (2004) Alkalithermophiles. Biochem Soc Trans 32:193–198

Wiegert T, Homuth G, Versteeg S, Schumann W (2001) Alkaline shock induces the *Bacillus subtilis* σ^W regulon. Mol Microbiol 41:59–71

Williams RJ (1978) The multifarious couplings of energy transduction Biochim. Biophys Acta 505:1–44

Ye Q, Roh Y, Carroll SL, Blair B, Zhou J, Zhang CL, Fields MW (2004) Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. Appl Environ Microbiol 70:5595–5602

Yumoto I (2007) Environmental and taxonomic biodiversities of Gram-positive alkaliphiles. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 295–310

Yumoto I, Yamazaki K, Hishinuma M, Nodasaka Y, Inoue N, Kawasaki K (2000) Identification of facultatively alkaliphilic *Bacillus* sp. strain YN-2000 and its fatty acid composition and cell-surface aspects depending on culture pH. Extremophiles 4:285–290

Yumoto I, Nakamura A, Iwata H, Kojima K, Kusumoto K, Nodasaka Y, Matsuyama H (2002) *Dietzia psychralcaliphila* sp. nov., a novel, facultatively psychrophilic alkaliphile that grows on hydrocarbons. Int J Syst Evol Microbiol 52:85–90

Yumoto I, Hirota K, Nodasaka Y, Yokota Y, Hoshino T, Nakajima N (2004) *Alkalibacterium psychrotolerans* sp. nov., a psychrotolerant obligate alkaliphile that reduces an indigo dye. Int J Syst Evol Microbiol 54:2379–2383

Zavarzin GA (1993) Epicontinental soda lakes are probable relict biotopes of terrestrial biota formation. Microbiology 62:473–479

Zhang H-M, Li Z, Tsudome M, Ito S, Takami H, Horikoshi K (2005) An alkali-inducible flotillin-like protein from *Bacillus halodurans* C125. Protein J 24:125–131

Zhao B, Mesbah NM, Dalin E, Goodwin LA, Nolan M, Pitluck S, Cherkov O, Brettin TS, Han J, Larimer FW, Land ML, Hauser LJ, Kyrpides N,

Wiegel J (2011) Complete genome sequence of the anaerobic, halophilic alkalithermophile *Natranaerobius thermophilus* JW/NM-WN-LF. J Bacteriol 193:4023–4024

Zhilina TN, Zavarzin GA, Rainey FA, Pikuta EN, Osipov GA, Kostrikina NA (1997) *Desulfonatronovibrio hydrogenovorans* gen. mov., sp. nov., an alkaliphilic sulfate-reducing bacterium. Int J Syst Bacteriol 47:144–149

Zhilina TN, Appel R, Probian C, Brossa EL, Harder J, Widdel F, Zavarzin GA (2004) *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. Arch Microbiol 182:244–253

Zhilina TN, Kevrin W, Turova TP, Lysenko AM, Kostrikina NA, Zavarzin GA (2005) *Clostridium alkalicellum* sp. nov., an obligately alkaliphilic cellulolytic bacterium from a soda lake in the Baikal region. Mikrobiologia 74:642–653

Zilberstein D, Schuldiner S, Padan E (1979) Proton electrochemical gradient in *Escherichia coli* cells and its relation to active transport of lactose. Biochemistry 18:669–673

21 Syntrophism Among Prokaryotes

Bernhard Schink[1] · *Alfons J. M. Stams*[2]
[1]Department of Biology, University of Konstanz, Constance, Germany
[2]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

Introduction: Concepts of Cooperation in Microbial Communities, Terminology

The study of pure cultures in the laboratory has provided an amazingly diverse diorama of metabolic capacities among microorganisms and has established the basis for our understanding of key transformation processes in nature. Pure culture studies are also prerequisites for research in microbial biochemistry and molecular biology. However, desire to understand how microorganisms act in natural systems requires the realization that microorganisms do not usually occur as pure cultures out there but that every single cell has to cooperate or compete with other micro- or macroorganisms. The pure culture is, with some exceptions such as certain microbes in direct cooperation with higher organisms, a laboratory artifact. Information gained from the study of pure cultures can be transferred only with great caution to an understanding of the behavior of microbes in natural communities. Rather, a detailed analysis of the abiotic and biotic life conditions at the microscale is needed for a correct assessment of the metabolic activities and requirements of a microbe in its natural habitat.

Most aerobic bacteria can degrade even fairly complex substrates to water and carbon dioxide without any significant cooperation with other organisms. Nutritional cooperation may exist but may be restricted to the transfer of minor growth factors, such as vitamins, from one organism to the other. However, we have to realize that this assumption is based on experience gained from pure cultures that were typically enriched and isolated in simple media, and the selection aimed at organisms that were easy to handle, independent of possible interactions with others. Estimations assume that we know only a small fraction of the microorganisms present in nature, perhaps 0.1–1.0 %. Thus, we cannot exclude that other bacteria out there might depend to a large extent on cooperation with partner microbes, and perhaps this is just one of the reasons why we failed so far to isolate them.

Anaerobic microorganisms, on the other hand, depend to a great extent on the cooperation of several metabolic types of bacteria in feeding chains. The complete conversion of complex organic matter, e.g., cellulose, to methane and carbon dioxide in a lake sediment is catalyzed by the concerted action of at least four different metabolic groups of bacteria, including primary fermenters, secondary fermenters, and at least two types of methanogenic archaea (Bryant 1979; McInerney 1988; Stams 1994; Schink 1991, 1997; Stams and Plugge 2009). The degree of mutual dependence among these different metabolic groups ("functional guilds") can vary considerably; whereas the latter

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_59,

members in the feeding line always depend on the former ones for substrate supply, they may also influence significantly the former chain members by removal of metabolic products. In an extreme case, this can mean that the fermenting bacterium depends entirely on cooperation with a methanogen to fulfill its function in, e.g., methanogenic fatty acid oxidation. This type of cooperation is called "syntrophic."

Mutual metabolic dependencies also can emerge from the cooperation of phototrophs with sulfur- or sulfate-reducing bacteria. Sulfur-reducing, acetate-oxidizing, chemotrophic bacteria such as *Desulfuromonas acetoxidans* and phototrophic green sulfide-oxidizing bacteria like *Chlorobium* sp. can cooperate closely in a phototrophic conversion of acetate plus CO_2 to bacterial cell mass using a sulfide/sulfur cycle as an electron shuttle system between both. The two partners cooperate very closely also in this system for which the term "syntrophy" was originally coined (Biebl and Pfennig 1978).

Syntrophy is a special case of symbiotic cooperation between two metabolically different types of bacteria which depend on each other for degradation of a certain substrate, typically through transfer of one or more metabolic intermediate(s) between the partners. The pool size of the shuttling intermediate has to be kept low to allow efficient cooperation.

The term "syntrophy" should be restricted to those cooperations in which partners depend on each other to perform the metabolic activity observed and in which the mutual dependence cannot be overcome by simply adding a cosubstrate or any type of nutrient. A classical example is the *Methanobacillus omelianskii* culture (Barker 1940), which was later shown to be a coculture of two partner organisms, the S-strain and the strain M.o.H. (Bryant et al. 1967). Both strains cooperate in the conversion of ethanol to acetate and methane by interspecies hydrogen transfer, as follows:

Strain S:

$$2CH_3CH_2OH + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 4H_2$$

$$\Delta G_0' = +19\,\text{kJ per 2 mol of ethanol}$$

Strain M.o.H.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$\Delta G_0' = -131\,\text{kJ per mol of methane}$$

Coculture:

$$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COO^- + 3H^+ + CH_4$$

$$\Delta G_0' = -112\,\text{kJ per mol of methane}$$

Thus, the fermenting bacterium cannot be grown with ethanol in the absence of the hydrogen-scavenging partner organism because it carries out a reaction that is endergonic under standard conditions. The first reaction can occur and provide energy for the first strain only if the hydrogen partial pressure is kept low enough ($>10^{-3}$ bar) by the methanogen. Therefore, neither partner can grow with ethanol alone, and the degradation of ethanol depends on the cooperating activities of both.

We avoid in this article the term "consortium" which is quite often used to describe any kind of enrichment cultures cooperating in whatever way. This term was originally coined for the structured phototrophic aggregates *Pelochromatium* and *Chlorochromatium*, etc., and should be restricted to such spatially well-organized systems (Pfennig 1980; Overmann 2002).

Electron Flow in Methanogenic and Sulfate-Dependent Degradation

The degradation of complex organic matter to methane and CO_2 is a process widespread in anoxic environments which receive only a limited supply of oxygen, nitrate, sulfate, or oxidized iron or manganese species. Methanogenesis is the typical terminal electron-accepting process in freshwater sediments rich in organic matter, in swamps or waterlogged soils such as rice paddies, or in anaerobic wastewater and sewage treatment plants. It is also an important process in fermentations occurring in the intestinal tract of animals, especially of ruminants. Methanogenic degradation is the least exergonic process in comparison to aerobic degradation or the alternative anaerobic respiration. Conversion of hexose to methane and carbon dioxide releases only 15% of the energy that would be available in aerobic degradation, and this small energy yield of methanogenic degradation may be the reason why methanogenesis is the last one to occur, after the other electron acceptors have been reduced.

The carbon and electron flow in methanogenic degradation of complex organic matter follows a rather simple pattern. Polymers (polysaccharides, proteins, nucleic acids, and also lipids) are first converted to oligo- and monomers (sugars, amino acids, purines, pyrimidines, fatty acids, and glycerol), typically through the action of extracellular hydrolytic enzymes. These enzymes are produced by the "classical" primary fermenting bacteria which ferment the monomers further to fatty acids, branched-chain fatty acids, succinate, lactate, alcohols, aromatic acids, etc. (group 1; ❯ *Fig. 21.1*). Some of these fermentation products, such as acetate, H_2, CO_2, and other one-carbon compounds, can be used directly by methanogens which convert them to methane and carbon dioxide (groups 2 and 3; ❯ *Fig. 21.1*). For methanogenic degradation of other fermentation products, e.g., fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, branched-chain and aromatic fatty acids, a further group of fermenting bacteria, the so-called secondary fermenters or obligate proton reducers (group 4; ❯ *Fig. 21.1*), is needed. These bacteria convert their substrates to acetate, carbon dioxide, hydrogen, perhaps also formate, which are subsequently used by the methanogens.

The situation is slightly different in sulfate-rich anoxic habitats such as marine sediments. There, the primary processes of polymer degradation are carried out by primary fermenting bacteria which form the classical fermentation products. Different from methanogens, sulfate-reducing bacteria are metabolically versatile, and a broad community of sulfate reducers can use all products of primary fermentations, and oxidize them to carbon dioxide, simultaneously reducing sulfate to sulfide (Widdel 1988). As a consequence, the complete oxidation of

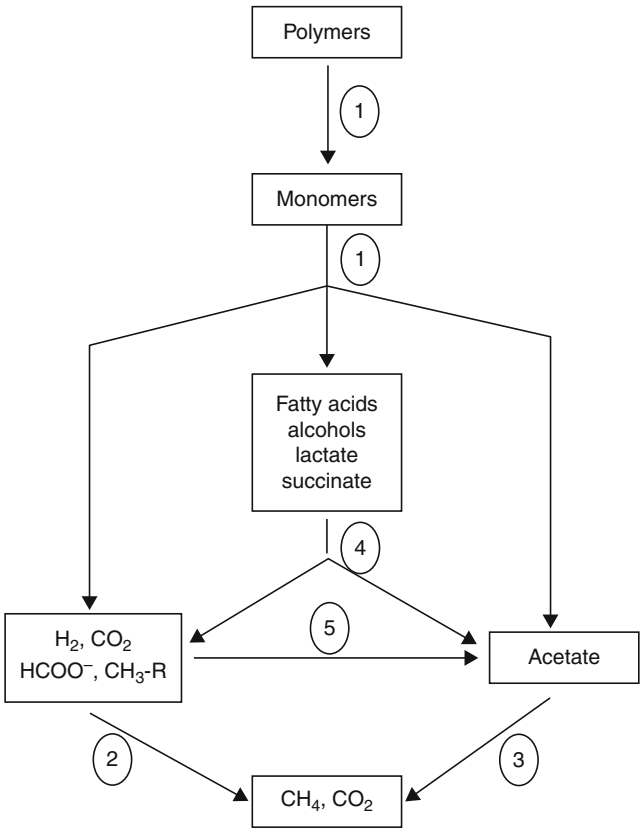


Fig. 21.1
Carbon and electron flow in the methanogenic degradation of complex organic matter. Groups of prokaryotes involved *1* primary fermentative bacteria, *2* hydrogen-oxidizing methanogens, *3* acetate-cleaving methanogens, *4* secondary fermenting bacteria (syntrophs), and *5* homoacetogenic bacteria

complex organic matter to carbon dioxide with simultaneous sulfate reduction proceeds in a two-step process and does not depend on syntrophic fermentations. One might add that this two-step scheme might be augmented also by cooperative sidepaths taken by different types of sulfate-reducing bacteria, e.g., completely and incompletely oxidizing ones (Widdel and Hansen 1991), but such cooperative activities are not required for complete sulfate-dependent oxidation of organic matter.

In methanogenic and sulfate-rich environments, the primary fermenting bacteria (group 1) profit from the activities of the hydrogen-oxidizing partners at the end of the degradation chain as well. A low hydrogen partial pressure ($<10^{-4}$ bar) allows electrons at the redox potential of NADH (−320 mV) to be released as molecular hydrogen, and fermentation patterns can shift to more acetate, CO_2, and hydrogen production rather than to ethanol or butyrate formation, thus allowing additional ATP synthesis via substrate-level phosphorylation, as opposed to production of reduced fermentation products (ethanol, lactate, and butyrate). Thus, such fermenting bacteria may profit from hydrogen-oxidizing partners, but they do not depend on such cooperation.

In a well-balanced anoxic sediment in which an active hydrogen-utilizing community maintains a low hydrogen partial pressure, the flux of carbon and electrons goes nearly exclusively through the "outer" paths of the electron flow scheme (*Fig. 21.1*), and therefore reduced fermentation intermediates play only a minor role. Recently a novel bacterium was described which ferments even sugars exclusively to acetate, CO_2, and hydrogen and depends for this process on a cooperation with methanogenic partners (Müller et al. 2008). Nonetheless, the flux through the "central" paths will never become zero because long-chain and branched-chain fatty acids and others are always produced in the fermentation of lipids and amino acids as well. The reduced intermediates of the central path become more important if the hydrogen pool increases for any reason, e.g., excess supply of fermentable substrate, inhibition of hydrogenotrophic methanogens due to a drop in pH (<6.0), or to the presence of toxic compounds, etc. Under such conditions, the pools of fatty acids increase and might even shift the pH further downward, thus inhibiting the hydrogenotrophic methanogens even further. The consequence may be that the whole system "turns over," meaning that methanogenesis ceases entirely and the fermentation stops with accumulation of huge amounts of foul-smelling fatty acids, as this is encountered with ill-balanced anaerobic sewage digestors. Obviously, the hydrogen/formate-utilizing methanogens act as the primary regulators in the total methanogenic conversion process (Bryant 1979; Zehnder 1978; Zehnder et al. 1982), and the syntrophically fatty acid-oxidizing bacteria are affected most severely by a failure in methanogenic hydrogen or formate removal.

The function of homoacetogenic bacteria (group 5; *Fig. 21.1*) in the overall process is less well understood. They connect the pool of one-carbon compounds and hydrogen with that of acetate. Owing to their metabolic versatility, they can participate also in sugar fermentation and degradation of special substrates such as *N*-methyl compounds or methoxylated phenols (Schink 1994). In certain environments, e.g., at lower pH or low temperature, they may even successfully compete with hydrogenotrophic methanogens and take over their function to a varying extent (see below).

Energetic Aspects

Anaerobes grow with small amounts of energy, and syntrophically cooperating anaerobes are extremely skilled in the exploitation of minimal energy spans. Synthesis of ATP as the general currency of metabolic energy in living cells requires +32 kJ per mol at equilibrium under standard conditions; under the conditions assumed to prevail in an actively growing cell ([ATP] = 10 mM; [ADP] = 1 mM; and $[P_i]$ = 10 mM), +49 kJ per mol is required (Thauer et al. 1977). In addition, part of the total energy budget is always lost in irreversible reaction steps as heat, thus rendering the overall metabolic process irreversible. This heat loss (on average about 20 kJ per mol ATP) has to be added to the above value, which gives a total of about 70 kJ per mol ATP synthesized irreversibly in the living cell. This is the

minimum amount of energy required for the synthesis of one mol of ATP in all known metabolic systems (Schink 1990). One may argue that (especially under conditions of energy limitation) an organism may waste less energy in heat production or that it may operate at an energy charge considerably lower than that quoted above for well-growing *Escherichia coli* cells. Nonetheless, one cannot expect the energy requirement for irreversible ATP synthesis to go substantially below about +60 kJ per mol.

The key postulate of the Mitchell theory of respirative ATP synthesis is that ATP formation is coupled to a vectorial transport of charged groups, typically protons, across a semipermeable membrane (Mitchell 1966). For several years, it was widely accepted that three protons cross the membrane (either of bacteria or mitochondria) per ATP hydrolyzed. As a consequence, the smallest quantum of metabolically convertible energy is that of an ion transported across the cytoplasmic membrane, equivalent to one-third of an ATP unit. Combined with the calculations above, this means that a bacterium needs a minimum of about −20 kJ per mol reaction to exploit a reaction's free energy change (Schink and Thauer 1988; Schink 1990).

On the basis of studies on the structure and function of F_1-F_0 ATPases in recent years, the stoichiometry of ATP synthesis versus proton translocation appears not to be as strictly fixed as suggested above. Rather, the system may operate like a sliding clutch, meaning that at very low energy input, the energy transfer into ATP synthesis may be substoichiometric. Moreover, the stoichiometry is not necessarily three protons per one ATP but is governed by the number of subunits arranged in the F_0 versus the F_1 complex. This concept would allow also stoichiometries of 4:1, perhaps even 5:1 (Engelbrecht and Junge 1997; Cherepanov et al. 1999; Stock et al. 1999; Dimroth 2000; Seelert et al. 2000; von Ballmoos et al. 2009). As a consequence, the minimum energy increment that can still be used for ATP synthesis may be as low as −15 or −12 kJ per mol reaction. In some cases, to make their living, bacteria cooperating in syntrophic fermentations are limited to this range of energy; Hoehler et al. (2001) calculated from metabolite concentrations in natural habitats for the partner bacteria cooperating in syntrophic conversions minimum amounts of exploitable energy in the range of −10 to −19 kJ per mol reaction.

Degradation of Amino Acids

During protein hydrolysis, a complex mixture of amino acids and small peptides is produced. These amino acids and peptides can serve as energy substrates for anaerobic microorganisms. Detailed information on the anaerobic fermentation of amino acids can be found in Barker (1981) and McInerney (1988). Many anaerobic amino acid-degrading bacteria require complex mixtures of amino acids, perhaps because a one-sided diet causes a serious imbalance in the internal amino acid metabolism of these cells, but growth by fermentation of single amino acids is also common. Mixtures of amino acids are often degraded by coupled fermentation of pairs of amino acids via the Stickland reaction. *Table 21.1* summarizes some oxidative conversions of amino acids. In the classical Stickland fermentation, these oxidation reactions are coupled within the same organism to reduction of other amino acids such as glycine or proline. Also phenylalanine, leucine, or compounds like sarcosine and betaine (Naumann et al. 1983; Gottschalk 1986) can act as electron acceptors, and oxidation of leucine coupled to the reduction of acetate to butyrate has been described as well (Girbal et al. 1997).

Table 21.1
Changes of Gibbs free energies under standard conditions in hydrogen-releasing reactions during fermentation of amino acids

Fermentation reaction	$\Delta G_0'$ (kJ per mol rct.)
Alanine + $2H_2O \rightarrow$ acetate$^-$ + CO_2 + NH_4^+ + $2H_2$	+2.7
Glycine + $2H_2O$ + $H^+ \rightarrow 2CO_2$ + NH_4^+ + $3H_2$	+17.8
Serine + $H_2O \rightarrow$ acetate$^-$ + CO_2 + NH_4^+ + H_2	−85.3[a]
Threonine + $H_2O \rightarrow$ propionate$^-$ + CO_2 + NH_4^+ + H_2	−83.0[a]
Histidine + $4H_2O$ + $H^+ \rightarrow$ glutamate$^-$ + CO_2 + $2NH_4^+$ + H_2	[b]
Proline + $2H_2O \rightarrow$ glutamate$^-$ + H^+ + $2H_2$	[b]
Glutamate$^-$ + $2H_2O$ + $H^+ \rightarrow$ propionate$^-$ + $2CO_2$ + NH_4^+ + $2H_2$	−16.6
Glutamate$^-$ + $2H_2O \rightarrow$ 2 acetate$^-$ + CO_2 + NH_4^+ + H_2	−38.6[a]
Aspartate$^-$ + $2H_2O$ + $H^+ \rightarrow$ acetate$^-$ + $2CO_2$ + NH_4^+ + $2H_2$	−24.1

All calculations are based on published tables (see Thauer et al. 1977; Dimroth 1983). For H_2S and CO_2, values for the gaseous state were used
[a]These fermentations may also allow growth in pure culture
[b]These reactions are always coupled to further fermentation of glutamate

These pure culture fermentations can also be catalyzed by cooperation of two different bacteria via interspecies hydrogen transfer according to the following reactions with a mixture of alanine and glycine:

$$CH_3CH(NH_3^+)COO^- + 2H_3O \rightarrow CH_3COO^- + CO_2 + NH_4^+ + 2H_2$$
$$\Delta G_0' = +27\ \text{kJ per mol}$$
$$CH_2(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+$$
$$\Delta G_0' = -78\ \text{kJ per mol}$$
$$CH_3CH(NH_3^+)COO^- + 2CH_2(NH_3^+)COO^- + 2H_2O \rightarrow 3CH_3COO^- + CO_2 + 3NH_4^+$$
$$\Delta G_0' = -153\ \text{kJ per mol}$$

Thus, the electrons derived in amino acid degradation by a fermenting bacterium can be used in glycine reduction as shown but can be transferred as well in the form of molecular hydrogen to sulfate-reducing, homoacetogenic, or methanogenic partner bacteria, depending on the availability of such partner bacteria and their respective electron acceptors. That amino acid oxidation and glycine reduction can be uncoupled from each other has been shown in detail with *Eubacterium*

acidaminophilum (Zindel et al. 1988). This bacterium can run either one of the first two reactions separately or combine them on its own, according to the third reaction, depending on the partner bacteria which act as hydrogen sources or sinks, and on the availability of selenium in the medium which is required for expression of the active glycine reductase complex.

In methanogenic environments, methanogens can act as scavengers of reducing equivalents in the oxidation of amino acids, thus taking over the role of the reductive part of the Stickland reaction. Nagase and Matsuo (1982) observed that in mixed methanogenic communities, the degradation of alanine, valine, and leucine was inhibited when methanogens were inhibited. Nanninga and Gottschal (1985) could stimulate the degradation of these amino acids by addition of hydrogen-scavenging sulfate reducers. These early observations indicated a functional role for hydrogen consumption also in the degradation of amino acids.

Influence of Methanogens

Methanogens can affect anaerobic oxidation in various ways. Fermentative oxidation of some amino acids can be coupled to hydrogen formation only if the hydrogen partial pressure is kept low, similar to the *Methanobacillus omelianskii* example described above. This results in an obligately syntrophic relationship between the fermenting microorganism and the methanogen. Some amino acids are degraded by the same fermenting bacterium either in pure culture or in mixed culture with methanogens; however, methanogens can cause a shift in metabolism resulting in a changed product formation pattern.

Clostridium sporogenes (Wildenauer and Winter 1986; Winter et al. 1987), *Eubacterium acidaminophilum* (Zindel et al. 1988), *Acidaminobacter hydrogenoformans* (Stams and Hansen 1984), *Aminomonas paucivorans* (Baena et al. 1999a), *Aminobacterium colombiense*, and *Aminobacterium mobile* (Baena et al. 1998, 2000), as well as strain PA-1 (Barik et al. 1985), are mesophilic bacteria which degrade one or more amino acids in syntrophic association with hydrogen-consuming anaerobes. Moderately thermophilic bacteria include *Caloramator coolhaasii* (Plugge et al. 2000), *Caloramator proteoclasticus* (Tarlera et al. 1997; Tarlera and Stams 1999), *Thermanaerovibrio acidaminovorans* (previously named *Selenomonas acidaminovorans*; Cheng et al. 1992; Baena et al. 1999b), *Gelria glutamica* (Plugge et al. 2002), and *Clostridium* P2 (Ørlygsson et al. 1993; Ørlygsson 1994). There are probably many other fermenting bacteria with the ability to grow syntrophically with certain amino acids and to release reducing equivalents as molecular hydrogen; however, such capacities are only rarely checked.

Obligately Syntrophic Amino Acid Deamination

The initial step in the oxidative degradation of alanine, valine, leucine, and isoleucine is an NAD(P)-dependent deamination to the corresponding α-keto acid, and the reaction, if coupled to reoxidation of NAD(P)H by proton reduction, would read as follows:

$$RCH(NH_4^+)COO^- + H_2O \rightarrow RCOCOO^- + NH_4^+ + H_2$$

The $\Delta G_0'$ of this reaction is about +55 kJ per mol; thus, very efficient hydrogen consumption would be required to pull this reaction. The α-keto acid is converted further via oxidative decarboxylation to a fatty acid:

$$RCOCOO^- + H_2O \rightarrow RCOO^- + CO_2 + H_2$$

a reaction which is much more favorable ($\Delta G_0'$ about −52 kJ per mol). Therefore, it is not surprising that one does not observe bacteria catalyzing the first reaction step only and that the organisms involved—as far as they have been tested—can grow in pure culture with α-keto acids. Such α-keto acids were used successfully for the isolation of amino acid-degrading proton-reducing bacteria (Stams and Plugge 1990). Conversion of the keto acids to the corresponding fatty acids likely proceeds through energy-rich CoA and phosphate derivatives, thus allowing ATP synthesis via substrate-level phosphorylation.

Eubacterium acidaminophilum degrades aspartate to acetate in syntrophic association with methanogens (Zindel et al. 1988). The degradation pathway is not known. Aspartate is either oxidized directly to oxaloacetate by means of an aspartate dehydrogenase or is degraded first to fumarate by aspartase activity. Fumarate is then converted to malate which is subsequently oxidized to oxaloacetate. In this pathway, the conversion of malate to oxaloacetate plus H_2 is most unfavorable; the $\Delta G_0'$ is +48 kJ per mol. Recently, a moderately thermophilic sugar-fermenting bacterium, *Gelria glutamica*, was isolated which can grow also by the analogous conversion of glutamate to propionate, provided that the hydrogen partial pressure is kept low by a methanogen (Plugge et al. 2002). In this bacterium, glutamate is first oxidatively deaminated to α-ketoglutarate, which is subsequently oxidatively decarboxylated to succinyl-CoA and further to propionate (Plugge et al. 2001). In coculture with methanogens, it is also able to oxidize proline to propionate. This is remarkable because proline is generally thought to be reductively degraded to aminovalerate. Similar to the above-mentioned amino acids, the aromatic amino acids phenylalanine, tryptophan, and tyrosine might also support growth by oxidative deamination leading to phenylacetate, indolylacetate, *p*-hydroxyphenylacetate, respectively, as products. This, however, has not yet been demonstrated for syntrophic amino acid-degrading bacteria.

Syntrophic Arginine, Threonine, and Lysine Fermentation

Acidaminobacter hydrogenoformans, *Aminomonas paucivorans*, and *T. acidaminovorans* can grow with histidine, ornithine, arginine, lysine, and threonine when cocultured with hydrogenotrophic bacteria (Stams and Hansen 1984; Cheng et al. 1992; Baena et al. 1999a). Remarkably, *T. acidaminovorans*

and *A. paucivorans* grow in pure culture with arginine, forming citrulline and/or ornithine as products. In this conversion, carbamyl phosphate is formed as an intermediate, which is further converted to yield ATP (Plugge and Stams 2001). Ornithine and citrulline could be degraded when these bacteria were cocultured with a hydrogenotrophic methanogen. It is not clear which pathway is used for ornithine degradation and which metabolic step is hampering growth of these bacteria in pure culture.

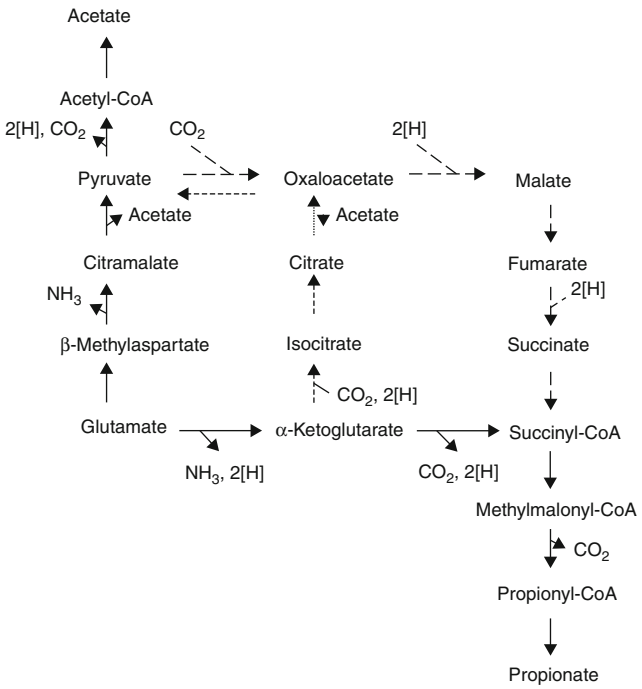
Acidaminobacter hydrogenoformans degrades threonine and lysine in syntrophic association with a hydrogen-consuming anaerobe. Acetate is the main product of the degradation of these two amino acids, and large amounts of hydrogen are formed. In pure culture, these compounds do not support growth. The stoichiometry of the fermentation is not exactly known, and the pathways involved in these fermentations have not been resolved as yet.

Also the biogenic amine cadaverine (the decarboxylation product of lysine) can be degraded to methane and CO_2 through a syntrophic association (Roeder and Schink 2009). Degradation is initiated by transamination with 2-oxoglutarate and proceeds via oxidation to a glutaryl residue.

Facultatively Syntrophic Growth with Amino Acids

The effect of methanogens on the metabolism of amino acid-fermenting anaerobes has been studied most extensively with glutamate. Glutamate fermentation is carried out by a variety of fastidious anaerobes, including a number of *Clostridium* species, *Peptostreptococcus asaccharolyticus*, and *Acidaminococcus fermentans* (Gottschalk 1986). These microorganisms ferment glutamate to acetate and butyrate by two different pathways, the β-methylaspartate or the hydroxyglutarate pathway (Buckel and Barker 1974). In this fermentation, reducing equivalents formed in the oxidation of glutamate to acetate are disposed of either partly or completely by reductive formation of butyrate from acetate (acetyl-CoA).

Anaeromusa acidaminophila (*Selenomonas acidaminophila*) ferments glutamate to acetate plus propionate (Nanninga et al. 1987; Baena et al. 1999b). In this bacterium, reducing equivalents are disposed of by reduction of pyruvate to propionate. However, in the last decade, several bacteria have been isolated which during growth on glutamate release reducing equivalents exclusively as hydrogen, both in the formation of acetate and the formation of propionate (◉ *Table 21.1*). These microorganisms ferment glutamate to acetate only (*Caloramator coolhaasii* [Plugge et al. 2000] and *Caloramator proteoclasticus* [Tarlera and Stams 1999]), propionate only (*Aminobacterium colombiense* [Baena et al. 1998] and *Gelria glutamica* [Plugge et al. 2002]), or acetate plus propionate (*A. hydrogenoformans* [Stams and Hansen 1984; Meijer et al. 1999], *T. acidaminovorans* [Cheng et al. 1992; Baena et al. 1999b], and *Aminomonas paucivorans* [Baena et al. 1999a]), with CO_2 as coproduct (◉ *Table 21.1*).



◘ Fig. 21.2
Pathways involved in the fermentation of glutamate to propionate and acetate in anaerobic bacteria. *Thin solid line* (*left* part): acetate formation by the commonly used β-methylaspartate pathway. *Thick solid line*: oxidative formation of propionate as catalyzed by, e.g., *Gelria glutamica*. *Dashed line*: reductive formation of propionate as performed by *Anaeromusa acidaminophila*. *Dotted line*: acetate formation via enzymes of the reductive tricarboxylic acid (TCA) cycle as used by *Acidaminobacter hydrogenoformans* when cocultured with hydrogenotrophic methanogens

Acidaminobacter hydrogenoformans ferments glutamate to 2 acetate, 1 CO_2, NH_3, and 1 H_2, or to 1 propionate, 2 CO_2, NH_3, and 2 H_2 (◉ *Table 21.1*; ◉ *Fig. 21.2*). In pure culture, *A. hydrogenoformans* can also form formate. However, in the presence of the hydrogen-utilizing *Methanobrevibacter arboriphilus*, formate is not formed (Stams and Hansen 1984). The ratio at which acetate and propionate are formed depends on the hydrogen partial pressure (Stams and Hansen 1984). Propionate formation is favored at low hydrogen partial pressure, whereas at high hydrogen pressure, mainly acetate is formed. Enzyme measurements revealed that the strain when grown in pure culture uses the β-methylaspartate pathway for acetate formation. However, if the bacterium is grown in coculture with a methanogen, both acetate and propionate appear to be formed via α-ketoglutarate (Stams et al. 1998). Reductive carboxylation of α-ketogluratate leads via isocitrate to citrate which is cleaved to acetate and pyruvate, and pyruvate is converted further to acetate. Propionate formation occurs via oxidative decarboxylation of α-ketoglutarate to succinyl-CoA, which is further converted to propionate. Apparently, a crucial step that determines the degradation pathway is the oxidative deamination of glutamate to

α-ketoglutarate which is highly endergonic if coupled to hydrogen formation. Under standard conditions, this conversion is energetically impossible ($\Delta G_0' = +60$ kJ per mol). However, at a hydrogen partial pressure of 10^{-5} atm, the $\Delta G_0'$ of this reaction would be +30 kJ per mol, and if the intracellular NH^{4+} concentration would be 10 mM and the glutamate/α-ketoglutarate ratio about 500, the $\Delta G'$ of this reaction would become about zero. A similar shift in product formation was described for *Aminomonas paucivorans* (Baena et al. 1999a).

Thermanaerovibrio acidaminovorans also forms acetate and propionate as products. Unlike the mesophilic organisms, this thermophile forms propionate already in pure culture. This bacterium uses the β-methylaspartate pathway for acetate formation both in pure culture and in coculture with a methanogen (Plugge et al. 2002). Some of the glutamate-fermenting bacteria also are able to grow with histidine, forming similar products and shifts in product formation as with glutamate. Histidine is supposed to be degraded through glutamate as an intermediate (Gottschalk 1986).

Stickland Reaction Versus Methanogenesis

Some of the bacteria which can degrade amino acids syntrophically, including *Clostridium sporogenes*, *Eubacterium acidaminophilum*, *Acidaminobacter hydrogenoformans*, and *Caloramator proteoclasticus*, are able to perform a Stickland reaction. The environmental conditions under which the Stickland reaction is favored and conditions under which reducing equivalents are disposed of as molecular hydrogen remain to be determined. Energetically, the reductive conversion of glycine is more favorable than methanogenesis, homoacetogenesis, or sulfate reduction (Table 21.2). It is likely that in environments rich in amino acids, the Stickland reaction will dominate, whereas in environments with low amino acid supply and high methanogenic activity, reducing equivalents may be preferentially channeled to methanogenesis. Unfortunately, up to now, little information was available on such comparative ecological aspects.

During growth of a coculture of *Caloramator proteoclasticus* and *Methanobacterium thermoautotrophicum* Z245 in a medium with alanine plus glycine, about 60% of the reducing equivalents were channeled to methanogenesis when a dense culture of the methanogen was inoculated with 0.2% of a culture of *C. proteoclasticus*. However, if 1% inoculum was applied, all the reducing equivalents were used to reduce glycine.

Table 21.2
Changes of Gibbs free energies under standard conditions in hydrogen-consuming reactions involved in interspecies hydrogen transfer

	$\Delta G_0'$ (kJ per mol rct.)	$\Delta G_0'$ (kJ per electron pair)
$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	−94.9	−23.8
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−131.0	−32.7
$H_2 + S^0 \rightarrow H_2S$	−33.9	−33.9
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	−151.0	−37.6
$H_2C(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+$	−78.0	−78.0
$Fumarate^{2-} + H_2 \rightarrow succinate^{2-}$	−86.0	−86.0

All calculations are based on published tables (see Thauer et al. 1977; Dimroth 1983). For H_2S and CO_2, values for the gaseous state were used

Syntrophic Degradation of Fermentation Intermediates

Syntrophic Ethanol Oxidation

The case of *Methanobacillus omelianskii* is the classical example of interspecies hydrogen transfer. Both partners operate in an overall reaction process which becomes exergonic for the first partner only through maintenance of a low hydrogen partial pressure by the second partner. After description of the cooperative nature of this process, the original S-strain was lost, but other syntrophically ethanol-oxidizing bacteria have been isolated, such as *Thermoanaerobium brockii* (Ben-Bassat et al. 1981) and various *Pelobacter* strains (Schink 1984, 1985a; Eichler and Schink 1986). Also certain ethanol-oxidizing sulfate reducers such as *Desulfovibrio vulgaris* are able to oxidize ethanol in the absence of sulfate by hydrogen transfer to a hydrogen-oxidizing methanogenic partner.

Unfortunately, the biochemistry of this syntrophic cooperation has not been resolved yet. The total reaction

$$2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COO^- + 2H^+ + CH_4$$

yields −112 kJ per 2 mol ethanol under standard conditions. On the side of the ethanol oxidizer, e.g., the "S-strain" of *Methanobacillus omelianskii* (Bryant et al. 1967) or other fermenting anaerobes with similar metabolic capacities such as *Pelobacter acetylenicus* (Schink 1985a), ethanol dehydrogenase, acetaldehyde ferredoxin oxidoreductase (acetyl-CoA-forming), phosphotransacetylase, and acetate kinase have been shown to be involved, forming one ATP per ethanol through substrate-level phosphorylation. Since the methanogenic hydrogen oxidizer requires at least one-third of an ATP unit for growth (−20 kJ per reaction run, see above), only about −45 kJ is available to the ethanol oxidizer per mol ethanol oxidized, which is too little energy to form one full ATP. It has to be postulated, therefore, that part of the energy bound in ATP has to be reinvested somewhere to push the overall reaction and balance the energy budget, but this reverse electron transport system has not yet been identified. In syntrophically ethanol-oxidizing *Desulfovibrio* strains, the pathway leads from acetaldehyde directly to acetate, without a phosphorylation step, which explains why *Desulfovibrio* cannot grow in this syntrophic association (Kremer et al. 1988).

Syntrophic Butyrate Oxidation

Similar cooperations have been described with syntrophic cultures degrading fatty acids. An overview of the reactions catalyzed is presented in *Table 21.3*; a list of described strains of syntrophically fermenting bacteria follows in *Table 21.4*. In general, degradation of fatty acids to acetate and hydrogen or, in the case of propionate, to acetate, hydrogen, and CO_2 are reactions far more endergonic under standard conditions than ethanol oxidation. Consequently, for fatty acid degradation, the hydrogen partial pressure has to be decreased to substantially lower values ($<10^{-4}$ bar) than with ethanol ($<10^{-3}$ bar).

The energetic situation of the partner bacteria involved in butyrate conversion to methane and CO_2 has been discussed in detail in earlier publications (Wallrabenstein and Schink 1994; Schink 1997). The overall reaction

$$2CH_3CH_2CH_2COO^- + 2H^+ + 2H_2O \rightarrow 5CH_4 + 3CO_2$$

yields under standard conditions a $\Delta G_0'$ of −177 kJ per 2 mol of butyrate. With concentrations better comparable to those prevailing in a natural habitat, e.g., a freshwater sediment or a sewage sludge digestor (butyrate: 10 μM, CH_4: 0.7 bar, and CO_2: 0.3 bar), the free energy of this process changes to −140 kJ per 2 mol of butyrate. Since the overall process is shared by seven partial reactions (two are involved in the butyrate oxidation, one in CO_2 reduction to methane, and four in acetate cleavage), the free energy change is about −20 kJ per mol for every partial reaction if the energy is shared by all reactions at equal rates. Measured partial pressures of hydrogen and acetate concentrations in active sewage sludge and various sediments (Zehnder et al. 1982) are in a range of 10^{-4} to 10^{-5} bar and 10–100 μM, respectively, which is in good agreement with the assumption of equal energy sharing in such a cooperative community. The corresponding energy yields of about −20 kJ per mol reaction are confirmed by growth yield determinations with pure cultures of methanogens (Schönheit et al. 1980; Zehnder et al. 1982).

On the side of the syntrophically fermenting partner, 1 ATP is synthesized by substrate-level phosphorylation through thiolytic acetoacetyl-CoA cleavage (Wofford et al. 1986), but part of this energy has to be reinvested into an assumed reverse electron transport to allow proton reduction with electrons from the butyryl-CoA dehydrogenase reaction at a hydrogen partial pressure of 10^{-4} to 10^{-5} bar (Thauer and Morris 1984). Experimental evidence of a reverse electron transport system between the crotonyl-CoA/butyryl-CoA couple ($E^{o\prime} = -125$ mV) and the H^+/H_2 couple has been provided with *Syntrophomonas wolfei* (Wallrabenstein and Schink 1994). In the meantime, several components of this electron transport system have been described in detail (Müller et al. 2009) on the basis of the sequenced genome of *Syntrophomonas wolfei* (McInerney et al. 2007). The present concept assumes that the menaquinone involved as electron carrier in this system translocates protons from the outside inward, thus pushing the endergonic reduction of protons with the electrons released in the butyryl-CoA dehydrogenase reaction. If two protons are transferred this way, one-third of the ATP synthesized by substrate-level phosphorylation (equivalent to −20 kJ per mol) would remain for growth and maintenance of the fatty acid-oxidizing bacterium, in accordance with the above assumptions.

The energetic situation of a binary mixed culture degrading butyrate to acetate and methane is considerably more difficult:

$$2CH_3CH_2CH_2COO^- + CO_2 + 2H_2O \rightarrow 4CH_3COO^- + 2H^+ + CH_4$$
$$\Delta G_0' = -35 \text{ kJ per 2 mol of butyrate}$$

This overall reaction has to feed two organisms in three partial reactions, so each step has only −12 kJ available under standard conditions, and the total changes to −46 kJ at butyrate and acetate concentrations in the range of 10 mM as used in laboratory cultures. Under these conditions, the energetic situation for the partners gets tough (−15 kJ per mol reaction), especially at the end of the substrate conversion process. Very slow, often nonexponential growth and substrate turnover as usually observed with such binary mixed cultures (Dwyer et al. 1988) indicates that the energy supply is insufficient. We have often observed, as did other authors, that accumulating acetate (>10 mM) inhibits butyrate degradation in such cultures substantially.

Table 21.3
Changes of Gibbs free energies under standard conditions in hydrogen-releasing reactions during oxidation of fermentation intermediates

	G_0' (kJ per mol rct.)	No. of electron pairs
Primary alcohols		
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6	2
Fatty acids		
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2H_2$	+48.3	2
$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2$	+76.0	3
$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9	4
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.2	1
Glycolic acid		
$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2$	+19.3	3
Aromatic compounds		
$C_6H_5COO^- + 6H_2O \rightarrow 3CH_3COO^- + 2H^+ + CO_2 + 3H_2$	+49.5	3
$C_6H_5OH + 5H_2O \rightarrow 3CH_3COO^- + 3H^+ + 2H_2$	+10.2	2
Amino acids		
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7	2

All calculations are based on published tables (see Thauer et al. 1977; Dimroth 1983). For H_2S and CO_2, values for the gaseous state were used

Table 21.4

Pure or defined mixed cultures of bacteria catalyzing syntrophic substrate oxidations via interspecies hydrogen transfer

Isolate	Substrate range	Gram type	Phylogenetic position	References
(a) Oxidation of primary alcohols				
S-strain	Ethanol	–	Unknown	Bryant et al. (1967)
Desulfovibrio vulgaris	Ethanol + sulfate	–	δ-Proteobacteria	Bryant et al. (1977)
Thermoanaerobacter brockii	Ethanol, sugars, etc.	+	Low G + C Gram positives	Ben-Bassat et al. (1981)
Pelobacter venetianus	Ethanol, propanol		δ-Proteobacteria	Schink and Stieb (1983)
Pelobacter acetylenicus	Ethanol, acetylene	–	δ-Proteobacteria	Schink (1985a)
Pelobacter carbinolicus	Ethanol, 2,3-butanediol		δ-Proteobacteria	Schink (1984)
(b) Oxidation of butyrate and higher homologues				
Syntrophomonas wolfei	C_4–C_8	–	Low G + C Gram positives	McInerney et al. (1979, 1981)
Syntrophomonas sapovorans	C_4–C_{18}	–	Low G + C Gram positives	Roy et al. (1986)
Syntrophospora bryantii	C_4–C_{11}, 2-methyl valerate	+	Low G + C Gram positives	Stieb and Schink (1985); Zhao et al. (1989)
Thermosyntropha lipolytica	C_4–C_{18}, crotonate, and betaine	+	Low G + C Gram positives	Svetlitshnyi et al. (1996)
Syntrophothermus lipocalidus	C_4–C_{10}, isobutyrate, and crotonate	+	Low G + C Gram positives	Sekiguchi et al. (2000)
(c) Oxidation of propionate				
Syntrophobacter wolinii	Propionate	–	δ-Proteobacteria	Boone and Bryant (1980)
Syntrophobacter pfennigii	Pyruvate	–	δ-Proteobacteria	Wallrabenstein et al. (1995a)
Syntrophobacter fumaroxidans	Propionate + fumarate	–	δ-Proteobacteria	Harmsen et al. (1998)
Smithella propionica	Propionate	–	δ-Proteobacteria	Liu et al. (1999)
(d) Oxidation of acetate				
Thermoacetogenium phaeum	Acetate, pyruvate, glycine, cysteine, formate, and H_2/CO_2	+	Low G + C Gram positives	Hattori et al. (2000)
Clostridium ultunense	Acetate, formate, and cysteine	+	Low G + C Gram positives	Schnürer et al. (1996)
(e) Oxidation of isovalerate				
Strain Gralva1	Isovalerate only	+	Unknown	Stieb and Schink (1986)
(f) Oxidation of glycolate				
Syntrophobotulus glycolicus	Glycolate and glyoxylate	+	Low G + C Gram positives	Friedrich et al. (1991, 1996)
(g) Oxidation of aromatic compounds				
Syntrophus buswellii	Benzoate and crotonate	–	δ-Proteobacteria	Mountfort and Bryant (1982)
Syntrophus gentianae	Benzoate, gentisate, and hydroquinone	–	δ-Proteobacteria	Wallrabenstein et al. (1995b)
Syntrophus aciditrophicus	Benzoate and crotonate	–	δ-Proteobacteria	Jackson et al. (1999)

The energetic difference between the ternary mixed culture and an artificial binary mixed culture demonstrates that the acetate-cleaving methanogens fill an important function in removal of acetate and, with this, "pull" the butyrate oxidation reaction. The above calculations also explain why addition of an acetate-cleaving methanogen to a defined binary mixed culture enhances growth and substrate turnover considerably (Ahring and Westermann 1988; Beaty and McInerney 1989).

Syntrophic oxidation of long-chain fatty acids from lipid hydrolysis probably proceeds via β-oxidation with concomitant

release of electrons as hydrogen via reverse electron transport, analogous to the process described above for butyrate oxidation. Long-chain dicarboxylic acids are degraded stepwise by β-oxidation, analogous to fatty acids, and decarboxylation occurs at the C5 or C4 state (glutarate or succinate; Matthies and Schink 1993).

Syntrophic Propionate Oxidation

For syntrophic propionate oxidation according to the equation

$$4CH_3CH_2COO^- + 4H^+ + 2H_2O \rightarrow 7CH_4 + 5CO_2$$
$$\Delta G_0' = -249 \text{ kJ per 4 mol of propionate}$$

a metabolic flow scheme can be drawn, leaving a free energy change in the range of −22 to −23 kJ per mol reaction (11 partial reactions) to all partners involved (Stams et al. 1989; Schink 1991). Studies in defined mixed cultures and in undefined communities in rice field soil have basically confirmed this assumption, although the amount of energy available to the propionate oxidizer may be sometimes substantially lower (Scholten and Conrad 2000; Fey and Conrad 2000).

The ability to grow on propionate in syntrophic association with methanogenic archaea is found in two phylogenetic groups of bacteria. Boone and Bryant (1980) described *Syntrophobacter wolinii*. Since then, several other mesophilic and thermophilic bacteria that grow in syntrophy with methanogens have been described as reviewed by McInerney et al. (2008) These include Gram-negative bacteria (*Syntrophobacter* and *Smithella*) and Gram-positive bacteria (*Pelotomaculum* and *Desulfotomaculum*). Phylogenetically, both groups are related to sulfate-reducing bacteria and some indeed grow by coupling propionate oxidation to sulfate reduction (Harmsen et al. 1993, 1995; Wallrabenstein et al. 1994, 1995a; Chen et al. 2005). Most syntrophically propionate-degrading bacteria are able to grow in pure culture by fermentation of fumarate or pyruvate. The exceptions are *Pelotomaculum schinkii* (de Bok et al. 2005) and *Pelotomaculum propionicicum* (Imachi et al. 2007), which seem to be true propionate-degrading syntrophs.

Two pathways for propionate metabolism are known, the methylmalonyl-CoA pathway and a dismutation pathway. In the latter pathway, two propionate molecules are converted to acetate and butyrate, the butyrate being degraded to acetate and hydrogen as described above. Thus far, this pathway is only found in *Smithella propionica* (Liu et al. 1999; de Bok et al. 2001). The methylmalonyl-CoA pathway is found in the other syntrophic propionate-oxidizing bacteria (McInerney et al. 2008). This pathway is basically a reversal of fermentative propionate formation, including methylmalonyl-CoA, succinate, malate, pyruvate, and acetyl-CoA as intermediates (Koch et al. 1983; Schink 1985b, 1991; Houwen et al. 1987, 1990; Kosaka et al. 2006). The initial substrate activation is accomplished by CoA transfer from acetyl-CoA (Houwen et al. 1990; Plugge et al. 1993) or succinyl-CoA. Of the redox reactions involved, succinate oxidation and malate oxidation are the most difficult ones to couple to proton reduction: Hydrogen partial pressures of 10^{-15} or 10^{-8} bar would be required, respectively, which are far lower concentrations than a methanogen can maintain. The enzymes and electron transfer components involved in propionate oxidation were initially studied with *Syntrophobacter wolinii* (Houwen et al. 1990; Plugge et al. 1993) and *Syntrophobacter pfennigii* (Wallrabenstein et al. 1995b). Genome-based studies have recently been done with *Syntrophobacter fumaroxidans* (Müller et al. 2010; Worm et al. 2011) and the thermophilic *Pelotomaculum thermopropionicum* (Kosaka et al. 2006, 2008).

Studies with *Syntrophobacter fumaroxidans* have shown that not only hydrogen but also formate could contribute to interspecies electron transport; syntrophic propionate oxidation was possible only in cooperation with formate- and hydrogen-oxidizing methanogens and not with *Methanobrevibacter* strains that are unable to use formate (Dong et al. 1994). This view was further supported by enzyme measurements; cells grown syntrophically with propionate contained tenfold higher formate dehydrogenase activity in comparison with cells grown in pure culture with fumarate; the hydrogenase activity was unchanged (de Bok et al. 2002). The complexity of interspecies electron transfer became clear in a recent study in which six different hydrogenases, four formate dehydrogenases, and two formate-hydrogen lyases were identified in *S. fumaroxidans*, and five formate dehydrogenases and three hydrogenases in the methanogenic partner *Methanospirillum hungatei* (Worm et al. 2011). All these enzymes are involved, but the relative importance of each enzyme is not yet clear.

In syntrophic propionate metabolism, menaquinone oxidation is linked to a membrane-bound hydrogenase or formate dehydrogenase. Genome and biochemical analysis of *S. fumaroxidans* revealed the presence of a membrane-integrated succinate dehydrogenase gene cluster containing menaquinone (Sfum 1998, 1999 and 2000) and several periplasmic and cytoplasmic hydrogenases and formate dehydrogenases (Müller et al. 2010; Worm et al. 2011). A requirement of 2/3 ATP for reversed electron transport was determined to push the endergonic succinate oxidation. A similar and phylogenetically related succinate dehydrogenase gene cluster was found in *P. thermopropionicum* (Kosaka et al. 2006, 2008). This indicates that succinate oxidation to fumarate requires a proton gradient over the membrane and the investment of ATP to form hydrogen or formate at the outside of the cytoplasmic membrane.

Smithella propionica (Liu et al. 1999) uses a different pathway to convert propionate. The labeling patterns of products formed from specifically labeled propionate indicate that propionate degradation by this bacterium proceeds through dimerization of propionate to a six-carbon intermediate that is subsequently cleaved to an acetyl and a butyryl moiety, with partial further oxidation (de Bok et al. 2001). This new pathway could also explain the results of labeling experiments with an enrichment culture which were first interpreted as indicative of a reductive carboxylation of propionate to a butyryl residue (Tholozan et al. 1988, 1990).

Syntrophic Acetate Oxidation

A special case is the syntrophic conversion of acetate to $2CO_2$ and $4H_2$ which was described first for a moderately thermophilic (58°C) bacterium, strain AOR (Zinder and Koch 1984). This syntrophic acetate oxidizer could be grown in pure culture like a homoacetogen by hydrogen-dependent reduction of CO_2 to acetate, thus reversing syntrophic acetate oxidation (Lee and Zinder 1988b, c). Biochemical studies revealed that it uses the carbon monoxide dehydrogenase pathway ("Wood-Ljungdahl pathway"), as do other homoacetogens (Lee and Zinder 1988a). The small energy span available in acetate conversion to methane and CO_2 is hardly sufficient to feed two bacteria:

$$\begin{aligned} CH_3COO^- + H^+ &\rightarrow CH_4 + CO_2 \\ \Delta G_0' &= -36 \text{ kJ per mol} \\ &= -8 \text{ kJ per partial reaction (at } 25^\circ C) \end{aligned}$$

The free energy change is slightly higher at 58°C (−42 kJ per mol) than under standard conditions (25°C). However, a syntrophic acetate-oxidizing culture has been described as well which operates at 35°C (Schnürer et al. 1994, 1996) and thus proves that this free energy change (−38°C) is sufficient for acetate degradation and (very slow) growth at this temperature. Another interesting feature of this type of metabolism is that these bacteria can run acetate formation and acetate degradation, in both directions, with probably the same biochemical reaction apparatus, just depending on the prevailing concentrations of substrates and products, and even can synthesize ATP both ways. This example shows how close to the thermodynamic equilibrium the energy metabolism of an anaerobic bacterium can operate.

The higher energy yield at elevated temperature may explain why at 35°C and lower this reaction is typically carried out by one single bacterium, e.g., *Methanosarcina barkeri* or *Methanosaeta soehngenii*, and why cooperations of syntrophic associations of the above-mentioned type are found nearly exclusively at elevated temperatures. Nonetheless, acetate conversion to methane at higher temperatures can as well be catalyzed by a single aceticlastic methanogen, e.g., *Methanosarcina thermophila*. Syntrophic acetate oxidation at lower temperatures is found only if further stress factors such as high ammonium concentrations inhibit aceticlastic methanogens (Schnürer et al. 1994).

Unfortunately, strain AOR was lost only few years after its description. Another thermophilic strain with similar properties, *Thermoacetogenium phaeum*, was isolated in Japan (Hattori et al. 2000) and was found to use the CO dehydrogenase pathway (the Wood-Ljungdahl pathway) for acetate oxidation, too (Hattori et al. 2005), and the same is true for the mesophilic acetate-oxidizing *Clostridium ultunense* (Schnürer et al. 1997). However, we do not know yet to what extent and in which steps energy is conserved by these bacteria: either in the acetate synthesis or the acetate oxidation reaction chain.

Syntrophic acetate oxidation was observed also in a coculture of the iron-reducing bacterium *Geobacter sulfurreducens* together with the nitrate-reducing anaerobe *Wolinella succinogenes* (Cord-Ruwisch et al. 1998). In this coculture, the hydrogen partial pressures were far below the levels observed in the cultures mentioned above. We found out later that the cysteine added to the cultures as reducing agent actually mediates interspecies electron transfer in this artificially composed syntrophic coculture through a cysteine/cystine cycle (Kaden et al. 2002), thus adding another means of electron transfer to our concepts of syntrophic cooperations.

Syntrophic Glycolate Oxidation

Glycolate is not a fermentation intermediate but an important excretion product of algal cells. It is syntrophically oxidized by homoacetogenic and methanogenic cocultures to two molecules of CO_2, and hydrogen is the electron carrier between the fermenting bacterium (*Syntrophobotulus glycolicus*) and a hydrogen-oxidizing partner bacterium (Friedrich et al. 1991, 1996). The primary fermentation is an endergonic process under standard conditions (Table 21.3) and needs coupling to, e.g., a methanogenic partner. The degradation pathway includes oxidation of glycolate to glyoxylate, condensation of glyoxylate with acetyl-CoA to form malyl-CoA, ATP formation in a malyl-CoA synthetase reaction, malate oxidation and decarboxylation by the malic enzyme, and oxidative decarboxylation of pyruvate to acetyl-CoA through pyruvate synthase, thus closing the chain for a new reaction cycle (Friedrich et al. 1991). In this cycle, the oxidation of glycolate to glyoxylate is the critical oxidation step ($E^{\circ\prime} = -92$ mV) which, if coupled to proton reduction, requires a reverse electron transport which has to be fueled by partial hydrolysis of the ATP formed in substrate-level phosphorylation. Proof of proton gradient-dependent hydrogen release was provided in experiments with membrane vesicles: Such vesicles converted glycolate stoichiometrically to glyoxylate and hydrogen in the presence of ATP. This hydrogen formation was abolished entirely by addition of CCCP and other protonophores, as well as by DCCD. Monensin and other sodium ionophores had no specific effect (Friedrich and Schink 1993). The process is even reversible: Membrane vesicles incubated in the presence of glyoxylate and hydrogen catalyzed a substrate-dependent net synthesis of ATP from ADP and P_i. The ratio of hydrogen-dependent glyoxylate reduction over ATP formation in isolated membrane vesicle preparations (0.2–0.5 mol per mol; Friedrich and Schink 1995) indicates that probably two-thirds of an ATP unit can be formed this way per reaction run. Thus, at least with this system, we have rather reliable data on the reaction stoichiometry.

Syntrophic Oxidation of Aromatic Compounds

So far, defined syntrophic cocultures for methanogenic degradation of aromatic substrates exist only for benzoate, gentisate, and hydroquinone (Table 21.3). The biochemistry of anaerobic degradation of aromatic compounds has been studied in most detail so far with benzoate, and a degradation pathway has been elaborated in the last years mainly with phototrophic and

nitrate-reducing bacteria (for reviews of this subject, see Fuchs et al. 1994; Heider and Fuchs 1997; Schink et al. 2000). The basic concept is an initial activation to benzoyl-CoA by an acyl-CoA synthetase reaction requiring two ATP equivalents, followed by partial ring saturation and subsequent ring opening by a mechanism analogous to β-oxidation of fatty acids. The resulting C_7-dicarboxylic acid undergoes further β-oxidation to form three acetate residues and one CO_2.

The reductive dearomatization of benzoyl-CoA in nitrate reducers requires two ATP equivalents and leads to cyclohexa-2,6-diene carboxyl-CoA which undergoes further hydration and oxidation (Heider and Fuchs 1997). This makes the initial steps in the total process rather ATP consuming (four ATP equivalents consumed before ring cleavage). Nitrate reducers will gain these ATP investments back during subsequent oxidation of the acetyl residues.

Syntrophically benzoate-oxidizing bacteria (Auburger and Winter 1992; Schöcke and Schink 1997; Elshahed et al. 2001) activate benzoate through a benzoyl-CoA ligase reaction, too, which consumes two ATP units and forms pyrophosphate as side product. Part of the energy invested can be regained through a membrane-bound, proton-translocating pyrophosphatase (Schöcke and Schink 1998), and another fraction of an ATP can be conserved by the action of a membrane-bound, sodium ion-translocating glutaconyl-CoA decarboxylase which stores the energy of the C_5 dicarboxylic acid decarboxylation to crotonate in a transmembrane sodium ion gradient (Schöcke and Schink 1999).

An alternative reaction for benzoyl-CoA reduction was described first in *Geobacter metallireducens* and appears to be used also in sulfate-reducing and syntrophically fermenting bacteria (Kung et al. 2009, 2010; Löffler et al. 2011). This enzyme (class II benzoyl-CoA reductase) does not require direct investment of ATP in the reduction but derives the necessary energy for the reduction step from a "bifurcation" reaction (Herrmann et al. 2008) in which the reduction of benzoyl-CoA with reduced ferredoxin is coupled with simultaneous electron transfer from ferredoxin to NAD. The energy gap has to be filled by ion translocation-dependent electron transfer from NADH to ferredoxin, e.g., through an Rnf transport system.

Although the energetic situation of syntrophic benzoate oxidizers in methanogenic cocultures is substantially better than that of butyrate oxidizers, it appears that these bacteria depend on efficient acetate removal as well. Benzoate was nearly completely converted to acetate, methane, and CO_2 in binary mixed cultures with *Methanospirillum hungatei* as hydrogen scavenger. Remnant benzoate concentrations at apparent equilibrium (in the range of 20–70 μM) increased in the presence of added acetate or propionate and decreased in the presence of a more efficient hydrogen consumer, e.g., a sulfate-reducing partner. The corresponding hydrogen concentrations measured in such cultures were in the range of $0.5 - 5 \times 10^{-5}$ bar, leaving a total ΔG of −30 to −45 kJ per mol reaction for the benzoate degrader. Similar results were obtained during studies on the kinetics of benzoate degradation with *Syntrophus aciditrophicus* in the presence of a sulfate-reducing partner bacterium (Warikoo et al. 1996).

Syntrophic Oxidation of Branched-Chain Fatty Acids

Branched-chain fatty acids are formed during fermentative degradation of the corresponding amino acids by oxidative deamination and decarboxylation, or reductive deamination (see above). The further degradation is not difficult with 2-methyl butyrate (neovalerate) because it can be β-oxidized, analogous to the degradation of butyrate to acetate and propionate by, e.g., *Syntrophospora bryantii* (Stieb and Schink 1985). Methanogenic degradation of isobutyrate proceeds via isomerization to butyrate and further β-oxidation (Stieb and Schink 1989); the isobutyrate/butyrate isomerization has been described in a defined culture as a coenzyme B_{12}-dependent rearrangement of the carbon skeleton (Matthies and Schink 1992). A similar isomerization may also occur as a side reaction with valerate, leading to formation of 2-methyl butyrate (Wu et al. 1994). Whether a similar isomerization occurs also during isobutyrate degradation by the thermophilic *Syntrophothermus lipocalidus* (Sekiguchi et al. 2000) still remains to be examined.

So far, only one defined culture has been described as capable of fermentative degradation of 3-methyl butyrate (isovalerate; Stieb and Schink 1986), and this culture specializes in degradation of this substrate, converting it with CO_2 as cosubstrate to three molecules of acetate and one molecule of hydrogen (*Tables 21.3* and *21.4*). The pathway is rather complicated and involves a carboxylation, a dehydrogenation of a saturated fatty acid residue, and one substrate-level phosphorylation step (Stieb and Schink 1986). The whole conversion is endergonic under standard conditions and depends on syntrophic hydrogen removal. Since three acetate residues are released per substrate molecule oxidized, acetate should have a far more pronounced influence on the total energetics. Details of the energetics of metabolism of this bacterium have been discussed earlier (Schink 1991).

Fermentation of Acetone

A special situation is the fermentative conversion of acetone to methane and CO_2, which is catalyzed by syntrophically cooperating bacteria as well. In this case, acetate is the only intermediate between both partners, as illustrated by the following equations:

$$CH_3COCH_3 + CO_2 + H_2O \rightarrow 2CH_3COO^- + 2H^+$$
$$\Delta G_0' = -25.8 \text{ kJ per mol}$$
$$2CH_3COO^- + 2H^+ \rightarrow 2CH_4 + 2CO_2$$
$$\Delta G_0' = -71.8 \text{ kJ per mol}$$
$$CH_3COCH_3 + H_2O \rightarrow 2CH_4 + CO_2$$
$$\Delta G_0' = -97.6 \text{ kJ per mol}$$

Although in this case all partial reactions are exergonic under standard conditions, the primary fermenting bacterium depends on the methanogenic partner, and acetone degradation in the mixed culture is substantially impaired in the presence of

acetylene as an inhibitor of methanogens (Platen and Schink 1987). Experiments with the primary acetone-fermenting bacterium in dialysis cultures revealed that acetate accumulation at concentrations higher than 10 mM inhibited growth and acetone degradation (Platen et al. 1994). Under these conditions, the free energy available to the acetone fermenter is still in the range of −40 kJ per mol. Since acetone metabolism by these bacteria starts with an endergonic carboxylation reaction, this might be the amount of energy that they need to invest into this primary substrate activation reaction, perhaps through a membrane-associated enzyme system (Dimroth 1987). Unfortunately, the acetone-fermenting bacterium has not been obtained in pure culture yet, and hence detailed studies on its biochemistry and energetics could not be performed yet.

Interspecies Metabolite Transfer

Although hydrogen due to its small size and fast diffusion appears to be an ideal carrier for electrons between bacteria of different metabolic types, formate also can act in a similar manner. A possible alternative involvement of formate in such electron transfer processes had been considered from the very beginning (Bryant et al. 1967; McInerney and Wofford 1992) because the original partner bacteria used could oxidize both hydrogen and formate. The standard redox potential of the CO_2/formate couple is nearly identical with that of H^+/H_2 at pH 7.0 (−420 vs. −414 mV), and hence the energetic problems are the same with both. Because both electron carrier systems couple inside the cell with similar, if not the same, electron transfer components, e.g., ferredoxins, most bacteria involved in interspecies electron transfer exchange hydrogen against formate and vice versa (e.g., Wu et al. 1993; Bleicher and Winter 1994). This renders a differentiation between both electron transfer systems rather difficult, and in many cases, both carriers may even be used simultaneously (Schink 1991). Thus, a formate/CO_2 shuttle could replace hydrogen transfer, and this idea has been brought up again on the basis of experiments with undefined floc cultures from methanogenic fermenters (Thiele and Zeikus 1988) and with pure cultures (Thiele and Zeikus 1988; Zindel et al. 1988), as well as on the basis of calculations of diffusion kinetics (Boone et al. 1989a, b). Biochemical and genomic information supports the combined occurrence of hydrogen and formate transfer (Müller et al. 2010). Even electron transfer via conductive nanowires has been proposed in cocultures of *P. thermopropionicum* (Gorby et al. 2006).

Exclusive action of hydrogen as electron carrier has been proven so far only for the butyrate-oxidizing coculture *Syntrophomonas wolfei* (Wofford et al. 1986), the glycolate-oxidizing *Syntrophobotulus glycolicus* (Friedrich and Schink 1993), and the thermophilic, syntrophically acetate-oxidizing strain AOR (Lee and Zinder 1988a, b, c); all these strains exhibit in coculture high hydrogenase and very little formate dehydrogenase activity. Syntrophic oxidation of propionate by *Syntrophobacter fumaroxidans* or of butyrate by *Syntrophospora bryantii* requires partner bacteria that are able to use both hydrogen and formate, indicating that both carriers are involved in interspecies electron transfer (Dörner 1992; Dong et al. 1994a, b; Dong and Stams 1995a, b). Nonetheless, hydrogenase activities in these cultures exceeded formate dehydrogenase activities substantially indicating that hydrogen played a dominant role as well. Similar conclusions were drawn from hydrogen and formate transfer experiments with thermophilic granular sludge preparations and different partner bacteria (Schnürer et al. 1994). In isobutyrate-degrading cocultures, formate appeared to play a role besides hydrogen as an electron carrier (Wu et al. 1996). On the basis of calculations of diffusion kinetics (Boone et al. 1989a, b), one can speculate that formate/CO_2 would be the preferred electron transfer system in suspended cultures of single cells where the carrier molecule has to diffuse over long distances through an aqueous phase, whereas hydrogen would be more efficient in densely packed aggregates which dominate in anaerobic digestors and probably also in sediments.

A key problem in an assessment of the relative importance of formate as electron shuttle is a reliable measurement of formate at low concentrations: At 0.3 bar CO_2 as typical of, e.g., sewage sludge or sediments, a hydrogen partial pressure of 10^{-4} to 10^{-5} bar (as required for the redox reactions discussed) is equivalent to formate concentrations of about 1–10 μM (Schink 1994). Whereas hydrogen partial pressures can today be measured reliably down to 10^{-7} bar with mercury oxide–based detectors, there is hardly an efficient method available which allows measurement of formate in concentrations of few micromolar and less. Moreover, most indications of formate formation were obtained in the presence of inhibitors that caused accumulation of measurable amounts of formate, but it remains doubtful whether such experiments describe adequately the electron flow in the undisturbed system.

Interestingly, besides the fact that methanogenic conversion can be driven by interspecies hydrogen and formate transfer, formate maybe a substrate for syntrophic growth as was demonstrated for mesophilic and thermophilic cocultures of anaerobic bacteria and hydrogen-consuming archaea (Dolfing et al. 2008).

Beyond hydrogen and formate, acetate is also excreted by syntrophically fermenting bacteria and is further metabolized by methanogens. The model used above shows that also acetate removal can have a profound influence on the total energetics of syntrophic degradation of fatty acids or benzoate. Its importance may be even higher with, e.g., isovalerate degradation, since three molecules of acetate and only one hydrogen molecule are formed (*Table 21.3*). Indeed, inhibition by acetate accumulations or by addition of acetate has been reported for syntrophic degradation of fatty acids and for benzoate (Ahring and Westermann 1988; Fukuzaki et al. 1990; van Lier et al. 1993; Warikoo et al. 1996; Schöcke and Schink 1997).

In the acetate-oxidizing coculture of *Geobacter sulfurreducens* with *Wolinella succinogenes* mentioned above, we observed that a cysteine/cystine cycle acts as electron carrier between both partners (Kaden et al. 2002). The use of this carrier system of higher redox potential ($E^{\circ\prime}$ = −270 mV) than the hydrogen or formate system makes sense in the coupling to the

comparably positive electron acceptor used by this coculture. Perhaps similar sulfur-based carriers are active also in other syntrophic associations which exhibit fast electron transfer to an acceptor system of comparably high redox potential (Daniel et al. 1999; Meckenstock 1999). In the natural environment, also exogenous electron carriers such as humic substances may play a role as interspecies electron carriers, as suggested for electron transfer from iron-reducing bacteria to insoluble iron minerals (Lovley et al. 1996). Cervantes et al. (2000) observed recently that the oxidation of phenolic compounds in methanogenic sewage sludge can be coupled to the reduction of the humic compound analogue anthraquinone-2,6-disulfonate, indicating that this acceptor could perhaps be reduced also by syntrophic phenol oxidizers.

Anaerobic Methane Oxidation

Anaerobic, sulfate-dependent methane oxidation is an important reaction in anoxic marine sediments, as documented on the basis of measurements of sulfate and methane gradients in such sediments (Reeburgh 1980; Iversen and Jørgensen 1985). The process could also be demonstrated with radiolabeled methane in anoxic incubations of sediment samples. Zehnder and Brock (1979) showed by similar experiments that methanogenic bacteria were responsible for this oxidation of labeled methane and that methane oxidation and formation proceeded simultaneously. However, in their assays, methane production always exceeded methane oxidation by two to three orders of magnitude, and thus this process could hardly explain the net methane oxidation observed in sediments.

From a thermodynamic point of view, sulfate-dependent methane oxidation is an exergonic reaction

$$CH_4 + SO_4^{2-} + 2H^+ \rightarrow CO_2 + H_2S + 2H_2O$$

which yields under standard conditions a $\Delta G_0'$ of -18 kJ per mol. Concentrations of the reaction partners in situ in the active sediment layers are in the range of 10^{-2} bar methane and 1–3 mM of both sulfate and free hydrogen sulfide. Thus, the overall energetics become only slightly more favorable if in situ conditions are taken into consideration. This amount of energy can feed only one bacterium, provided that the bacterium is able to exploit this biological minimum energy quantum. Based on the observation that methanogens can catalyze an oxygen-independent methane oxidation (Zehnder and Brock 1979) and the description of a reversal of homoacetogenic fermentation by strain AOR and others (see above; *Table 21.3*), it was speculated that "reversed methanogenesis" may be the key to an understanding of this process (Hoehler et al. 1994; Schink 1997). If the overall reaction is actually a syntrophic cooperation involving a methanogen-running methane formation backward and a sulfate-reducing bacterium, it is obvious that only one of the partners can gain metabolic energy from the reaction, and the other one has to run this process only as a cometabolic activity. This would explain at least why scientists have always failed to enrich for methane-oxidizing sulfate reducers in the past, simply because one cannot enrich for a bacterium on the basis of a cometabolic activity.

Through the last 10 years, our picture of anaerobic methane oxidation has developed dramatically (Valentine and Reeburgh 2000). Analysis of lipids of marine archaea and sulfate-reducing bacteria in anoxic sediment layers indicated that these organisms fed on (^{13}C-depleted) methane (Pancost et al. 2000), and similar findings, combined with molecular population analysis, were reported for archaeal/bacterial communities in marine sediments and close to submarine methane seeps and gas hydrates (Hinrichs et al. 1999; Orphan et al. 2001; Thomsen et al. 2001). In sediments overlying methane hydrates off the coast of Oregon, United States, active anaerobic methane oxidation was found to be associated with discrete, spherical microbial aggregates which consisted, according to fluorescent in situ hybridization analysis (FISH), of *Methanosarcina*-like archaea in the center, surrounded by *Desulfosarcina*-related sulfate-reducing bacteria (Boetius et al. 2000). The energetics of sulfate-dependent methane oxidation at these gas hydrate sites (with methane pressures of about 80 bar) are considerably more favorable than in deep-lying marine sediments, and the overall free energy change of the reaction in situ (-40 kJ per mol) may really allow sufficient energy conservation and growth for both partners in this cooperation. In the meantime, thus, these aggregates represent a first model system to understand sulfate-dependent methane oxidation as a syntrophic cooperation phenomenon, but it still needs to be proven whether this model can also be applied to methane oxidation in deep-lying, methane-poor marine sediments that are being cultivated in the lab at high methane pressures (Nauhaus et al. 2007), and several new examples of syntrophically methane-oxidizing associations have been described which are grouped in two different groups, ANME-I and ANME-II (Nauhaus et al. 2005). The question remains open how the partners involved cooperate. Evidence available so far indicates that neither hydrogen, nor formate, acetate, or methanol is transferred.

"Obligately Syntrophic" Bacteria: Cultivation and Biochemical Studies

The mutual dependence of partner bacteria in syntrophic associations has caused severe difficulties in the cultivation of such organisms; nonetheless, defined cocultures have been obtained in numerous cases. For isolation, pure cultures of known methanogenic or sulfate-reducing partner bacteria are usually provided in excess as a background "lawn" during the cultivation and dilution process to isolate the syntrophically fermenting bacterium in defined binary or ternary mixed culture. Today, all well-described syntrophically fermenting bacteria can be cultivated also in pure culture with different substrate combinations (see below). Hence, we should no longer talk about "obligately syntrophic bacteria" (because they are not obligately syntrophic) but only about syntrophic relationships or syntrophic conversion processes.

Many efforts have been made to grow syntrophically fermenting bacteria in the absence of partner bacteria. Removal of hydrogen by nonbiological procedures (low pressure and gas diffusion through thin membranes) had only little success with ethanol oxidation, and no success at all with fatty acid oxidation. In other cases, hydrogen removal by palladium catalysts spread on either charcoal or $CaCO_3$ surfaces, with alkenes or alkynes as oxidant have shown some success (Mountfort and Kaspar 1986), as did efforts to couple hydrogen release to reoxidation by electrochemically controlled platinum electrodes. Whether a cultivation apparatus maintaining the hydrogen partial pressure at subnanomolar concentrations (Valentine et al. 2000a, b) will hold more promise has still to be proven. More successful was the use of fumarate as external electron acceptor in cultivation of syntrophic propionate degraders (Stams et al. 1993). Today, pure cultures of syntrophically fermenting bacteria of all known metabolic types have been isolated. Typically, this has been accomplished with substrates that are more oxidized than the original one and can be fermented by dismutation. As an example, ethanol-oxidizing syntrophs can be grown in pure culture with acetaldehyde analogues such as acetoin or acetylene (Schink 1985a; Eichler and Schink 1986), butyrate- or benzoate-degrading syntrophs with crotonate (Beaty and McInerney 1987; Zhao et al. 1989; Wallrabenstein et al. 1995a) or with pentenoate as external electron acceptor (Dong et al. 1994a), and syntrophically propionate-degrading bacteria with pyruvate (Wallrabenstein et al. 1994) or propionate plus fumarate (Stams et al. 1993). Beyond that, all syntrophic propionate oxidizers (with the exception of *Smithella propionica*) have been shown to be able also to reduce sulfate, and can be isolated in pure culture with propionate plus sulfate, although they grow only very slowly with this substrate combination (Harmsen et al. 1993, 1995; Wallrabenstein et al. 1994, 1995b).

Biochemical studies with defined cocultures of syntrophically fermenting bacteria have been carried out successfully with cell-free extracts prepared by, e.g., lysozyme (Wofford et al. 1986) or mutanolysin (Wallrabenstein and Schink 1994) treatment, which opens selectively only the fermenting bacterium and leaves the methanogenic partner intact, owing to its archaeal cell wall chemistry. In another approach, the partner organisms were separated by centrifugation in Percoll gradients before cell disruption and enzyme assays (Beaty et al. 1987).

Homoacetogenic Versus Methanogenic Coupling and the Effect of Temperature

Under standard conditions, methanogenic hydrogen oxidation yields more energy than homoacetogenic hydrogen oxidation (*Table 21.2*), and one would therefore assume that homoacetogens have little chance to compete successfully against methanogens for hydrogen at limiting concentrations. The function of homoacetogens in the complex electron flow scheme depicted in *Fig. 21.1* remains unclear, therefore, and the general assumption is that they take advantage of their metabolic versatility, which allows them to compete with several partners of various metabolic types, and consume two or more substrates simultaneously (Schink 1994). Such simultaneous utilization of more than one substrate may increase the effective affinity for every single substrate, as studies with *Escherichia coli* in continuous cultures with multiple substrate supply have proven (Egli 1995; Lendenmann et al. 1996). However, there are exceptional situations in which homoacetogens may definitively outcompete methanogens in their function as hydrogen consumers in sulfate-poor anoxic environments. One such situation may be slightly acidic lake sediments such as that found in Knaack Lake, Wisconsin, United States, where at pH 6.1 the total electron flow goes through the acetate pool, and no methane is formed by direct CO_2 reduction (Phelps and Zeikus 1984). Obviously, hydrogen-oxidizing methanogens do not perform sufficiently well under these conditions. Thus, homoacetogens take over their function, however, only at low acetate concentration (at 10^{-4} bar H_2 and 10 μM acetate); homoacetogenic hydrogen oxidation yields a ΔG of -26 kJ per mol of acetate and has to be maintained by aceticlastic methanogens.

Temperature is a further effector which improves the ability of homoacetogens to compete successfully against methanogens for hydrogen. At temperatures lower than 20°C, homoacetogens appear to take over significant parts of hydrogen oxidation in paddy soil and lake sediments (Conrad et al. 1989; Conrad and Wetter 1990). The known species of hydrogen-oxidizing methanogens are not significantly active at such temperatures (Zeikus and Winfrey 1976), and homoacetogens appear to be less restricted in this respect. Dominance of homoacetogenesis in the total electron flow is even more expressed in tundra wetland soils at temperatures lower than 10°C (Kotsyurbenko et al. 1996). This effect becomes understandable from a look at the temperature dependence of hydrogen-dependent methanogenesis and homoacetogenesis. At hydrogen partial pressures lower than 10^{-4} bar and acetate concentrations at 10 mM, homoacetogenesis reaches at 5°C the same energy gain as hydrogen-dependent methanogenesis does at 35°C. Thus, the general scheme of electron flow in methanogenic environments (*Fig. 21.1*) has to be modified for slightly acidic or for low-temperature habitats (*Fig. 21.3a*). Under these conditions, there is no significant hydrogen-dependent methanogenesis (group 2), and the electrons flow nearly exclusively via acetogenesis and aceticlastic methanogenesis (groups 5 and 3).

The opposite situation emerges at high-temperature habitats as discussed already above in the context of syntrophic acetate oxidation. Under these conditions, homoacetogenesis can operate in the opposite direction, and the electron flow goes from acetate through the C1 pool and hydrogen toward methane (*Fig. 21.3b*). Thus, the general flow scheme in *Fig. 21.1* represents kind of an intermediate situation that probably describes the situation of a sewage sludge digestor correctly but has to be modified for high- and low-temperature situations in the way indicated.

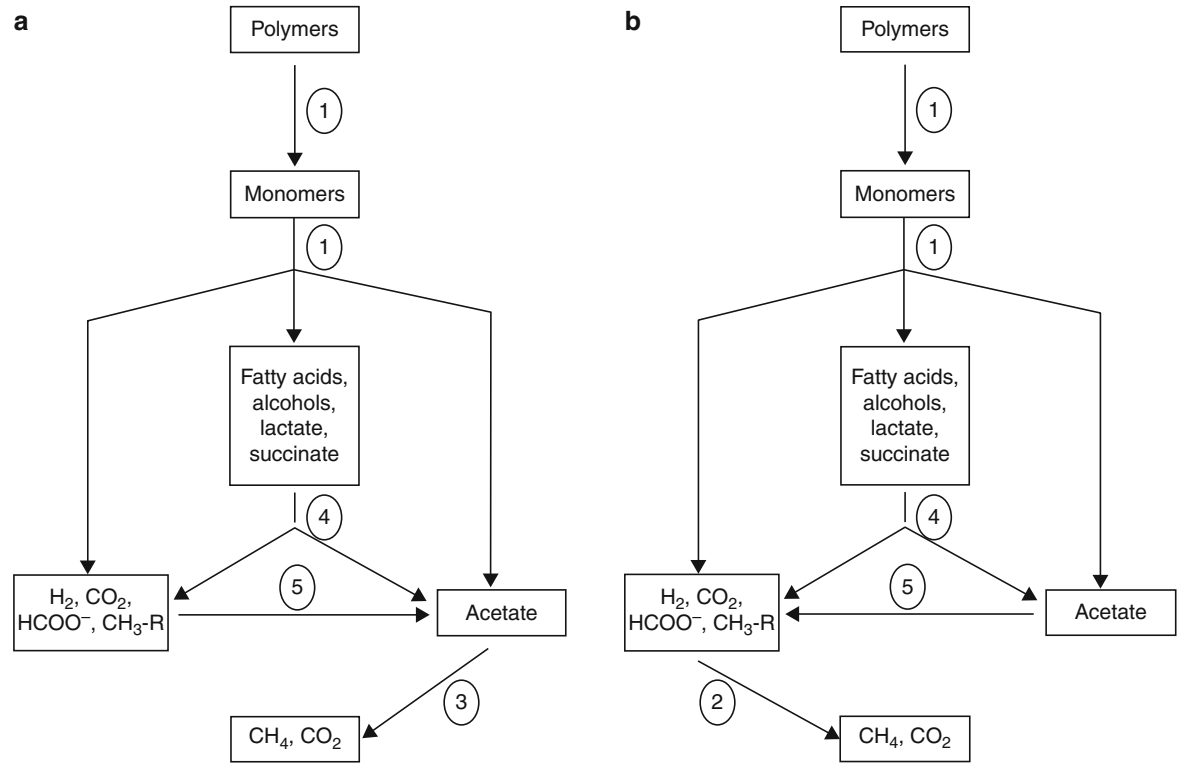


Fig. 21.3
Carbon and electron flow in methanogenic degradation of complex organic matter. Groups of prokaryotes involved: *1* primary fermentative bacteria, *2* hydrogen-oxidizing methanogens, *3* acetate-cleaving methanogens, *4* secondary fermenting bacteria (syntrophs), and *5* homoacetogenic bacteria. Carbon and electron flow (a) at low temperature and (b) at high temperature

Structure and Growth Dynamics of Syntrophic Associations

The separation of metabolic functions and their distribution within metabolically different microorganisms, i.e., substrate oxidation and hydrogen formation in the one and hydrogen oxidation and CO_2 reduction in the other organism, is a rather unusual strategy that we do not observe, at least not to this extent, in oxic environments. It may have its advantages because it allows a high degree of metabolic specialization to the single organisms concerned, which requires very little effort into refined regulation of energy metabolism. However, the metabolic efficiency of such cooperating communities depends on an efficient metabolite transfer between the partners involved: The flux of, e.g., hydrogen between the hydrogen-forming fermenter and the hydrogen-consuming methanogen is inversely proportional to the distance between both (Schink and Thauer 1988). Optimal metabolite transfer can be achieved best when both partners are in close contact (i.e., directly attached to each other, forming an aggregate or floc). Such flocs form preferentially in anaerobic digestors in which fatty acids are degraded, although the establishment of stable floc formation may require substantial amounts of time, sometimes even several months after start-up (Lettinga et al. 1988). Indirect evidence of aggregate formation in sediments and sludges has been obtained on the basis of hydrogen exchange measurements (Conrad et al. 1985, 1986), and the recently discovered aggregates involved in sulfate-dependent methane formation (Boetius et al. 2000) exhibit even a high degree of spatial organization.

Efforts to synthesize stable aggregates (granules) of microbiologically defined composition from pure cultures have been made, and the success depended very specifically on the type of partners used. Butyrate-degrading aggregates with only two partners were stable only with *Methanobacterium formicicum*, not with *Methanospirillum hungatei*, whereas propionate-degrading granules depended on the additional presence of an acetate-degrading partner (Wu et al. 1996). In suspended cultures, *M. hungatei* is often the dominant hydrogen utilizer, but it is obviously not the ideal partner for formation of efficient granules. This experiment demonstrates again that our enrichment and cultivation techniques influence to a high degree the results obtained and may give a rather incorrect picture of the situation prevailing in the natural or seminatural system. The composition and stability of the formed granules are further influenced by the fact that methanogenic granules in waste-degrading reactors have to deal with mixed substrate supplies that add further microbial constituents into the architecture of the resulting granule, including, e.g., sulfate-reducing bacteria (Wu et al. 1991). In any case, optimal cooperation will be secured in granules in which the partner organisms are randomly mixed to near homogeneity, rather than in situations in which the partners form "nests" of identical subpopulations.

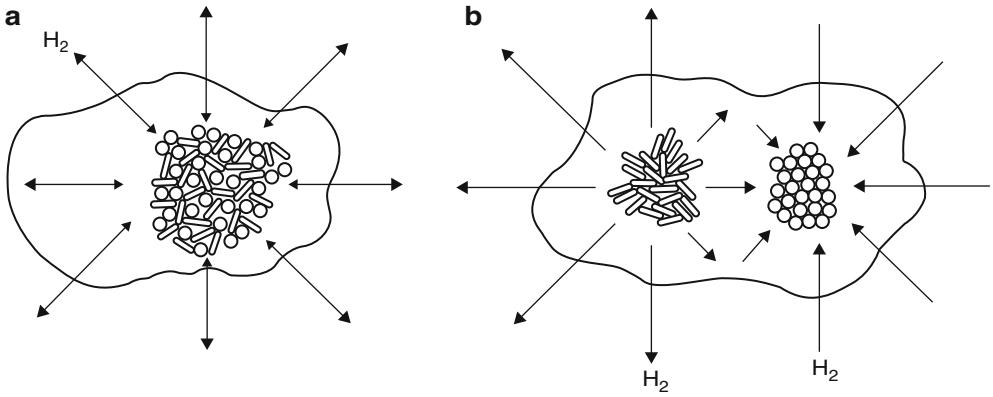


Fig. 21.4
Exchange of hydrogen (or other intermediates) in anaerobic bacterial flocs containing (a) a homogenously mixed community of hydrogen formers and hydrogen consumers or (b) hydrogen producers and hydrogen consumers in spatially separated nests

A basic problem remains in that each partner bacterium multiplies and produces offspring only of its own kind. Thus, the situation given in *Fig. 21.4a* will change after several generations into one similar to that depicted in *Fig. 21.4b*, and the efficiency of metabolite transfer will decrease with age of the aggregates unless there are ways of internal mixing of the aggregates. Thin sections of methanogenic granules have shown examples of fully mixed as well as of nest structures (Dubourgier et al. 1988), and similar pictures were recently obtained with thin sections in which the partner bacteria were identified by specifically RNA-directed probes (Harmsen et al. 1996). The largely segregated arrangement of the two partners in the spherical aggregates active in syntrophic sulfate-dependent methane oxidation (Boetius et al. 2000) probably represents a terminal stage in the growth of these partners, and the rather regular size of these aggregates indicates that it is limited by metabolite diffusion kinetics. The dynamics of growth and internal structure development in syntrophic aggregates appears to be an interesting object of research now that gene probes provide excellent tools for direct in situ identification of the various microbial components involved. First efforts in this respect have shown that there are clear functional separations between the subpopulations in granular sludge, especially if also some sulfate is available for fatty acid oxidation (Oude Elferink et al. 1998; Santegoeds et al. 1999). A similar study on syntrophic propionate oxidizers in thermophilic granular sludge revealed that these organisms were localized mainly in the centers of the granules, closely associated with hydrogen-scavenging methanogens (Imachi et al. 2000).

It may be worth mentioning in this context that the observed maximum specific growth rates of syntrophic amino acid-degrading associations are much lower than those of other amino acid-fermenting bacteria. For example, the μ_{max} of *Clostridium* sp. growing on glutamate is 0.3–0.6 h^{-1} (Laanbroek et al. 1979) and that of *S. acidaminophila* is 0.13 h^{-1} (Nanninga et al. 1987). These bacteria use a butyric acid and propionic acid fermentation for the degradation of glutamate, respectively. However, the μ_{max} of *A. hydrogenoformans* growing on glutamate in syntrophic association with a hydrogenotrophic anaerobe is only 0.10 h^{-1}. The μ_{max} of a *Campylobacter* sp. growing on aspartate is about 0.17 h^{-1} (Laanbroek et al. 1978), whereas the μ_{max} of a coculture of *E. acidaminophilum* and a methanogen is below 0.1 h^{-1} (Zindel et al. 1988). Nevertheless, in environments with a high methanogenic activity, e.g., granular sludge from anaerobic bioreactors, high numbers of bacteria can be counted which grow syntrophically with methanogens. Three explanations can be given for this: (1) the growth rates of the syntrophic associations at low substrate concentrations are higher than those of other amino acid-fermenting bacteria, i.e., the syntrophic associations have a higher affinity for the substrate; (2) the syntrophic associations grow on mixtures of substrates rather than on single substrates; and (3) the growth rates of the syntrophic associations are higher than the ones that have been measured. The reported growth rates of the cocultures refer to suspended growth, whereas in methanogenic biofilms and aggregates, growth rates might be much higher because of the shorter interbacterial distances. These observations give further support to the assumption that the growth rates of syntrophic associations are limited by the rate of interspecies metabolite transfer.

Cooperation with Protozoa, Hydrogenosomes

The function of the primary fermenting bacteria (group 1) in conversion of complex organic matter to methane and CO_2 (*Fig. 21.1*) may be taken over also by eukaryotic organisms. Anaerobic fungi, ciliates and flagellates, are known to thrive in entirely anoxic environments under reducing conditions (Finlay and Fenchel 1992; Fenchel and Finlay 1995), and some of them are extremely oxygen sensitive. Since aerobic respiration is not possible in such habitats, anaerobic protozoa do not contain mitochondria. Instead, intracellular organelles are present which release hydrogen and have been called "hydrogenosomes." The metabolism of these protozoa is fermentative; particles, especially bacterial cells, are ingested into food vacuoles and digested

by hydrolysis and further fermentation, and acetate is probably the most important fermentation product.

Anaerobic protozoa can be associated with symbiotic methanogens, either extracellularly or intracellularly. Ciliates living in strictly anoxic, eutrophic sediments carry methanogenic partner bacteria inside the cell (van Bruggen et al. 1983, 1985), often closely associated with the hydrogenosomes. The advantage of this cooperation with hydrogenotrophic methanogens for the protozoan host is obvious: Removal of hydrogen and maintenance of a low hydrogen/formate concentration in the cell allow fermentation of complex organic matter mainly to acetate and CO_2. Thus, waste of organic precursors into reduced end products such as ethanol, fatty acids, etc. can be avoided, and the fermenting protozoon obtains a maximum ATP yield. The symbiotic methanogen takes over part of the function that mitochondria have in aerobic higher cells. Reducing equivalents are removed by the symbiotic partner, and the eukaryotic host cell runs a fermentative metabolism with maximum ATP yield.

It is assumed that the hydrogen released by hydrogenosomes stems mainly from pyruvate oxidation to acetyl-CoA (pyruvate synthase reaction; Müller 1988). Hydrogenosomes contain this enzyme, as well as ferredoxin and hydrogenase. In some cases, especially with the larger types of anaerobic protozoa, close associations of methanogenic endosymbionts with hydrogenosomes have been observed. Smaller protozoa may achieve the same effect of hydrogen release also with extracellular partner bacteria because the diffusion distance to the surface may be short enough. The same applies to the rumen ciliates which in their comparably rich habitat cooperate only occasionally with symbiotic partners on their cell surface (Stumm et al. 1982).

Hydrogenosome and methanogenic endosymbiont together form a functional entity. In some cases, especially with the comparably big ciliates such as *Plagiopyla frontata*, hydrogenosomes and methanogens are organized in an alternating sandwich arrangement that allows optimal hydrogen transfer in highly refined structures (Finlay and Fenchel 1992). It has been speculated that hydrogenosomes of strictly anaerobic protozoa have evolved from the mitochondria of their aerobic predecessors; other speculations assume a relationship of hydrogenosomes to clostridia. The high structural development of hydrogenosomes in some protozoa may suggest that such arrangements could operate also in transfer of hydrogen from less easily available electron donors than the pyruvate synthase system represents. Unfortunately, detailed studies on the cooperation of methanogenic endosymbionts with their protozoan hosts have been hampered so far by extreme difficulties in handling defined cultures of strictly anaerobic protozoa.

Taxonomy of Syntrophs

The survey of described bacteria active in syntrophic oxidation of alcohols, fatty acids, and aromatic compounds (*Table 21.4*) documents that these bacteria are found only in two groups within the taxonomic system based on sequence similarities of the 16S rRNA, namely, the Gram-positive bacteria with low G + C content and the Δ-proteobacteria. Some metabolic specializations are clustered in certain taxonomic groups, e.g., ethanol, propionate, and benzoate oxidation in the Δ-proteobacteria (with the exception of *Thermoanaerobium brockii*), or fatty acid β-oxidation and acetate oxidation in the Gram-positive bacteria with low G+C content. Nonetheless, these groups are not really homogenous but encompass representatives of many other metabolic types, e.g., sulfate reducers that do not exhibit any tendency to transfer electrons to partner organisms. Finally, as pointed out above, the fermentative degradation of amino acids includes so many different expressions of obligate and facultative syntrophy within taxonomically extremely different groups of organisms that syntrophy can hardly be assumed to be associated with a single evolutionary trait. Rather, syntrophy appears to be kind of a lifestyle that is experienced and perfected by many different organisms to varying extents.

Conclusions

The energetics of syntrophic fatty acid and alcohol-oxidizing processes represent exciting examples of energy metabolism based on the smallest energy quantum that, to our present understanding, can be exploited by living cells. This minimum amount of energy which can be converted into ATP in the living cell is in the range of −20 kJ per reaction run or even lower, and this is the amount of energy available to the respective partners in most of the degradation processes discussed here. Models of metabolic cooperation and energy sharing between syntrophic partners can be based on this assumption, and experimental evidence of reverse electron transport systems to balance the energy requirements for hydrogen release have been obtained in several instances.

Recent studies on the biochemistry of syntrophic fatty acid oxidizers revealed that these bacteria are by no means "primitive" but actually admirable creatures from the point of view of energy conservation and efficient energy utilization. Most of these bacteria grow in plain mineral media and synthesize all their cellular components on the basis of only the minimum quantum of energy which can be exploited by living cells at all. They are spectacular examples of how diligently nature has organized the components of global energy flux down to those environments where very little energy is available to their living inhabitants.

Acknowledgments

The authors express their gratitude to Marvin P. Bryant, Ralph Wolfe, Norbert Pfennig, Rudolf Thauer, Fritz Widdel, Alex Zehnder, and Ralf Conrad for numerous fruitful discussions about the biology and energetics of syntrophic anaerobes and to their former and present coworkers for their dedicated work on these extremely fastidious bacteria.

References

Ahring BK, Westermann P (1988) Product inhibition of butyrate metabolism by acetate and hydrogen in a thermophilic coculture. Appl Environ Microbiol 54:2393–2397

Auburger G, Winter J (1992) Purification and characterization of benzoyl-CoA ligase from a syntrophic, benzoate-degrading, anaerobic mixed culture. Appl Microbiol Biotechnol 37:789–795

Baena S, Fardeau M-L, Labat M, Ollivier B, Garcia J-L, Patel BKC (1998) *Aminobacterium colombiense*, gen. nov., sp. nov., an amino acid degrading anaerobe isolated from anaerobic sludge. Anaerobe 4:241–250

Baena S, Fardeau M-L, Ollivier B, Labat M, Thomas P, Garcia J-L, Patel BKC (1999a) *Aminomonas paucivorans* gen. nov., sp. nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. Int J Syst Bacteriol 49:975–982

Baena S, Fardeau M-L, Woo THS, Ollivier B, Labat M, Patel BKC (1999b) Phylogenetic relationships of three amino-acid-utilizing anaerobes, *Selenomonas acidaminovorans*, "*Selenomonas acidaminophila*" and *Eubacterium acidaminophilum*, as inferred from partial 16S rDNA nucleotide sequences and proposal of *Thermanaerovibrio acidaminovorans* gen. nov., comb. nov. and *Anaeromusa acidaminophila* gen. nov, comb. nov. Int J Syst Bacteriol 49:969–974

Baena S, Fardeau M-L, Labat M, Ollivier B, Garcia BJ-L, Patel BKC (2000) *Aminobacterium mobile*, sp. nov., a new anaerobic amino-acid-degrading bacterium. Int J Syst Evol Microbiol 50:259–264

Barik S, Brulla WJ, Bryant MP (1985) PA-1, a versatile anaerobe obtained in pure culture, catabolizes benzenoids and other compounds in syntrophy with hydrogenotrophs, and P-2 plus *Wolinella* sp. degrades benzenoids. Appl Environ Microbiol 50:304–310

Barker HA (1940) Studies upon the methane fermentation. IV: The isolation and culture of *Methanobacterium omelianskii*. Ant v Leeuwenhoek 6:201–220

Barker HA (1981) Amino acid degradation by anaerobic bacteria. Annu Rev Biochem 50:23–40

Beaty PS, McInerney MJ (1987) Growth of *Syntrophomonas wolfei* in pure cultures on crotonate. Arch Microbiol 147:389–393

Beaty PS, McInerney MJ (1989) Effect of organic acid anions on the growth and metabolism of *Syntrophomonas wolfei* in pure culture and in defined consortia. Appl Environ Microbiol 55:977–983

Beaty PS, Wofford NQ, McInerney MJ (1987) Separation of *Syntrophomonas wolfei* from *Methanospirillum hungatei* in syntrophic cocultures by using Percoll gradients. Appl Environ Microbiol 53:1183–1185

Ben-Bassat A, Lamed R, Zeikus JG (1981) Ethanol production by thermophilic bacteria: metabolic control of end product formation in *Thermoanaerobium brockii*. J Bacteriol 146:192–199

Biebl H, Pfennig N (1978) Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Arch Microbiol 117:9–16

Bleicher K, Winter J (1994) Formate production and utilization by methanogens and by sewage sludge consortia—interference with the concept of interspecies formate transfer. Appl Microbiol Biotechnol 40:910–915

Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Giesecke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Boone DR, Bryant MP (1980) Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. Appl Environ Microbiol 40:626–632

Boone DR, Johnson RL, Liu Y (1989a) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems, and implications in the measurement of K_M for H_2 or formate uptake. Appl Environ Microbiol 55:1735–1741

Boone DR, Johnson RL, Liu Y (1989b) Microbial ecology of interspecies hydrogen and formate transfer in methanogenic ecosystems. In: Hattori T, Ishida Y, Maruyama Y, Morita RY, Uchida A (eds) Recent advances in microbial ecology. Japan Scientific Society Press, Tokyo, pp 450–453

Bryant MP (1979) Microbial methane production—theoretical aspects. J Anim Sci 48:193–201

Bryant MP, Wolin EA, Wolin MJ, Wolfe RS (1967) *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch Mikrobiol 59:20–31

Bryant MP, Campbell LL, Reddy CA, Crabill MR (1977) Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl Environ Microbiol 33:1162–1169

Buckel W, Barker HA (1974) Two pathways of glutamate fermentation by anaerobic bacteria. J Bacteriol 117:1248–1260

Cervantes FJ, van der Velde S, Lettinga G, Field JA (2000) Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds. Biodegradation 11:313–321

Chen SY, Liu XL, Dong XZ (2005) *Syntrophobacter sulfatireducens* sp. nov., a novel syntrophic, propionate-oxidizing bacterium isolated from UASB reactors. Int J Syst Evol Microbiol 55:1319–1324

Cheng G, Plugge CM, Roelofsen W, Houwen FP, Stams AJM (1992) *Selenomonas acidaminovorans* sp. nov., a versatile thermophilic proton-reducing anaerobe able to grow by decarboxylation of succinate to propionate. Arch Microbiol 157:169–175

Cherepanov DA, Mulkidjanian AY, Junge W (1999) Transient accumulation of elastic energy in proton translocating ATP synthase. FEBS Lett 449:1–6

Conrad R, Wetter B (1990) Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria. Arch Microbiol 155:94–98

Conrad R, Phelps TJ, Zeikus JG (1985) Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. Appl Environ Microbiol 50:595–601

Conrad R, Schink B, Phelps TJ (1986) Thermodynamics of H_2-consuming and H_2-producing metabolic reactions in diverse methanogenic environments under in situ conditions. FEMS Microbiol Ecol 38:353–360

Conrad R, Bak F, Seitz HJ, Thebrath B, Mayer HP, Schütz H (1989) Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. FEMS Microbiol Ecol 62:285–294

Cord-Ruwisch R, Lovley DR, Schink B (1998) Growth of *Geobacter sulfurreducens* with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. Appl Environ Microbiol 64:2232–2236

Daniel R, Warnecke F, Potekhina JS, Gottschalk G (1999) Identification of the syntrophic partners in a coculture coupling anaerobic methanol oxidation to Fe(III) reduction. FEMS Microbiol Lett 180:197–203

De Bok FAM, Stams AJM, Dijkema C, Boone DR (2001) Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. Appl Environ Microbiol 67:1800–1804

de Bok FAM, Luijten MLGC, Stams AJM (2002) Biochemical evidence for formate transfer in syntrophic propionate-oxidizing cocultures of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Appl Environ Microbiol 68:4247–4252

de Bok FAM, Harmsen HJM, Plugge CM, de Vries MC, Akkermans ADL, de Vos WM, Stams AJM (2005) The first true obligately syntrophic propionate-oxidizing bacterium, *Pelotomaculum schinkii* sp. nov., co-cultured with *Methanospirillum hungatei*, and emended description of the genus *Pelotomaculum*. Int J Syst Evol Microbiol 55:1697–1703

Dimroth K (1983) Thermochemische Daten organischer Verbindungen. In: Synowietz C (ed) D'Ans-Lax Taschenbuch für Chemiker und Physiker, vol 2. Springer, Berlin, pp 997–1038

Dimroth P (1987) Sodium transport decarboxylases and other aspects of sodium ion cycling in bacteria. Microbiol Rev 51:320–340

Dimroth P (2000) Operation of the F0 motor of the ATP synthase. Biochem Biophys Acta 1458:374–386

Dolfing J, Jiang B, Henstra AM, Stams AJM, Plugge CM (2008) Syntrophic growth on formate: a new microbial niche in anoxic environments. Appl Environ Microbiol 74:6126–6131

Dong X, Stams AJM (1995a) Evidence for H_2 and formate formation during syntrophic butyrate and propionate degradation. Anaerobe 1:35–39

Dong X, Stams AJM (1995b) Localization of enzymes involved in H_2 and formate metabolism in *Syntrophospora bryantii*. Ant v Leeuwenhoek 67:345–350

Dong X, Cheng G, Stams AJM (1994a) Butyrate oxidation by *Syntrophospora bryantii* in coculture with different methanogens and in pure culture with pentenoate as electron acceptor. Appl Microbiol Biotechnol 42:647–652

Dong X, Plugge CM, Stams AJM (1994b) Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in coculture and triculture with different methanogens. Appl Environ Microbiol 60:2834–2838

Dörner C (1992) Biochemie und Energetik der Wasserstofffreisetzung in der syntrophen Vergärung von Fettsäuren und Benzoat. Thesis, Universität Tübingen, Tübingen, 58–61

Dubourgier HC, Prensier G, Albagnac G (1988) Structure and microbial activities of granular anaerobic sludge. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff LW (eds) Granular anaerobic sludge: microbiology and technology. Pudoc, Wageningen, pp 18–33

Dwyer DF, Weeg-Aerssens E, Shelton DR, Tiedje JM (1988) Bioenergetic conditions of butyrate metabolism by a syntrophic, anaerobic bacterium in coculture with hydrogen-oxidizing methanogenic and sulfidogenic bacteria. Appl Environ Microbiol 54:1354–1359

Egli T (1995) The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. In: Jones JG (ed) Advances in microbial ecology, vol 14. Plenum, New York, pp 305–386

Eichler B, Schink B (1986) Fermentation of primary alcohols and diols, and pure culture of syntrophically alcohol-oxidizing anaerobes. Arch Microbiol 143:60–66

Elshahed MS, Bhupathiraju VK, Wofford NQ, Nanny MA, McInerney MJ (2001) Metabolism of benzoate, cyclohex-1-ene carboxylate, and cyclohexane carboxylate by "*Syntrophus aciditrophicus*" strain SB in syntrophic association with H_2-using microorganisms. Appl Environ Microbiol 67:1728–1738

Engelbrecht S, Junge W (1997) ATP synthase: a tentative structural model. FEBS Lett 414:485–491

Fenchel T, Finlay BJ (1995) Ecology and evolution in anoxic worlds. Oxford University Press, Oxford, UK, pp 108–171

Fey A, Conrad R (2000) Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. Appl Environ Microbiol 66:4790–4797

Finlay BJ, Fenchel T (1992) Methanogens and other bacteria as symbionts of free-living anaerobic ciliates. Symbiosis 14:375–390

Friedrich M, Schink B (1993) Hydrogen formation from glycolate driven by reversed electron transport in membrane vesicles of a syntrophic glycolate-oxidizing bacterium. Eur J Biochem 217:233–240

Friedrich M, Schink B (1995) Electron transport phosphorylation driven by glyoxylate respiration with hydrogen as electron donor in membrane vesicles of a glyoxylate-fermenting bacterium. Arch Microbiol 163:268–275

Friedrich M, Laderer U, Schink B (1991) Fermentative degradation of glycolic acid by defined syntrophic cocultures. Arch Microbiol 156:398–404

Friedrich M, Springer N, Ludwig W, Schink B (1996) Phylogenetic position of *Desulfofustis glycolicus* gen. nov. sp. nov. and *Syntrophobotulus glycolicus* gen. nov. sp. nov., two strict anaerobes growing with glycolic acid. Int J Syst Bacteriol 46:1065–1069

Fuchs GM, Mohamed ES, Altenschmidt U, Roch J, Lach A, Brackmann R, Lockmeyer C, Oswald B (1994) Biochemistry of anaerobic biodegradation of aromatic compounds. In: Ratledge C (ed) Biochemistry of microbial degradation. Kluwer, Dordrecht, pp 513–553

Fukuzaki S, Nishio N, Shobayashi M, Nagai S (1990) Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate. Appl Environ Microbiol 56:719–723

Girbal L, Ørlygsson J, Reinders BJ, Gottschal JC (1997) Why does *Clostridium acetireducens* not use interspecies hydrogen transfer for growth on leucine? Curr Microbiol 35:155–160

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc Natl Acad Sci USA 103:11358–11363

Gottschalk G (1986) Bacterial metabolism, 2nd edn. Springer, New York

Harmsen H, Wullings B, Akkermans ADL, Ludwig W, Stams AJM (1993) Phylogenetic analysis of *Syntrophobacter wolinii* reveals a relationship with sulfate-reducing bacteria. Arch Microbiol 160:238–240

Harmsen HJM, Kengen HMP, Akkermans ADL, Stams AJM (1995) Phylogenetic analysis of two syntrophic propionate-oxidizing bacteria in enrichment cultures. Syst Appl Microbiol 18:67–73

Harmsen HJM, Kengen HMP, Akkermans ADL, Stams AJM, de Vos WM (1996) Detection and localization of syntrophic propionate-oxidizing bacteria in granular sludge by in situ hybridization using 16S rRNA-based oligonucleotide probes. Appl Environ Microbiol 62:1656–1663

Harmsen HJ, Van Kuijk BL, Plugge CM, Akkermans AD, De Vos WM, Stams AJ (1998) *Syntrophobacter fumaroxidans* sp. nov., a syntrophic propionate-degrading sulfate-reducing bacterium. Int J Syst Bacteriol 48:1383–1387

Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. Int J Syst Evol Microbiol 50:1601–1609

Hattori S, Galushko AS, Kamagata Y, Schink B (2005) Operation of the CO dehydrogenase/acetyl-CoA pathway in both acetate oxidation and acetate formation by the syntrophically acetate-oxidizing bacterium *Thermacetogenium phaeum*. J Bacteriol 187:3471–3476

Heider J, Fuchs G (1997) Anaerobic metabolism of aromatic compounds. Eur J Biochem 243:577–596

Herrmann G, Jayamani E, Mai G, Buckel W (2008) Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. J Bacteriol 190:784–791

Hinrichs KU, Hayes JM, Sylva SP, Brewer PG, DeLong EF (1999) Methane–consuming archaebacteria in marine sediments. Nature 398:802–805

Hoehler TM, Alperin MJ, Albert DB, Martens CS (1994) Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate reducer consortium. Global Biochem Cycles 8:451–463

Hoehler TM, Alperin MJ, Albert DB, Martens CS (2001) Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. FEMS Microbiol Ecol 38:33–41

Houwen FP, Dijkema C, Schoenmakers CHH, Stams AJM, Zehnder AJB (1987) ^{13}C-NMR study of propionate degradation by a methanogenic coculture. FEMS Microbiol Lett 41:269–274

Houwen FP, Plokker J, Stams AJM, Zehnder AJB (1990) Enzymatic evidence for involvement of the methylmalonyl-CoA pathway in propionate oxidation by *Syntrophobacter wolinii*. Arch Microbiol 155:52–55

Imachi H, Sekiguchi Y, Kamagata Y, Ohashi A, Harada H (2000) Cultivation and in situ detection of a thermophilic bacterium capable of oxidizing propionate in syntrophic association with hydrogenotrophic methanogens in a thermophilic methanogenic granular sludge. Appl Environ Microbiol 66:3608–3615

Imachi H, Sakai S, Ohashi A, Harada H, Hanada S, Kamagata Y, Sekiguchi Y (2007) *Pelotomaculum propionicicum* sp. nov., an anaerobic, mesophilic, obligately syntrophic, propionate-oxidizing bacterium. Int J Syst Evol Microbiol 57:1487–1492

Iversen N, Jørgensen BB (1985) Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). Limnol Oceanogr 30:944–955

Jackson BE, Bhupathiraju VK, Tanner RS, Woese CR, McInerney MJ (1999) *Syntrophus aciditrophicus* sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. Arch Microbiol 171:107–114

Kaden J, Galushko AS, Schink B (2002) Cysteine-mediated electron transfer in syntrophic acetate oxidation by cocultures of *Geobacter sulfurreducens* and *Wolinella succinogenes*. Arch Microbiol 178(1):53–58

Koch M, Dolfing J, Wuhrmann K, Zehnder AJB (1983) Pathway of propionate degradation by enriched methanogenic cultures. Appl Environ Microbiol 45:1411–1414

Kosaka T, Kato S, Shimoyama T, Ishii S, Abe T, Watanabe K (2008) The genome of *Pelotomaculum thermopropionicum* reveals niche-associated evolution in anaerobic microbiota. Genome Res 18:442–448

Kosaka T, Uchiyama T, Ishii S, Enoki M, Imachi H, Kamagata Y, Ohashi A, Harada H, Ikenaga H, Watanabe K (2006) Reconstruction and regulation of the central catabolic pathway in the thermophilic propionate-oxidizing syntroph *Pelotomaculum thermopropionicum*. J Bacteriol 188:202–210

Kotsyurbenko OR, Nozhevnikova AN, Soloviova TI, Zavarzin GA (1996) Methanogenesis at low temperatures by microflora of tundra wetland soil. Ant v Leeuwenhoek 69:75–86

Kremer DR, Nienhuis-Kuiper HE, Hansen TA (1988) Ethanol dissimilation in *Desulfovibrio*. Arch Microbiol 150:552–557

Kung JW, Löffler C, Dörner K, Heintz D, Gallien S, Van Dorsselaer A, Friedrich T, Boll M (2009) Identification and characterization of the tungsten-containing class of benzoyl-coenzyme A reductases. Proc Natl Acad Sci USA 106:17687–17692

Kung JW, Baumann S, von Bergen M, Müller M, Hagedoorn PL, Hagen WR, Boll M (2010) Reversible biological Birch reduction at an extremely low redox potential. J Am Chem Soc 132:9850–9856

Laanbroek HJ, Stal LJ, Veldkamp H (1978) Utilization of hydrogen and formate by *Campylobacter* spec. under aerobic and anaerobic conditions. Arch Microbiol 119:99–102

Laanbroek HJ, Smit AJ, Klein-Nulend G, Veldkamp H (1979) Competition for glutamate between specialized and versatile *Clostridium* species. Arch Microbiol 120:330–335

Lee MJ, Zinder SH (1988a) Carbon monoxide pathway enzyme activities in a thermophilic anaerobic bacterium grown acetogenically and in a syntrophic acetate-oxidizing coculture. Arch Microbiol 150:513–518

Lee MJ, Zinder SH (1988b) Hydrogen partial pressures in a thermophilic acetate-oxidizing methanogenic cocultures. Appl Environ Microbiol 54:1457–1461

Lee MJ, Zinder SH (1988c) Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H_2-CO H_2. Appl Environ Microbiol 54:124–129

Lendenmann U, Snozzi M, Egli T (1996) Kinetics of the simultaneous utilization of sugar mixtures by *Escherichia coli* in continuous culture. Appl Environ Microbiol 62:1493–1499

Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff LW (eds) (1988) Granular anaerobic sludge: microbiology and technology. Pudoc, Wageningen

Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. Int J Syst Bacteriol 49:545–556

Löffler C, Kuntze K, Vazquez JR, Rugor A, Kung JW, Böttcher A, Boll M (2011) Occurrence, genes and expression of the W/Se-containing class II benzoyl-coenzyme A reductases in anaerobic bacteria. Environ Microbiol 13:696–709

Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC (1996) Humic substances as electron acceptors for microbial respiration. Nature 382:445–448

Matthies C, Schink B (1992) Reciprocal isomerization of butyrate and isobutyrate by strain WoGl3, and methanogenic isobutyrate degradation by a defined triculture. Appl Environ Microbiol 58:1435–1439

Matthies C, Schink B (1993) Anaerobic degradation of long-chain dicarboxylic acids by methanogenic enrichment cultures. FEMS Microbiol Lett 111:177–182

McInerney MJ (1988) Anaerobic hydrolysis and fermentation of fats and proteins. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 373–415

McInerney MJ, Wofford NQ (1992) Enzymes involved in crotonate metabolism in *Syntrophomonas wolfei*. Arch Microbiol 158:344–349

McInerney MJ, Bryant MP, Pfennig N (1979) Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch Microbiol 122:129–135

McInerney MJ, Bryant MP, Hespell RB, Costerton JW (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. Appl Environ Microbiol 41:1029–1039

McInerney MJ, Rohlin L, Mouttaki H, Kim U, Krupp RS, Rios-Hernandez L, Sieber J, Struchtemeyer CG, Bhattacharyya A, Campbell JW, Gunsalus RP (2007) The genome of *Syntrophus aciditrophicus*: life at the thermodynamic limit of microbial growth. Proc Natl Acad Sci USA 104:7600–7605

McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B, Rohlin L, Gunsalus RP (2008) Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. Ann N Y Acad Sci 1125:58–72

Meckenstock RU (1999) Fermentative toluene degradation in anaerobic defined syntrophic cocultures. FEMS Microbiol Lett 177:67–73

Meijer WG, Nienhuis-Kuiper ME, Hansen TA (1999) Fermentative bacteria from estuarine mud: phylogenetic position of *Acidaminobacter hydrogenoformans* and description of a new type of Gram-negative, propionigenic bacterium as *Propionibacter pelophilus* gen. nov., sp. nov. Int J Syst Bacteriol 49:1039–1044

Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev Camb Phil Soc 41:445–502

Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch Microbiol 133:249–256

Mountfort DO, Kaspar HF (1986) Palladium-mediated hydrogenation of unsaturated hydrocarbons with hydrogen gas released during anaerobic cellulose degradation. Appl Environ Microbiol 52:744–750

Müller M (1988) Energy metabolism of protozoa without mitochondria. Annu Rev Microbiol 42:465–488

Müller N, Stingl U, Griffin BM, Schink B (2008) Dominant sugar utilizers in sediment of Lake Constance depend on syntrophic cooperation with methanogenic partner organisms. Environ Microbiol 10:1501–1511

Müller N, Schleheck D, Schink B (2009) Involvement of NADH: acceptor oxidoreductase and butyryl-CoA dehydrogenase in reversed electron transport during syntrophic butyrate oxidation by *Syntrophomonas wolfei*. J Bacteriol 191:6167–6177

Müller N, Worm P, Schink B, Stams AJM, Plugge CM (2010) Syntrophic butyrate and propionate oxidation processes: from genomes to reaction mechanisms. Environ Microbiol Rep 2:489–499

Nagase M, Matsuo T (1982) Interaction between amino-acid degrading bacteria and methanogenic bacteria in anaerobic digestion. Biotechnol Bioeng 24:2227–2239

Nanninga HJ, Gottschal JC (1985) Amino acid fermentation and hydrogen transfer in mixed cultures. FEMS Microbiol Ecol 31:261–269

Nanninga HJ, Drent WJ, Gottschal JC (1987) Fermentation of glutamate by *Selenomonas acidaminophila* sp. nov. Arch Microbiol 147:152–157

Nauhaus K, Treude T, Boetius A, Kruger M (2005) Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. Environ Microbiol 7:98–106

Nauhaus K, Albrecht M, Elvert M, Boetius A, Widdel F (2007) In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulphate. Environ Microbiol 9:187–196

Naumann E, Hippe H, Gottschalk G (1983) Betaine: new oxidant in the Stickland reaction and methanogenesis from betaine and L-alanine by a *Clostridium sporogenes-Methanosarcina barkeri* coculture. Appl Environ Microbiol 45:474–483

Ørlygsson J (1994) The role of interspecies hydrogen transfer on thermophilic protein and amino acid metabolism. PhD thesis, Swedish University of Agricultural Sciences, Uppsala (Chap 4)

Ørlygsson J, Houwen FP, Svensson BH (1993) Anaerobic degradation of protein and the role of methane formation in steady state thermophilic enrichment cultures. Swed J Agric Res 23:45–54

Orphan VJ, Hinrichs K-U, Ussler W, Paull CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF (2001) Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922–1934

Oude Elferink SJWH, Vorstman WJC, Sopjes A, Stams AJM (1998) Characterization of the sulfate-reducing and syntrophic population in granular sludge from a full-scale anaerobic reactor treating papermill wastewater. FEMS Microbiol Ecol 27:185–194

Overmann J (2002) Phototrophic consortia: a tight cooperation between non-related eubacteria. In: Seckbach J (ed) Symbiosis: mechanisms and model systems. Kluwer, Dordrecht, pp 239–255

Pancost RD, Damsté JSS, de Lint S, van der Maarel MJEC, Gottschal KC, The Medinaut Shipboard Scientific Party (2000) Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. Appl Environ Microbiol 66:1126–1132

Pfennig N (1980) Syntrophic mixed cultures and symbiotic consortia with phototrophic bacteria: a review. In: Gottschalk G, Pfennig N, Werner H (eds) Anaerobes and anaerobic infections. Fischer, Stuttgart/New York, pp 127–131

Phelps TJ, Zeikus JG (1984) Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. Appl Environ Microbiol 48:1088–1095

Platen H, Schink B (1987) Methanogenic degradation of acetone by an enrichment culture. Arch Microbiol 149:136–141

Platen H, Janssen PH, Schink B (1994) Fermentative degradation of acetone by an enrichment culture in membrane-separated culture devices and in cell suspensions. FEMS Microbiol Lett 122:27–32

Plugge CM, Stams AJM (2001) Arginine catabolism by *Thermanaerovibrio acidaminovorans*. FEMS Microbiol Lett 195:259–262

Plugge CM, Dijkema C, Stams AJM (1993) Acetyl-CoA cleavage pathway in a syntrophic propionate oxidizing bacterium growing on fumarate in the absence of methanogens. FEMS Microbiol Lett 110:71–76

Plugge CM, Zoetendal EG, Stams AJM (2000) *Caloramator coolhaasii*, sp. nov. a glutamate-degrading, moderately thermophilic anaerobe. Int J Syst Bacteriol 50:1155–1162

Plugge CM, van Leeuwen JM, Hummelen T, Balk M, Stams AJM (2001) Elucidation of the pathways of catabolic glutamate conversion in three thermophilic anaerobic bacteria. Arch Microbiol 176:29–36

Plugge CM, Balk M, Zoetendal EG, Stams AJM (2002) *Gelria glutamica*, gen. nov., sp. nov., a thermophilic obligate syntrophic glutamate-degrading anaerobe. Int J Syst Evol Microbiol 52(Pt 2):401–407

Reeburgh WS (1980) Anaerobic methane oxidation: rate distributions in Skan Bay sediments. Earth Planet Sci Lett 47:345–352

Roeder J, Schink B (2009) Syntrophic degradation of cadaverine by a defined methanogenic coculture. Appl Environ Microbiol 75:4821–4828

Roy F, Samain E, Dubourgier HC, Albagnac G (1986) *Syntrophomonas sapovorans* sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. Arch Microbiol 145:142–147

Santegoeds CM, Damgaard LR, Hesselink G, Zopfi J, Lens P, Muyzer G, de Beer D (1999) Distribution of sulfate-reducing and methanogenic bacteria in anaerobic aggregates determined by microsensor and molecular analyses. Appl Environ Microbiol 65:4618–4629

Schink B (1984) Fermentation of 2.3-butanediol by *Pelobacter carbinolicus* sp. nov., and *Pelobacter propionicus*, sp. nov., and evidence for propionate formation from C_2 compounds. Arch Microbiol 137:33–41

Schink B (1985a) Fermentation of acetylene by an obligate anaerobe, *Pelobacter acetylenicus* sp. nov. Arch Microbiol 142:295–301

Schink B (1985b) Mechanism and kinetics of succinate and propionate degradation in anoxic freshwater sediments and sewage sludge. J Gen Microbiol 131:643–650

Schink B (1990) Conservation of small amounts of energy in fermenting bacteria. In: Finn RK, Präve P (eds) Biotechnology: focus 2. Hanser, New York, pp 63–89

Schink B (1991) Syntrophism among prokaryotes. In: Balows A, Trüper HG, Dworkin M, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer, New York, pp 276–299

Schink B (1994) Diversity, ecology, and isolation of acetogenic bacteria. In: Drake HL (ed) Acetogenesis. Chapman and Hall, New York, pp 197–235

Schink B (1997) Energetics of syntrophic cooperations in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280

Schink B, Friedrich M (1994) Energetics of syntrophic fatty acid degradation. FEMS Microbiol Rev 15:85–94

Schink B, Stieb, M (1983) Fermentative degradation of polyethylene glycol by a new strictly anaerobic Gram-negative non-sporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl Environ Microbiol 45:1905–1913

Schink B, Thauer RK (1988) Energetics of syntrophic methane formation and the influence of aggregation. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff LW (eds) Granular anaerobic sludge: microbiology and technology. Pudoc, Wageningen, pp 5–17

Schink B, Philipp B, Müller J (2000) Anaerobic degradation of phenolic compounds. Naturwissenschaften 87:12–23

Schnürer A, Houwen FP, Svensson BH (1994) Mesophilic syntrophic acetate oxidation during methane formation by a triculture at high ammonium concentration. Arch Microbiol 162:70–74

Schnürer A, Schink B, Svensson BH (1996) *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. Int J Syst Bacteriol 46:1145–1152

Schnürer A, Svensson BH, Schink B (1997) Enzyme activities in and energetics of acetate metabolism by the mesophilic syntrophically acetate-oxidizing anaerobe *Clostridium ultunense*. FEMS Microbiol Lett 154:331–336

Schöcke L, Schink B (1997) Energetics of methanogenic benzoate degradation by *Syntrophus gentianae* in syntrophic coculture. Microbiology 143:2345–2351

Schöcke L, Schink B (1998) Membrane-bound proton-translocating pyrophosphatase of *Syntrophus gentianae*, a syntrophically benzoate-degrading fermenting bacterium. Eur J Biochem 256:589–594

Schöcke L, Schink B (1999) Biochemistry and energetics of fermentative benzoate degradation by *Syntrophus gentianae*. Arch Microbiol 171:331–337

Scholten JCM, Conrad R (2000) Energetics of syntrophic propionate oxidation in defined batch and chemostat cocultures. Appl Environ Microbiol 66:2934–2942

Schönheit P, Moll J, Thauer RK (1980) Growth parameters (Ks, vmax, Ys) of Methanobacterium thermoautotrophicum. Arch Microbiol 127:59–65

Seelert H, Poetsch A, Dencher NA, Engel A, Stahlberg H, Müller DJ (2000) Proton-powered turbine of a plant motor. Nature 405:418–419

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (2000) *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. Int J Syst Evol Microbiol 50:771–779

Stams AJM (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. Ant v Leeuwenhoek 66:271–294

Stams AJM, Hansen TA (1984) Fermentation of glutamate and other compounds by *Acidaminobacter hydrogenoformans* gen. nov., sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and mixed cultures with sulfate-reducing and methanogenic bacteria. Arch Microbiol 137:329–337

Stams AJM, Plugge CM (1990) Isolation of syntrophic bacteria on metabolic intermediates. In: Belaich JP, Bruschi M, Garcia JL (eds) Microbiology and biochemistry of strict anaerobes involved in interspecies hydrogen transfer. Plenum, New York, pp 473–476

Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol 7:568–577

Stams AJM, Grotenhuis JTC, Zehnder AJB (1989) Structure-function relationship in granular sludge. In: Hattori T, Ishida Y, Maruyama Y, Morita RY, Uchida A (eds) Recent advances in microbial ecology. Japan Scientific Society Press, Tokyo, pp 440–445

Stams AJM, van Dijk JB, Dijkema C, Plugge CM (1993) Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. Appl Environ Microbiol 59:1114–1119

Stams AJM, Dijkema C, Plugge CM, Lens P (1998) Contribution of ^{13}C-NMR spectroscopy to the elucidation of pathways of propionate formation and degradation in methanogenic environments. Biodegradation 9:463–473

Stieb M, Schink B (1985) Anaerobic oxidation of fatty acids by *Clostridium bryantii* sp. nov., a spore-forming, obligately syntrophic bacterium. Arch Microbiol 140:387–390

Stieb M, Schink B (1986) Anaerobic degradation of isovalerate by a defined methanogenic coculture. Arch Microbiol 144:291–295

Stieb M, Schink B (1989) Anaerobic degradation of isobutyrate by methanogenic enrichment cultures and by a *Desulfococcus multivorans* strain. Arch Microbiol 151:126–132

Stock D, Leslie AGW, Walker JE (1999) Molecular architecture of the rotary motor in ATP synthase. Science 286:1700–1705

Stumm CK, Gijzen HJ, Vogels GD (1982) Association of methanogenic bacteria with ovine rumen ciliates. Br J Nutr 47:95–99

Svetlitshnyi V, Rainey F, Wiegel J (1996) *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. Int J Syst Bacteriol 46:1131–1137

Tarlera S, Stams AJM (1999) Degradation of proteins and amino acids by *Caloramator proteoclasticus* in pure culture and in coculture with *Methanobacterium thermoautotrophicum* Z245. Appl Microbiol Biotechnol 53:133–138

Tarlera S, Muxi L, Soubes M, Stams AJM (1997) *Caloramator proteoclasticus* sp. nov., a new moderately thermophilic anaerobic proteolytic bacterium. Int J Syst Bacteriol 47:651–656

Thauer RK, Morris JG (1984) Metabolism of chemotrophic anaerobes: old views and new aspects. In: Kelly DP, Carr NG (eds) The microbe 1984. Part II: prokaryotes and eukaryotes. Cambridge University Press, Cambridge, UK, pp 123–168

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Thiele JH, Zeikus JG (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl Environ Microbiol 54:20–29

Tholozan JL, Samain E, Grivet JP, Moletta R, Dubourguier HC, Albagnac G (1988) Reductive carboxylation of propionate to butyrate in methanogenic ecosystems. Appl Environ Microbiol 54:441–445

Tholozan JL, Samain E, Grivet JP, Albagnac G (1990) Propionate metabolism in a methanogenic enrichment culture: direct reductive carboxylation and acetogenesis pathways. FEMS Microbiol Ecol 73:291–298

Thomsen TR, Finster K, Ramsing NB (2001) Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. Appl Environ Microbiol 67:1646–1656

Valentine DL, Reeburgh WS (2000) New perspectives on anaerobic methane oxidation. Environ Microbiol 2:477–484

Valentine DL, Blanton DC, Reeburgh WS (2000a) Hydrogen production by methanogens under low-hydrogen conditions. Arch Microbiol 174: 415–421

Valentine DL, Reeburgh WS, Blanton DC (2000b) A culture apparatus for maintaining H_2 at sub-nanomolar concentrations. J Microbiol Methods 39:243–251

van Bruggen JJA, Stumm CK, Vogels GD (1983) Symbiosis of methanogenic bacteria and sapropelic protozoa. Arch Microbiol 136:89–95

van Bruggen JJA, Stumm CK, Zwart KB, Vogels GD (1985) Endosymbiotic methanogenic bacteria of the sapropelic amoeba *Mastigella*. FEMS Microbiol Ecol 31:187–192

van Lier JB, Grolle KC, Frijters CT, Stams AJM, Lettinga G (1993) Effects of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludge and defined cultures. Appl Environ Microbiol 59:1003–1011

Von Ballmoos C, Wiedenmann A, Dimroth P (2009) Essentials for ATP synthesis by F_1F_0 ATP synthases. Annu Rev Biochem 78:649–672

Wallrabenstein C, Schink B (1994) Evidence of reversed electron transport involved in syntrophic butyrate and benzoate oxidation by *Syntrophomonas wolfei* and *Syntrophus buswellii*. Arch Microbiol 162:136–142

Wallrabenstein C, Hauschild E, Schink B (1994) Pure culture and cytological properties of *Syntrophobacter wolinii*. FEMS Microbiol Lett 123:249–254

Wallrabenstein C, Gorny N, Springer N, Ludwig W, Schink B (1995a) Pure culture of *Syntrophus buswellii*, definition of its phylogenetic status, and description of *Syntrophus gentianae* sp. nov. Syst Appl Microbiol 18:62–66

Wallrabenstein C, Hauschild E, Schink B (1995b) *Syntrophobacter pfennigii* sp. nov., a new syntrophically propionate-oxidizing anaerobe growing in pure culture with propionate and sulfate. Arch Microbiol 164:346–352

Warikoo V, McInerney MJ, Robinson JA, Suflita JM (1996) Interspecies acetate transfer influences the extent of anaerobic benzoate degradation by syntrophic consortia. Appl Environ Microbiol 62:26–32

Widdel F (1988) Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 469–585

Widdel F, Hansen T (1991) The dissimilatory sulfate and sulfur-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer-Verlag, New York, pp 583–624.

Wildenauer FX, Winter J (1986) Fermentation of isoleucine and arginine by pure and syntrophic cultures of *Clostridium sporogenes*. FEMS Microbiol Ecol 38:373–379

Winter J, Schindler F, Wildenauer FX (1987) Fermentation of alanine and glycine by pure and syntrophic cultures of *Clostridium sporogenes*. FEMS Microbiol Ecol 45:153–161

Wofford NQ, Beaty PS, McInerney MJ (1986) Preparation of cell-free extracts and the enzymes involved in fatty acid metabolism in *Syntrophomonas wolfei*. J Bacteriol 167:179–185

Worm P, Stams AJM, Cheng X, Plugge CM (2011) Growth- and substrate-dependent transcription of formate dehydrogenase and hydrogenase coding genes in *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Microbiology 157:280–289

Wu W-M, Jain MK, Hickey RF, Zeikus JG (1991) Characterization of metabolic performance of methanogenic granules treating brewery wastewater: role of sulfate-reducing bacteria. Appl Environ Microbiol 57:3438–3449

Wu W-M, Hickey RF, Jain M, Zeikus JG (1993) Energetics and regulations of formate and hydrogen metabolism by *Methanobacterium formicicum*. Arch Microbiol 159:57–65

Wu W-M, Jain MK, Hickey RF, Zeikus JG (1994) Anaerobic degradation of normal- and branched-chain fatty acids with four or more carbons to methane by a syntrophic methanogenic triculture. Appl Environ Microbiol 57:2220–2226

Wu W-M, Jain MK, Hickey RF, Zeikus JG (1996) Perturbation of syntrophic isobutyrate and butyrate degradation with formate and hydrogen. Biotechnol Bioeng 52:404–411

Zehnder AJB (1978) Ecology of methane formation. In: Mitchell R (ed) Water pollution microbiology, vol 2. Wiley, London, pp 349–376

Zehnder AJB, Brock TD (1979) Methane formation and methane oxidation by methanogenic bacteria. J Bacteriol 137:420–432

Zehnder AJB, Ingvorsen K, Marti T (1982) Microbiology of methane bacteria. In: Hughes DE, Stafford DA, Wheatley BI, Baader W, Lettinga G, Nyns EJ, Verstraete W (eds) Anaerobic digestion. Elsevier Biomedical Press, Amsterdam, pp 45–68

Zeikus JG, Winfrey M (1976) Temperature limitation of methanogenesis in aquatic sediments. Appl Environ Microbiol 31:99–107

Zhao H, Yang D, Woese CR, Bryant MP (1989) Assignment of *Clostridium bryantii* to *Syntrophospora bryantii* gen. nov., nov. comb., based on 16S rRNA sequence analysis of its crotonate-grown pure culture. Int J Syst Bacteriol 40:40–44

Zindel U, Freudenberg W, Rieth M, Andreesen JR, Schnell J, Widdel F (1988) *Eubacterium acidaminophilum* sp. nov., a versatile amino acid-degrading anaerobe producing or utilizing H_2 or formate: description and enzymatic studies. Arch Microbiol 150:254–266

Zinder SH, Koch M (1984) Non-aceticlastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. Arch Microbiol 138:263–272

22 Quorum Sensing

Bonnie L. Bassler[1] · *Melissa B. Miller*[2]
[1]Department of Molecular Biology, Princeton University, Princeton, NJ, USA
[2]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Introduction

Bacteria have an exquisite ability to sense and adapt to a constantly fluctuating environment. They have evolved the capacity to detect a variety of temporal and spatial cues, and in response to such stimuli, bacteria initiate signal transduction cascades that culminate in changes in gene expression. The ability to rapidly alter gene expression, and consequently behavior, in response to a dynamic environment presumably gives bacteria the plasticity to survive in rich, neutral, and hostile situations.

One changing parameter that bacteria encounter is cell population density. Bacteria experience situations in which they exist essentially alone (low cell density) and also situations in which they exist in a community (high cell density). Furthermore, in the high-cell-density situation, bacteria can be in either a monoculture or in a mixed species consortium. Bacteria sense and respond to fluctuations in cell population density as well as changes in the species composition of the community, using a cell-cell communication system that is called "quorum sensing."

Quorum-sensing bacteria produce, release, detect, and respond to small hormonelike molecules called "autoinducers." As a population of autoinducer-producing bacteria grows, the concentration of released autoinducer increases. When a critical threshold concentration of the signal molecule is achieved, the bacteria are able to detect its presence and initiate a signaling cascade that results in changes in target-gene expression. Therefore, regulation of gene expression by quorum sensing allows bacteria to behave differently when they exist alone versus when they exist in a community. Furthermore, communication via quorum sensing enables bacteria to coordinate the gene expression of the entire community, thereby allowing the bacteria to behave as a multicellular organism.

Bacteria use quorum sensing to communicate both within and between species. Both species-specific and species-nonspecific autoinducers exist. As mentioned, these signals enable bacteria to distinguish low from high cell population density, but, further, independent responses to the species-specific and species-nonspecific signaling molecules allow the bacteria to behave differently when they exist in a pure culture versus when they exist in a consortium. Presumably this facet of quorum sensing allows mixed populations to act synergistically to take advantage of metabolic or other processes that are not common to all the species in the mixture. Therefore, quorum sensing could allow species in the mixed population to succeed better than each species could in isolation. Conversely, interspecies quorum sensing could also allow bacteria to measure and respond appropriately to increases in numbers of competitor bacteria. Detection of the presence of competitors coupled with the initiation of defensive behaviors could allow a population of quorum-sensing bacteria to slow or stop the growth of competing species.

This chapter describes several different model bacterial quorum-sensing signaling circuits and their uses. The first quorum-sensing circuit, that of the bioluminescent marine bacterium *Vibrio fischeri*, was identified and reported in 1983 (Engebrecht et al. 1983). At that time, cell-cell communication in bacteria was assumed to be a very limited phenomenon. Therefore, intercellular communication in *V. fischeri* was considered an interesting anomaly of no real significance. However, in the last decade, dozens of other species of Gram-negative

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_60,

bacteria have been identified that use a very similar quorum-sensing circuit to that of *V. fischeri* (De Kievit and Iglewski 2000). Quorum sensing has also now been described in numerous Gram-positive bacterial species, and an interspecies quorum-sensing system has also been discovered that is shared by both Gram-negative and Gram-positive bacteria. In these latter cases, the signal molecules and the detection machinery are different from that of *V. fischeri* and other Gram-negative bacteria (Bassler 1999b; Kleerebezem et al. 1997). However, all quorum-sensing systems allow bacteria to accomplish the same task, i.e., to count one another and regulate gene expression in response to cell number. The findings of the last 10 years indicate that quorum sensing is a widespread, fundamental signaling process that is critical for bacterial life in the wild. As this is a burgeoning field of research, we suspect that novel signals, unique detection, and response apparatuses and additional, as yet undescribed, quorum-sensing behaviors await discovery.

Quorum Sensing in Gram-Negative Bacteria: The LuxI/LuxR Paradigm

Initial investigations of quorum sensing centered on *Vibrio fischeri*, a bioluminescent marine bacterium that exists as a symbiont inhabiting specialized light organs of several animal hosts, including the squid *Euprymna scolopes* and the fish *Monocentris japonicus* (for review see Visick and McFall-Ngai 2000). In the eukaryote-*V. fischeri* association, the animal host provides a nutrient-rich environment for the bacteria, and the bacteria provide light (bioluminescence) to the host. The hosts use the light produced by the bacteria for different purposes including attraction of mates and escape from predators.

In the case of the *E. scolopes*-*V. fischeri* symbiosis, *V. fischeri* exists in pure culture and grows to extremely high cell densities (approximately 10^{11} cells/ml) in the squid light organ (Ruby and McFall-Ngai 1992). As *V. fischeri* grows, it produces an autoinducer hormone that accumulates in the light organ. Presumably the buildup of autoinducer communicates to the bacteria that they exist "inside" a light organ as opposed to "outside" in the ocean, an environment where the autoinducer would diffuse away and therefore never accumulate to any significant concentration. In the squid light organ, when a critical autoinducer concentration is achieved, a signaling cascade is initiated that results in induction of the expression of the genes required for light production. These genes, *luxCDABE* (*lux*), encode the structural components of the luciferase enzyme complex (Engebrecht and Silverman 1984). Therefore, *V. fischeri* only produces light at high cell density and only in the light organ of the host.

The squid *E. scolopes*-*V. fischeri* association is fascinating. *Euprymna scolopes* is a nocturnal animal that lives in shallow coastal waters, and it uses the light made from *V. fischeri* for counter-illumination at night. The light organ inhabited by *V. fischeri* resides on the underside of the squid. At night, the squid senses the ambient starlight or moonlight penetrating the water and shining onto its back. By opening and closing a shutter beneath the specialized light organ, the squid is able to modulate the amount of light emanating from the symbiotic *V. fischeri* culture. The squid appropriately opens and closes this shutter to make the amount of light shining down from the light organ exactly match the amount of light shining onto its back from the stars and moon. Therefore, using the light from *V. fischeri*, *E. scolopes* manages to avoid casting a shadow beneath itself and thereby avoids predation (Ruby and McFall-Ngai 1992).

In summary, *V. fischeri* exists at high cell density only in the light organ of the squid, and this is the only niche where autoinducer concentration is above the required threshold for *lux* expression. Therefore, under this condition, the bacteria make light. Conversely, when the bacteria are shed from the light organ into the seawater (which occurs at sunrise and is regulated by the circadian rhythm of the squid), both the bacterial cell density and the autoinducer diminish to below the required level for signaling, and the bacteria make no light (Lee and Ruby 1994). Quorum sensing thus enables *V. fischeri* to determine when it exists in a symbiotic association with a eukaryotic host versus when it exists free-living in the ocean. This sensory transduction system thereby grants *V. fischeri* the benefits of life as a symbiont.

The *Vibrio fischeri* LuxI/LuxR System

In *V. fischeri*, quorum sensing is regulated by two proteins called LuxI and LuxR (Engebrecht and Silverman 1987). The LuxI protein is the autoinducer synthase, and it is responsible for production of the autoinducer signal molecule. The autoinducer is an acylated homoserine lactone (AHL, described in LuxI-directed autoinducer biosynthesis), and it freely diffuses through the cell membrane (Kaplan and Greenberg 1985). The second protein, LuxR, is a regulatory protein that binds both the autoinducer and DNA (Stevens and Greenberg 1999; Salmond et al. 1995).

Engebrecht and Silverman discovered and cloned both the regulatory components (*luxI* and *luxR*) and the luciferase structural genes (*luxCDABE*) from *V. fischeri* (Engebrecht and Silverman 1984, 1987). They also determined how this first quorum-sensing circuit functioned. Their work demonstrated that the bioluminescence structural and regulatory genes were arranged in two divergently transcribed units, *luxR* and *luxICDABE* (Fig. 22.1). In dilute culture, the *luxICDABE* operon has weak constitutive expression, and *V. fischeri* produces almost no light. Conversely, significant transcription of *luxR* occurs at low cell density. As the cell density increases, autoinducer accumulates due to the low-level expression of the *luxI* gene in the *luxICDABE* operon. Therefore, when a critical concentration of the autoinducer molecule is reached, LuxR binds it and together they activate expression of the *luxICDABE* operon. This action results in a positive-feedback circuit. Specifically, an exponential increase in autoinducer production occurs (from the increase in *luxI* transcription), and because the luciferase structural genes *luxCDABE* reside downstream of *luxI*, an exponential increase in light production occurs. Furthermore, the LuxR-autoinducer complex, while acting positively on

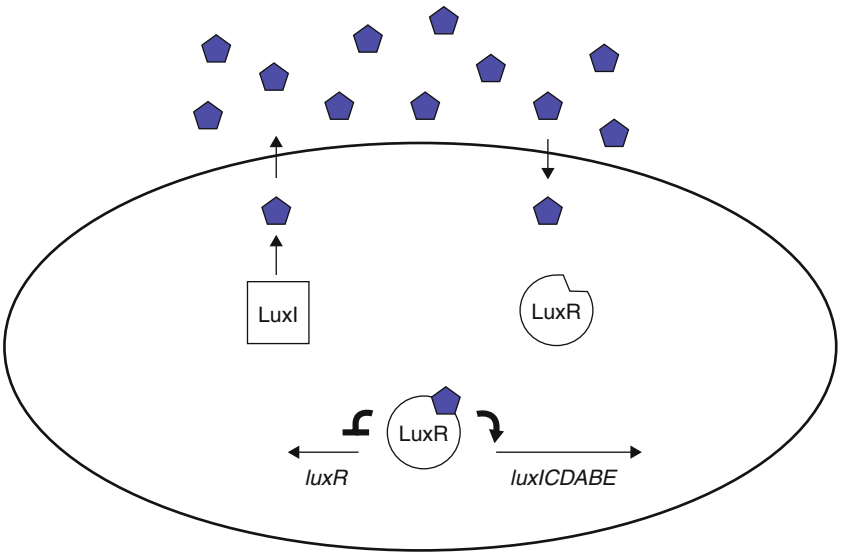


Fig. 22.1

The LuxI/R quorum-sensing paradigm – *V. fischeri*. This figure depicts the prototypical *V. fischeri* quorum-sensing system. LuxI is the protein responsible for autoinducer production, and LuxR is the protein necessary for detecting and responding to autoinducer. Following LuxI-directed synthesis, autoinducer molecules (blue pentagons) accumulate thereby allowing interaction with LuxR. The LuxR-autoinducer complex is a transcriptional activator of the *luxICDABE* operon. Activation of the *luxICDABE* operon establishes a positive-feedback loop, increasing the level of autoinducer production (*via luxI*) and the amount of light the bacterium emits (*via luxCDABE*). Conversely, the LuxR-autoinducer complex inhibits the transcription of *luxR*, which provides a compensatory mechanism for the regulation of light production. The *oval* represents a bacterium; the *square* and *circle* demarcate the proteins LuxI and LuxR, respectively

luxICDABE transcription, acts negatively to control *luxR* expression. Negative regulation of *luxR* transcription by the LuxR-autoinducer complex is a compensatory mechanism for modulating *luxICDABE* expression. Together, these two autoregulatory loops tightly control light production in response to autoinducer concentration and therefore in response to increasing cell population density (Engebrecht et al. 1983).

Homologues of the *V. fischeri* LuxI and LuxR proteins have now been identified in over 25 species of Gram-negative bacteria (Fuqua et al. 1996; De Kievit and Iglewski 2000). In each documented case, the LuxI enzymes control the synthesis of an acylated homoserine lactone autoinducer. The LuxR proteins bind a specific partner autoinducer, and together the cognate pair activates the transcription of some target gene(s) in response to increasing cell population density. Although the genetic arrangement of the regulatory genes and target genes differs, in every case the mechanism of regulation is conserved. A variety of functions are controlled by LuxI-R quorum-sensing systems, as described in The *Pseudomonas aeruginosa* LasI/LasR-RhlI/RhlR Systems, The *Agrobacterium tumefaciens* TraI/TraR System, and The *Erwinia carotovora* ExpI/ExpR System.

LuxI-Directed Autoinducer Biosynthesis: Autoinducer Homoserine Lactone

The autoinducer synthesized by LuxI in *V. fischeri* is *N*-(3-oxohexanoyl)-homoserine lactone (AHL; Eberhard et al. 1981). *S*-Adenosyl methionine (SAM) and acyl-acyl carrier protein (acyl-ACP), an intermediate in fatty acid biosynthesis, are the substrates for AHL synthesis (Hanzelka and Greenberg 1996; Val and Cronan 1998). The LuxI enzyme promotes the formation of an amide bond joining the acyl side chain from the acyl-ACP to SAM (More et al. 1996). Lactonization of the ligated intermediate with the concomitant release of methylthioadenosine (MTA) results in AHL. The complete biosynthetic pathway for AHL autoinducers is shown in ❯ *Fig. 22.2*.

The biochemical mechanism of using SAM and a fatty acid acyl-ACP as substrates for AHL-autoinducer synthesis has been demonstrated for several autoinducers produced by LuxI homologues, indicating that this biosynthetic pathway is likely conserved among the entire family of LuxI autoinducer synthases (Parsek et al. 1999). Although not proven, because the AHL class of autoinducers are very similar, it is assumed that most AHL autoinducers are freely permeable to the Gram-negative cell membrane, similar to what has been demonstrated for the *V. fischeri* AHL (Kaplan and Greenberg 1985). However, in the case of the *Pseudomonas aeruginosa* autoinducer *N*-(3-oxododecanoyl)-HSL, there is evidence suggesting that the MexAB-OprM multidrug efflux pump is involved in export of that particular AHL signal (Evans et al. 1998). Structurally, AHLs differ only in the acylated side chains, suggesting that the LuxI interaction with a particular acyl-ACP provides the specificity in AHL-autoinducer biosynthesis (Fuqua and Eberhard 1999). However, because AHL autoinducers act by binding to a particular LuxR protein, the LuxR homologues

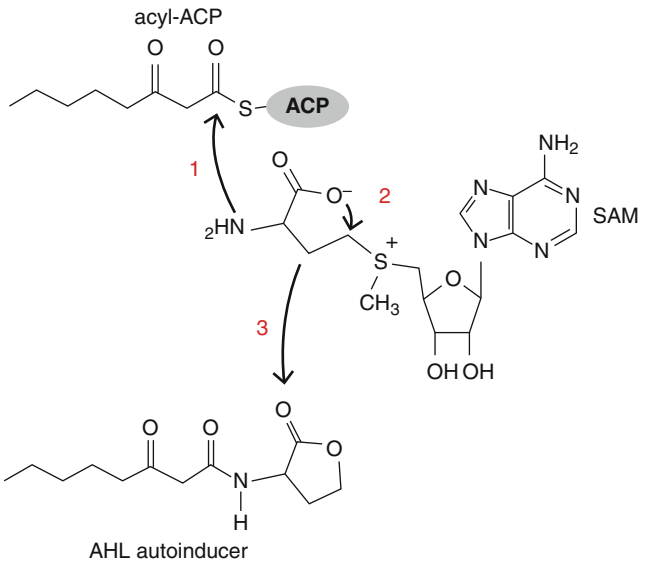


Fig. 22.2

The biosynthetic pathway for acylated homoserine lactone (AHL) autoinducers. *S*-adenosyl methionine (SAM) and acyl-acyl carrier proteins (acyl-ACP) are the substrates in autoinducer synthesis by LuxI-like enzymes. LuxI promotes the formation of an amide bond between SAM and the acyl side chain from acyl-ACP (1). This intermediate subsequently undergoes lactonization (2) and releases methylthioadenosine (MTA) as a side product. The result is the formation of an acylated homoserine lactone (3). The autoinducer structure shown in the model is N-(3-oxooctanoyl)-homoserine lactone, the autoinducer of *Agrobacterium tumefaciens* (Adapted and reprinted with permission of S.C. Winans)

also contribute to the specificity inherent in quorum-sensing systems by binding to their cognate autoinducer at a higher affinity than to other autoinducers.

Specificity appears crucial to the simple signal-response quorum-sensing systems of the LuxI-R type. Whereas the LuxI's produce a highly similar family of signaling molecules, the autoinducers are typically not cross-reactive (Gray et al. 1994). The selectivity of the LuxRs for their partner autoinducers presumably makes the LuxI bacterial language quite species specific. *Table 22.1* lists the bacterial species known to possess LuxI proteins, the structures of the autoinducers, and the regulated functions.

The *Pseudomonas aeruginosa* LasI/LasR-RhlI/RhlR Systems

Quorum sensing in the opportunistic pathogen *P. aeruginosa* is controlled by a more complex LuxI/LuxR quorum-sensing circuit than that described for *Vibrio fischeri* (De Kievit and Iglewski 2000). Specifically, two pairs of LuxI/LuxR homologues have been identified in *P. aeruginosa*, LasI/LasR and RhlI/RhlR. LasI and RhlI are autoinducer synthases that produce the AHL signals *N*-(3-oxododecanoyl)-homoserine lactone and *N*-(butyryl)-homoserine lactone, respectively (Winson et al. 1995; Pearson et al. 1995). These two quorum-sensing systems function in tandem to control virulence in *P. aeruginosa* (Pesci and Iglewski 1997).

The LasI/LasR system was the first quorum-sensing system identified in *P. aeruginosa*, and as in the *V. fischeri* system, the transcriptional activator LasR was shown to bind to its cognate AHL autoinducer whose synthesis was dependent on LasI (Passador et al. 1993). The LasR-autoinducer complex is responsible for activation of several target virulence genes, the products of which are secreted and are involved in host tissue destruction during the establishment of infection (Jones et al. 1993). These virulence targets include *lasB*, encoding elastase; *lasA*, encoding a protease; *toxA*, encoding exotoxin A; and *aprA*, encoding an alkaline phosphatase. In addition to these virulence factors, and similar to the prototypical *V. fischeri* LuxI/LuxR system, the *P. aeruginosa* LasI/LasR system also activates *lasI* to establish an autoregulatory circuit.

However, in contrast to the *V. fischeri* system, the LasI/LasR system also activates a second quorum-sensing system, composed of RhlI/RhlR, by promoting the expression of the transcriptional activator *rhlR* (Ochsner and Reiser 1995). Again, like LasI/LasR, in the RhlI/RhlR system, the RhlI-synthesized AHL autoinducer binds to the transcriptional activator RhlR to regulate specific genes that display density-dependent expression. The genes regulated by RhlR bound to its cognate autoinducer include *lasB* and *aprA*, which are also under the control of the LasI-R system; *rpoS*, encoding the stationary phase σ factor required for stress response; *rhlAB*, encoding rhamnosyltransferase that is involved in the production of the biosurfactant/hemolysin rhamnolipid; pyocyanin, a phenazine antibiotic; and *rhlI*, encoding the autoinducer synthase (Pesci and Iglewski 1999).

In addition to the activation of *rhlR* by the LasI/LasR system, the LasI-dependent AHL autoinducer also acts to inhibit the RhlI autoinducer from binding RhlR, when the concentration of the LasI-dependent autoinducer is significantly higher than the RhlI-dependent autoinducer. It is hypothesized that this dual regulation by the LasI-R quorum-sensing system ensures that the RhlI-R quorum-sensing system will only be activated once the LasI-R-controlled regulon has been established. Presumably, this hierarchy allows *P. aeruginosa* to precisely and specifically time expression of the different density-dependent target genes (*Fig. 22.3*).

A third autoinducer has recently been identified in *P. aeruginosa*. This autoinducer is especially interesting because it is not an AHL, but rather 2-heptyl-3-hydroxy-4-quinolone (Pesci et al. 1999). This third signal is referred to as the *Pseudomonas* quinolone signal (PQS). Similar to the Las and Rhl quorum-sensing systems, the PQS regulates the expression of *lasB*, the gene encoding the virulence factor elastase. Recent evidence suggests that the PQS could be the link between the Las and Rhl quorum-sensing hierarchies, because PQS production requires LasR and also because PQS significantly stimulates rhlI expression (McKnight et al. 2000). It is interesting that this

Table 22.1

Summary of the LuxI/LuxR-like quorum-sensing systems that have been described

Organism	LuxI/LuxR homologue(s)	Autoinducer identity[a]	Target genes and functions	References
Vibrio fischeri	LuxI/LuxR	*N*-(3-oxohexanoyl)-HSL	*luxICDABE* (bioluminescence)	Engebrecht et al. (1983) Eberhard et al. (1981)
Aeromonas hydrophila	AhyI/AhyR	*N*-butanoyl-HSL	Serine protease and metalloprotease production	Swift et al. (1997)
Aeromonas salmonicida	AsaI/AsaR	*N*-butanoyl-HSL	*aspA* (exoprotease)	Swift et al. (1999)
Agrobacterium tumefaciens	TraI/TraR	*N*-(3-oxooctanoyl)-HSL	*tra, trb* (Ti plasmid conjugal transfer)	Piper et al. (1993) Zhang et al. (1993)
Burkholderia cepacia	CepI/CepR	*N*-octanoyl-HSL	Protease and siderophore production	Lewenza et al. (1999)
Chromobacterium violaceum	CviI/CviR	*N*-hexanoyl-HSL	Violacein pigment, hydrogen cyanide, antibiotics, exoproteases, and chitinolytic enzymes	McClean et al. (1997) Chernin et al. (1998)
Enterobacter agglomerans	EagI/EagR	*N*-(3-oxohexanoyl)-HSL	Unknown	Swift et al. (1993)
Erwinia carotovora	(1) ExpI/ExpR	*N*-(3-oxohexanoyl)-HSL	(1) Exoenzyme synthesis	(1) Pirhonen et al. (1993)
	(2) CarI/CarR		(2) Carbapenem antibiotic synthesis	Jones et al. (1993) (2) Bainton et al. (1992)
Erwinia chrysanthemi	ExpI/ExpR	*N*-(3-oxohexanoyl)-HSL	*PecS* (regulator of pectinase synthesis)	Nasser et al. (1998) Reverchon et al. (1998)
Erwinia stewartii	EsaI/EsaR	*N*-(3-oxohexanoyl)-HSL	Capsular polysaccharide biosynthesis, virulence	Beck von Bodman and Farrand (1995)
Escherichia coli	?/SdiA	?	*ftsQAZ* (cell division), chromosome replication	Sitnikov et al. (1996) Garcia-Lara et al. (1996) Withers and Nordstrom (1998)
Pseudomonas aureofaciens	PhzI/PhzR	*N*-hexanoyl-HSL	*phz* (phenazine antibiotic biosynthesis)	Pierson et al. (1994) Wood et al. (1997)
Pseudomonas aeruginosa	(1) LasI/LasR	(1) *N*-(3-oxododecanoyl)-HSL	(1) *lasA*, *lasB*, *aprA*, *toxA* (exoprotease virulence factors), biofilm formation	(1) De Kievit and Iglewski (2000) Pearson et al. (1994) Davies et al. (1998)
	(2) RhlI/Rh1R	(2) *N*-butyryl-HSL	(2) *lasB*, *rhlAB* (rhamnolipid), *rpoS* (stationary phase)	(2) Pearson et al. (1995) Latifi et al. (1996) De Kievit and Iglewski (2000)
Ralstonia solanacearum	SolI/SolR	*N*-hexanoyl-HSL, *N*-octanoyl-HSL	Unknown	Flavier et al. (1997)
Rhizobium etli	RaiI/RaiR	Multiple, unconfirmed	Restriction of nodule number	Rosemeyer et al. (1998)
Rhizobium leguminosarum	(1) RhiI/RhiR	(1) *N*-hexanoyl-HSL	(1) *rhiABC* (rhizosphere genes) and stationary phase	(1) Cubo et al. (1992) Gray et al. (1996) Rodelas et al. (1999)
	(2) CinI/CinR	(2) *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-HSL	(2) Quorum-sensing regulatory cascade	(2) Lithgow et al. (2000)
Rhodobacter sphaeroides	CerI/CerR	7,8-*cis*-*N*-(tetradecanoyl)-HSL	Prevents bacterial aggregation	Puskas et al. (1997)
Salmonella typhimurium	?/SdiA	?	*rck* (resistance to competence killing), ORF on *Salmonella* virulence plasmid	Ahmer et al. (1998)

Table 22.1 (continued)

Organism	LuxI/LuxR homologue(s)	Autoinducer identity[a]	Target genes and functions	References
Serratia liquefaciens	SwrI/?	*N*-butanoyl-HSL	Swarmer cell differentiation, exoprotease	Eberl et al. (1996)
				Givskov et al. (1997)
Vibrio anguillarum	VanI/VanR	*N*-(3-oxodecanoyl)-HSL	Unknown	Milton et al. (1997)
Yersinia enterocolitica	YenI/YenR	*N*-hexanoyl-HSL, *N*-(3-oxohexanoyl)-HSL	Unknown	Throup et al. (1995)
Yersinia pseudotuberculosis	(1) YpsI/YpsR	(1) *N*-(3-oxohexanoyl)-HSL	Hierarchical quorum-sensing cascade regulating bacterial aggregation and motility	Atkinson et al. (1999)
	(2) YtbI/YtbR	(2) *N*-octanoyl-HSL		

[a]Generally, only the primary autoinducers are listed, but it should be noted that in a few species, multiple autoinducers have been demonstrated to be synthesized by the one LuxI-type protein
Adapted from De Kievit and Iglewski (2000)

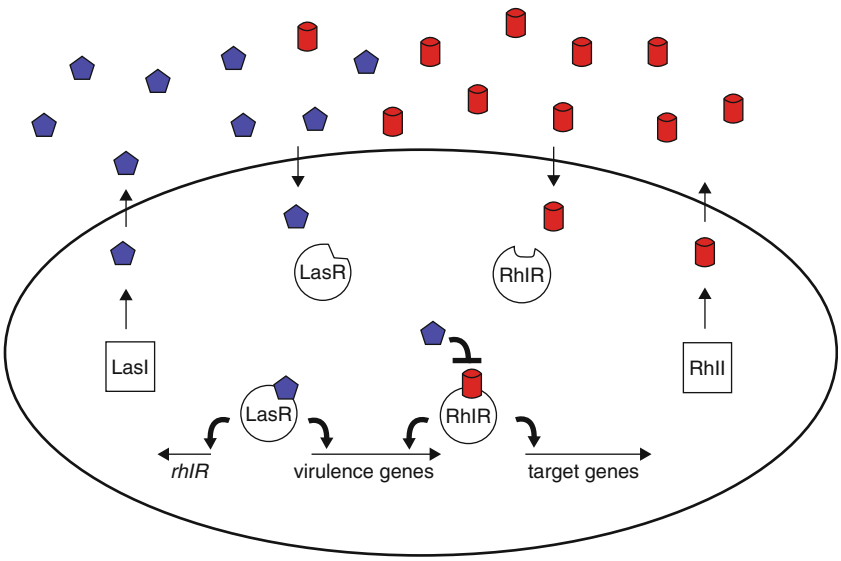


Fig. 22.3
Hierarchical quorum sensing in *P. aeruginosa*. Similar to many Gram-negative bacteria, *P. aeruginosa* uses a LuxI/R-type quorum-sensing system. *P. aeruginosa* has two LuxI/LuxR homologue pairs (LasI/LasR and RhlI/RhlR) that function in tandem to regulate gene expression in response to cell number. As in the *V. fischeri* model, the AHL autoinducers of *P. aeruginosa* are produced by the synthases LasI and RhlI. As the cell population density increases, and autoinducer concentration increases, the AHL autoinducers bind their corresponding transcriptional activators (LasR and RhlR). The transcriptional activator-autoinducer complexes then positively regulate target-gene expression. The target genes for the LasR-autoinducer complex include secreted virulence factors and the *rhlR* gene encoding the activator RhlR of the second quorum-sensing system. Upon activation by the LasI/R quorum-sensing system, the RhlI/R system induces the transcription of a subset of the LasI/R-regulated virulence genes as well as RhlI/R-specific target genes. In addition to activating the RhlI/R system, the LasI/R system also negatively regulates the binding of the RhlI-dependent autoinducer to RhlR through competitive inhibition by the LasI-dependent autoinducer. The Pseudomonas quinolone signal (PQS) also links the regulation of the Las and Rhl systems (see text). The *oval* represents a bacterium. The LasI-dependent autoinducer is represented by a *blue hexagon*, while the RhlI-dependent autoinducer is depicted as a *red cylinder*. The *squares* represent the autoinducer synthases LasI and RhlI; the *circles* represent the transcriptional activators LasR and RhlR

autoinducer is a member of the quinolone family because halogenated quinolones are commonly used as potent antibiotics against both Gram-negative and Gram-positive bacteria. It is possible that *P. aeruginosa* uses this compound both for intercellular communication and as an antimicrobial agent.

The *Agrobacterium tumefaciens* TraI/TraR System

The plant pathogen *A. tumefaciens* causes crown gall tumors in part by the transfer of its tumor-inducing (Ti) plasmid to the plant host nuclei. Though quorum sensing is not directly

involved in the transfer of the Ti plasmid to the plant host, it does control the interbacterial specific transfer of the Ti plasmid by conjugation (Zhang et al. 1993; Piper et al. 1993). Conjugation in *A. tumefaciens* requires two signals: one from the host plant (opine) and an AHL-autoinducer signal produced by the autoinducer synthase TraI. Opines produced at the tumor site in plants are a nutritive source for the infecting bacteria, but opines also indirectly activate expression of the quorum-sensing regulator *traR* via an opine-specific regulator. Therefore, in *A. tumefaciens*, bacterial conjugation is jointly controlled by both plant and bacterial signals.

The quorum-sensing system of *A. tumefaciens* is comprised of TraI/TraR, both of which are encoded on the transmissible Ti plasmid. The autoinducer synthase, TraI, produces *N*-(3-oxooctanoyl)-homoserine lactone. As in *Vibrio fischeri*, *traI* is expressed at a low basal level, so only low amounts of autoinducer are produced. After activation of the expression of *traR* by plant opines, TraR binds the autoinducer and together the complex activates the transcription of traI to establish the characteristic autoinduction loop. In addition to activating *traI*, the TraR-autoinducer complex activates the *tra* operon required for mobilization of the Ti plasmid, the *trb* operon that encodes the mating pore, and an additional regulator encoded by *traM* (Winans et al. 1999). The TraM protein, although activated by TraI/TraR, acts as a negative regulator of quorum sensing by inhibiting TraR-autoinducer-specific target activation by binding directly to TraR and inhibiting the DNA-binding and target-gene activation functions of TraR (Luo et al. 2000). This additional layer of regulation in the *A. tumefaciens* system that apparently does not exist in the *V. fischeri* or the *P. aeruginosa* system indicates that the different quorum-sensing bacteria have evolved specific regulatory controls to precisely adapt their specific density-dependent regulons to particular niches.

The *Erwinia carotovora* ExpI/ExpR System

Erwinia carotovora is a plant pathogen that causes soft rot in its host. Virulence in *E. carotovora* depends on several factors, many of which are exoenzymes that act to degrade plant tissue, enabling the bacterium to successfully establish an infection (Jones et al. 1993). The LuxI/LuxR homologues ExpI/ExpR are hypothesized to be involved in the regulation of many of the secreted enzymes (Pirhonen et al. 1993). An *expI* mutant displays pleiotropic defects in exoenzyme production, but a distinct role for ExpR (and therefore quorum sensing) in regulation of exoenzyme production has not been demonstrated.

The role of a second quorum-sensing system in *E. carotovora*, CarI/CarR, is less ambiguous. The CarI/CarR system positively regulates the biosynthesis of carbapenem antibiotics (Bainton et al. 1992; Williams et al. 1992). Carbapenem production is density dependent and, furthermore, occurs simultaneous with exoenzyme production. It is theorized that, during infection, *E. carotovora* not only destroys the plant tissue for nutrients but it also kills competing/invading bacteria of other species with antibiotics. In addition, CarI/CarR activate the production of exoenzymes, and this activity is suggested to be coupled to the activity of ExpR through the *rex* (regulation of exoenzymes) gene product (Pierson et al. 1999). Interestingly, both ExpI and CarI produce the same AHL signaling molecule, *N*-(3-oxohexanoyl)-homoserine lactone. It is noteworthy that ExpI and CarI were identified in separate isolates of *E. carotovora*, and whereas the proteins show only 70% identity, they may prove to be functionally identical in the independent isolates. Although the understanding of ExpI and CarI is limited, it is apparent that the functions of the two transcriptional activators, ExpR and CarR, are somehow integrated through the use of the same AHL signal molecule.

Gram-Positive Quorum Sensing: Peptide Signals and Two-Component Signal Transduction

Like Gram-negative bacteria, quorum sensing also occurs in Gram-positive bacterial species. Although the fundamental purpose of quorum sensing in Gram-negative and Gram-positive bacteria is identical, i.e., the density-dependent expression of target genes via the secretion and detection of an autoinducer signaling molecule, the signaling molecules, mechanism of their synthesis, and the secretion and detection apparatus used by Gram-positive bacteria are not similar to those of Gram-negative bacteria (see section "Quorum Sensing in Gram-Negative Bacteria: The LuxI/LuxR Paradigm").

Gram-positive quorum-sensing bacteria use a secreted peptide as the autoinducer. Typically, the peptide signal molecule is secreted by a dedicated ATP-binding cassette (ABC) transporter (Kleerebezem et al. 1997). Also in contrast to the simple detection/response mechanism of the LuxR-like transcriptional regulators, autoinducer detection and response are mediated by two-component adaptive response circuits in Gram-positive bacteria (Kleerebezem et al. 1997). Two-component systems consist of a family of homologous proteins that exist in a wide variety of both Gram-negative and Gram-positive bacteria. These systems enable bacteria to adapt to alterations in a wide variety of environmental conditions. Two-component systems relay sensory information by phosphorylation/dephosphorylation cascades. The two components are a membrane-bound sensor kinase protein that initiates information transfer by autophosphorylation and a response-regulator protein, which, following phosphotransfer from a cognate sensor kinase, typically controls transcription of downstream target genes. (For a detailed review of two-component systems, see Stock et al. 1989 and Parkinson 1995.) Gram-positive quorum-sensing bacteria use two-component systems to detect and respond to the accumulation of a threshold concentration of a peptide autoinducer. A general scheme for Gram-positive quorum sensing is shown in *Fig. 22.4*, and several Gram-positive quorum-sensing regulatory systems and the targets they control are described in The *Streptococcus pneumoniae* Competence System, The *Bacillus subtilis* Competence System, and The *Staphylococcus aureus* Agr System.

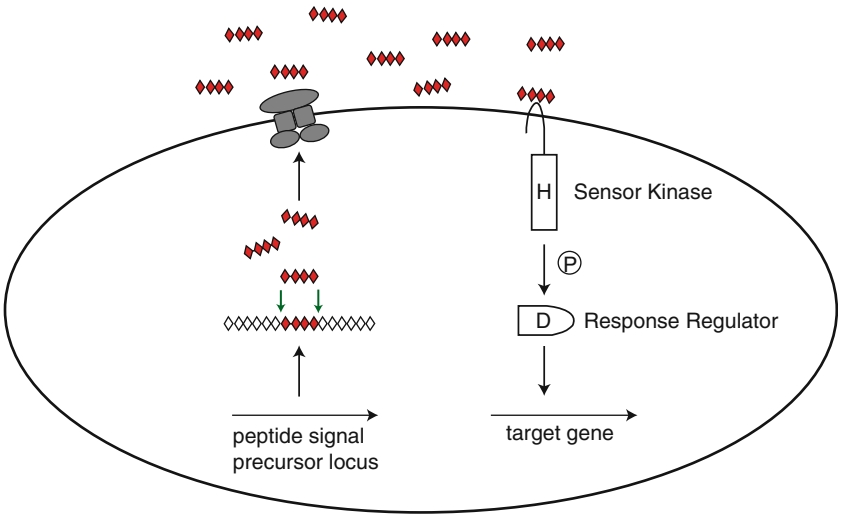


Fig. 22.4

A General model for Gram-positive quorum sensing. In Gram-positive bacteria, the autoinducer is a peptide signal that is processed from a larger precursor peptide. The peptide signal precursor locus is translated into a precursor protein, which is subsequently cleaved to produce the peptide signal. In most cases, the peptide signal is transported out of the cell via an ATP-binding cassette (ABC) transporter. As the bacterial population grows, the peptide signal accumulates extracellularly, where the signal can then be detected by a two-component system. Following interaction with the peptide signal, the sensor kinase protein of the two-component system autophosphorylates on a conserved histidine residue (H). This autophosphorylation event initiates a phosphorelay cascade that results in phosphorylation of the cognate response-regulator protein on a conserved aspartic acid residue (D). The phosphorylated response regulator activates the transcription of the target gene(s). The *oval* represents a bacterium; *diamonds* are units of the precursor protein with the *red diamonds* representing the signal peptide. The *green arrows* indicate the processing of the precursor protein into the peptide signal. The *gray* proteins represent an ABC transporter. The P in the circle represents the phosphorylation cascade. Note that the length of the precursor and processed peptides do not imply a specific number of amino acids

The *Streptococcus pneumoniae* Competence System

Genetic transformation of bacteria was first described in *S. pneumoniae* (see Havarstein and Morrison 1999 and references therein). Transformation by foreign DNA requires the bacterium to possess the ability to take up exogenous DNA. This ability is known as "competence" for transformation. *Streptococcus pneumoniae* is a naturally competent bacterium, and it uses peptide quorum sensing to regulate development of the competent state. Unlike most other naturally competent organisms, *S. pneumoniae* can assimilate DNA regardless of sequence and, thus, regardless of the species of origin of the DNA. By using quorum sensing to regulate competence genes, these promiscuous organisms are presumed to enhance their likelihood of acquiring DNA from a variety of sources that could contain a vast assortment of genes specifying beneficial functions that have not evolved within their own species.

The peptide signal for density-dependent competence development in *S. pneumoniae* is called the "competence-stimulating peptide" (CSP), which contains 17 amino acids and is produced by the cleavage of a 41-residue peptide precursor called "ComC" (Tortosa and Dubnau 1999). The CSP is secreted by the ABC transporter encoded by *comAB*. Similar to AHL autoinducers, the concentration of CSP molecule increases in the extracellular environment as the bacterial population grows. Accumulated CSP is detected by the two-component sensor kinase ComD, which initiates a phosphorylation cascade that results in phosphorylation of the cognate response-regulator protein ComE (Kleerebezem et al. 1997). Phosphorylation of ComE activates it, and phospho-ComE, in turn, activates transcription of the gene *comX*. The protein encoded by *comX* is an alternative σ factor that is required for the downstream expression of genes necessary for development of the competent state (Havarstein and Morrison 1999). Competence in *S. pneumoniae* occurs only during exponential growth, and competence is transient. The benefit *S. pneumoniae* derives from a temporary competent state is unclear, but it is likely that additional regulatory mechanisms exist to eliminate the transient density-dependent expression of competence.

The *Bacillus subtilis* Competence System

Bacillus subtilis is a commensal soil organism that, like *Streptococcus pneumoniae*, uses quorum sensing to control genes required for the acquisition of extracellular DNA (Lazazzera and Grossman 1998). The development of competence in *B. subtilis* occurs in about 10% of the bacterial population at the transition between logarithmic growth and stationary phase, when cell lysis and the concomitant release of DNA likely occur. Therefore, competence for uptake of exogenous DNA in a small

fraction of the population could allow this subpopulation to use these fragments of DNA as a repository for repair of mutated and broken chromosomes (Lazazzera et al. 1999). Unlike *S. pneumoniae*, which is postulated to use competence in early log phase to acquire heterologous DNA, *B. subtilis* is proposed to use quorum sensing and competence at higher cell density to inherit its own species' DNA.

Density-dependent control of competence in *B. subtilis* is mediated by two peptide signals called "ComX" and "CSF" (competence and sporulation factor). These two autoinducer signals are secreted as the cell population density increases. The ComX peptide is translated as a 55-amino-acid precursor protein, but is subsequently posttranslationally modified on a tryptophan residue, and the precursor protein is cleaved (Lazazzera and Grossman 1998). The final exported ComX signal molecule is a modified decapeptide. A protein called "ComQ" is required for production of the ComX peptide. The ComQ protein is hypothesized to be involved in the processing, modification, and/or secretion of the ComX peptide; however, the exact function of ComQ has not been established (Lazazzera et al. 1999). The concentration of external ComX increases as the culture grows, and detection of the peptide is via the two-component system ComP/ComA. The ComP protein is the sensor kinase and ComA is the response regulator. Phosphorylated ComA is responsible for the activation of *comS*. The function of the ComS protein is to protect another protein called "ComK" from proteolytic degradation (Tortosa and Dubnau 1999). The ComK protein is the transcriptional activator of competence genes. Finally, this complicated quorum-sensing circuit allows *B. subtilis* to become competent for transformation by exogenous DNA only at high cell density.

As mentioned, two peptide signals (ComX and CSF) are involved in quorum sensing and competence development in *B. subtilis*. The CSF pentapeptide is produced by the processing of the C-terminus of a peptide precursor called "PhrC" (Lazazzera and Grossman 1998). Although the CSF peptide signal is produced in a density-dependent manner, its mechanism of action is different than that of other peptide quorum-sensing autoinducers. Specifically, secreted CSF is internalized via an oligopeptide permease, and intracellular CSF acts to modulate the levels of phosphorylated ComA by inhibiting the activity of a phosphatase called "RapC" (Lazazzera et al. 1999). As described, ComA is a response-regulator protein, and the inhibition of a specific ComA phosphatase results in an increase in the level of phospho-ComA in the cell. Phospho-ComA activates expression of a set of genes required for competence development. Thus, whereas CSF may not itself be a typical quorum-sensing signaling molecule, it is clearly involved in the modulation of competence gene expression in a density-dependent manner.

The *Staphylococcus aureus* Agr System

Staphylococcus aureus is an invasive pathogen that can cause disease in almost any tissue or organ in the human body, primarily in compromised individuals. Staphylococcal infections such as pneumonia, endocarditis, septicemia, toxic shock syndrome, and food poisoning require several virulence factors, most of which are secreted enzymes or toxins. Expression of many of the virulence factors of *S. aureus* is dependent on cell density and peptide quorum sensing.

Density-dependent virulence in *S. aureus* is regulated by an RNA molecule called "RNAIII" (Kleerebezem et al. 1997). Levels of RNAIII are controlled by three loci, one of which encodes a peptide quorum-sensing system. The *agrBDCA* operon encodes the quorum-sensing components, and this operon is divergently transcribed from the *hld* locus which encodes the RNAIII transcript. In the *S. aureus* quorum-sensing circuit, the 46-residue signal-peptide precursor is encoded by *agrD* (Morfeldt et al. 1996). The AgrD precursor protein is subsequently cleaved to an octapeptide, and this processing step requires the product of the *agrB* gene (Novick 1999). The processed autoinducing peptide (AIP) is unique in that it contains a thiolactone ring (Mayville et al. 1999). The products of the *agrC* and *agrA* genes, AgrC and AgrA, respectively, comprise the two-component sensor kinase and response-regulator signaling pair. Following the buildup and detection of AIP, the AgrC/AgrA two-component phosphorylation cascade culminates in phosphorylation of AgrA. Phospho-AgrA is responsible for increasing RNAIII levels in the cell, although the exact mechanism of activation has not been determined. The RNAIII, through another unknown mechanism, subsequently functions to activate the expression of a variety of exported virulence factors in *S. aureus*.

Quorum Sensing in *Vibrio harveyi*: Integration of AHL and Two-Component Signaling

Vibrio harveyi is a marine bacterium that uses quorum sensing, among other environmental cues, to modulate bioluminescence (Bassler 1999a). However, unlike *Vibrio fischeri*, *V. harveyi* is not known to exist in symbiotic relationships, and the benefit it receives from producing light remains a mystery. Although *V. harveyi* is a Gram-negative bacterium, its quorum-sensing circuit possesses features reminiscent of both Gram-negative (see section ❯ "Quorum Sensing in Gram-Negative Bacteria: The LuxI/LuxR Paradigm") and Gram-positive bacteria (see section ❯ "Gram-Positive Quorum Sensing: Peptide Signals and Two-Component Signal Transduction"; Bassler 1999b). For this reason, we have chosen to discuss the *V. harveyi* quorum-sensing circuit separately from the other Gram-negative systems.

Analogous to other Gram-negative bacteria, *V. harveyi* produces and responds to an AHL autoinducer (Bassler et al. 1993; Cao and Meighen 1989). In contrast to Gram-negative bacteria, but similar to Gram-positive bacteria, detection of and response to autoinducer are carried out by a two-component circuit (Bassler et al. 1993, 1994a, b). Additionally, *V. harveyi* possesses a novel autoinducer signaling molecule, called "AI-2" (Bassler et al. 1993; Surette and Bassler 1998). The AI-2 molecule and the

gene required for its production have recently been demonstrated to occur in a wide variety of Gram-negative and Gram-positive bacteria (Surette and Bassler 1998; Surette et al. 1999). The AI-2 molecule could be the common link that connects the evolution of the two major classes of quorum-sensing circuits.

Multiple Systems Regulate Quorum Sensing in *Vibrio harveyi*

Vibrio harveyi was the first bacterium in which the use of multiple autoinducers was described (Bassler et al. 1994a). Specifically, two parallel quorum-sensing systems converge to regulate *luxCDABE*, the luciferase structural operon in *V. harveyi*. System 1 is comprised of autoinducer-1 (AI-1) and Sensor 1 (LuxN); System 2 consists of autoinducer-2 (AI-2) and Sensor 2 (LuxPQ) (Bassler et al. 1993, 1994a). While the two sensors detect independent autoinducer signals, the parallel signaling systems converge at a downstream integrator protein called "LuxU" (Freeman and Bassler 1999b). Integration of the two autoinducer cues allows light production in *V. harveyi* to be modulated by multiple inputs (Fig. 22.5).

Both LuxN and LuxQ are members of a family of two-component proteins called "hybrid kinases." Members of the hybrid sensor kinase family contain multiple signaling modules. In the case of LuxN and LuxQ, each possesses both a sensor kinase domain and an attached response-regulator domain. In *V. harveyi*, at low cell density, when little autoinducer is present, the hybrid sensor kinases LuxN and LuxQ initiate a phosphorelay cascade that results in phosphorylation of the signal integrator protein LuxU, and finally the phosphoryl group is transferred to a response-regulator protein called "LuxO" (Freeman et al. 2000). When LuxO is phosphorylated, the luciferase operon (*luxCDABE*) is not transcribed, and the bacteria do not make light (Bassler et al. 1994b; Freeman and Bassler 1999a). Conversely, at high cell density, when autoinducer is abundant, the sensors switch from being kinases to being phosphatases. Phosphatase activity leads to the dephosphorylation of LuxO and subsequent transcription of *luxCDABE*. As in *V. fischeri*, transcription of *luxCDABE* in *V. harveyi* results in luciferase production and light emission. Additionally, a transcriptional activator, LuxR, is absolutely required for the transcription of the *V. harveyi luxCDABE* operon (Martin et al. 1989; Showalter et al. 1990). However, the *V. harveyi* LuxR protein shares no homology to the *V. fischeri* family of LuxR transcriptional activators (The *Vibrio fischeri* LuxI/LuxR System).

Apparently *V. harveyi* does not possess LuxI/LuxR homologues. This finding is surprising because the *V. harveyi* AI-1 is an AHL, *N*-(3-hydroxybutanoyl)-homoserine lactone (Cao and Meighen 1989). Synthesis of AI-1 however is not dependent on a *luxI* gene. Rather synthesis of the *V. harveyi* AHL autoinducer is dependent on the *luxLM* locus (Bassler et al. 1993). The genes

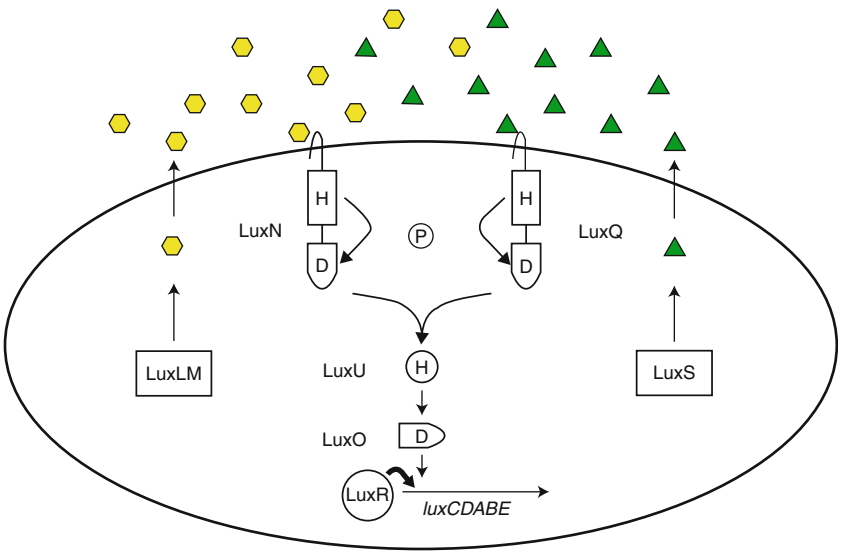


Fig. 22.5

Quorum sensing in *V. harveyi*. This model demonstrates how *V. harveyi* uses both an AHL (AI-1, *yellow hexagons*) and a novel autoinducer signal (AI-2, *green triangles*) as autoinducers to regulate quorum sensing. *V. harveyi* has two parallel two-component signaling circuits. LuxN is the hybrid sensor kinase for AI-1 (synthesized by LuxLM), and LuxQ senses and responds to AI-2 (synthesized by LuxS). At low cell density and low autoinducer concentration, LuxN and LuxQ autophosphorylate and initiate phosphoryl flow through LuxU to the response regulator, LuxO. When LuxO is phosphorylated, *luxCDABE* is not transcribed and the bacteria make no light. Conversely, at high cell density and high autoinducer concentration, the sensor kinases switch from being kinases to phosphatases, which results in the draining of phosphate out of the system. When LuxO is dephosphorylated, *luxCDABE* is transcribed and the bacteria make light. Additionally, the transcriptional activator LuxR (not homologous to the *V. fischeri* LuxR) is required for the transcription of *luxCDABE*. The *oval* represents a bacterium; *H* histidine, *D* aspartic acid. The P in the *circle* represents the phosphorylation cascade

luxL and *luxM* share no homology with the *luxI* gene family. The second *V. harveyi* autoinducer, AI-2, is not an AHL, as its structure has recently been determined (Schauder and B.L. Bassler, manuscript in preparation). Synthesis of AI-2 is dependent on the gene *luxS* (Surette et al. 1999). Again, the *luxS* gene is not similar to the *luxI* gene.

Although no quorum-sensing components similar to *V. fischeri* LuxI and LuxR have been identified in *V. harveyi*, the opposite is not the case. A LuxM homologue (called "AinS") and homologues of the two-component proteins LuxU and LuxO have recently been identified in *V. fischeri* (Kuo et al. 1994; Gilson et al. 1995; Miyamoto et al. 2000). The AinS protein directs the synthesis of an AHL autoinducer, and LuxU and LuxO play a role in density-dependent regulation of *lux* expression in *V. fischeri*. It is now becoming apparent that multiple signaling circuits could be involved in quorum sensing in *V. fischeri* similar to what is known about quorum-sensing regulation in *V. harveyi*.

Recently many Gram-negative and Gram-positive bacteria have been shown to produce a *V. harveyi* AI-2-like activity, whereas only one closely related species, *Vibrio parahaemolyticus*, has been identified to produce a *V. harveyi* AI-1-like activity (Bassler et al. 1997). This finding led to the hypothesis that, in *V. harveyi*, AI-1 and System 1 are involved in intraspecies quorum sensing, while AI-2 and System 2 could be used by *V. harveyi* for interspecies cell-cell communication. The convergence of these two quorum-sensing systems allows *V. harveyi* to regulate light production in response to its own high cell density and also in response to the presence of other species of bacteria. This observation is noteworthy because, in its natural habitat, *V. harveyi* is expected to exist in mixed populations containing many species of bacteria. Multiple autoinducer languages could grant *V. harveyi* a selective advantage in the wild. If *V. harveyi* can detect its own species as well as the presence of other species of bacteria, this ability could allow *V. harveyi* to determine when it is likely to be in intense competition for scarce nutrients. In addition, *V. harveyi* could specifically and appropriately modulate gene expression in response to the presence of other species of bacteria. Distinct roles for the two *V. harveyi* quorum-sensing systems are further supported by the recent discovery that *V. harveyi* regulates many different genes in addition to *lux* by these two autoinducers. In addition, AI-1- and AI-2-specific targets have now been identified in *V. harveyi* indicating that not all of the quorum-sensing information is channeled to LuxU and LuxO to control gene expression (B.L. Bassler, unpublished data).

LuxS and AI-2: The Language of Interspecies Communication

As mentioned, a gene called *luxS* is required for the production of AI-2 in *V. harveyi* (Surette et al. 1999). DNA database analysis revealed that highly conserved homologues of this novel gene are present in over 30 species of both Gram-negative and Gram-positive bacteria, including, but not limited to, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella paratyphi*, *Haemophilus influenzae*, *Helicobacter pylori*, *B. subtilis*, *Borrelia burgdorferi*, *Neisseria meningitidis*, *Yersinia pestis*, *Campylobacter jejuni*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Enterococcus faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, *Clostridium perfringens*, *Clostridium difficile*, and *Klebsiella pneumoniae*. Most of these species have now been shown to produce an AI-2 activity, and mutation of *luxS* in a number of these species, including *V. harveyi*, *S. typhimurium*, and *E. coli*, eliminates AI-2 production. These results suggest that *luxS* is responsible for AI-2 production in all of these bacteria. Because both Gram-negative and Gram-positive bacterial species are represented, and because preliminary evidence indicates that many of these bacteria are producing an identical signaling molecule, AI-2 is considered a universal bacterial language that bacteria could use for interspecies communication (Bassler 1999b). Therefore, in contrast to AHL and peptide quorum-sensing systems, which represent species-specific bacterial languages, AI-2 and LuxS could be the foundation of a species-nonspecific bacterial language.

The identities of the genes regulated by the AI-2 signal are being actively sought in a number of bacteria. Initial evidence in several bacterial species indicates that AI-2 is involved in regulation of pathogenicity. For example, AI-2-specific activation of the locus of enterocyte effacement (LEE) pathogenicity island, encoding a type III secretion system in *E. coli* O157, has been reported (Sperandio et al. 1999). Secretion of virulence factors in *Vibrio vulnificus* has been shown to be controlled by AI-2, and furthermore, the LD50 of a *V. vulnificus luxS* mutant is greatly increased (Kim et al. 2000). Presently there are only a few reports of AI-2 regulated target-gene expression, but this is most likely because the *luxS* gene and its widespread nature have only recently been discovered. It will be interesting to determine how bacteria that live in diverse habitats have adapted the use of the AI-2 quorum-sensing language to enhance survival in their particular niches.

Quorum Sensing in *Myxococcus xanthus*: A Unique Sensory System

Myxococcus xanthus is a Gram-negative soil bacterium that displays complex social behaviors. *Myxococcus xanthus* moves by gliding. Specifically, it glides over and colonizes solid surfaces such as decaying plant material that it subsequently uses for nutrients. The *M. xanthus* bacteria hunt for food in swarms, a behavior that allows the individual cells to take advantage of secreted hydrolytic enzymes produced by neighboring cells (Dworkin 1973; Dworkin and Kaiser 1985). At high cell density under nutrient-limiting conditions, *M. xanthus* forms complex structures called fruiting bodies. Bacterial cells inside the fruiting body undergo a developmental process that leads to spore formation. Spore formation is partially controlled by a quorum-sensing circuit. However, in contrast to other Gram-negative quorum-sensing bacteria, in *M. xanthus*, quorum sensing is not dependent on an AHL autoinducer. Therefore, because

M. xanthus has a unique quorum-sensing system, as in the *Vibrio harveyi* case (Quorum Sensing in *Vibrio harveyi*: Integration of AHL and Two-Component Signaling), this system is discussed separately.

A secreted signal called "A-signal" is required for quorum sensing in *M. xanthus* (Hagen et al. 1978; LaRossa et al. 1983). The A-signal is a mixture of amino acids that are produced as a consequence of the enzymatic action of extracellular proteases (Kupsa et al. 1992a, b; Plamann et al. 1992). Similar to other quorum-sensing bacteria, the extracellular concentration of the signal increases as the cell population density increases. Three genes are necessary for production of A-signal. These genes are called *asgA*, *asgB*, and *asgC*. The AsgA protein is a two-component sensor kinase, AsgB is a DNA-binding transcriptional regulator, and AsgC encodes the housekeeping σ factor for *M. xanthus* (Davis et al. 1995; Plamann et al. 1994, 1995). These proteins, as well as others that remain to be identified, function in a signaling circuit that activates the expression of genes encoding the secreted proteases required for A-signal generation.

Detection of A-signal is via a two-component sensor kinase called "SasS." The SasS protein transfers phosphate to a response regulator called "SasR" (Kaplan et al. 1991; Yang and Kaplan 1997). Phosphorylated-SasR, in conjunction with the alternative sigma factor σ^{54}, activates downstream target genes. These genes are hypothesized to encode structural and possibly regulatory proteins that are required for the spore differentiation process. A negative regulatory protein, called "SasN," must also be inactivated for *M. xanthus* to respond to A-signal. The function of SasN is not known, and SasN is not homologous to any other identified protein (Gorski et al. 2000; Xu et al. 1998). This system is remarkable because it is so different from every other described quorum-sensing system. Apparently, some facet(s) of the complicated social lifestyle of *M. xanthus* warrants the use of a distinct quorum-sensing mechanism.

Eukaryotic Interference with Quorum Sensing

Outlined in this chapter (sections ❯ "Quorum Sensing in Gram-Negative Bacteria: The LuxI/LuxR Paradigm," ❯ "Gram-Positive Quorum Sensing: Peptide Signals and Two-Component Signal Transduction," ❯ "Quorum Sensing in *Vibrio harveyi*: Integration of AHL and Two-Component Signaling," ❯ "Quorum Sensing in *Myxococcus xanthus*: A Unique Quorum Sensing System") are just a few examples of the many known quorum-sensing systems. Identification of new quorum-sensing systems continues at a rapid pace, in part owing to the use of PCR amplification of quorum-sensing genes based on homology to known quorum-sensing regulators and also owing to the development of easy-to-use bioassays that facilitate the cloning and identification of new quorum-sensing genes (Swift et al. 1993). However, in general, defining and understanding the regulons controlled by new quorum-sensing regulatory proteins typically lag behind the identification of the autoinducer/sensor pair. Although we know that over 25 LuxI/LuxR systems exist, and at least as many species of bacteria produce AI-2 and possess a LuxS homologue, in most cases we do not yet know what targets these autoinducer systems control.

It is clear, however, that several quorum-sensing systems have been demonstrated to modulate the expression of virulence factors, and interest in designing and implementing novel antimicrobial strategies that target quorum sensing in pathogenic bacteria is high (Zhu et al. 1998). In addition to synthetic strategies for drug design based on autoinducers, it seemed likely that eukaryotes that are susceptible to infection by quorum-sensing bacteria could have already evolved natural therapies to thwart bacterial invasion by inhibiting quorum sensing. One such example of the evolution of a naturally occurring antibacterial agent that specifically counteracts invasion of a eukaryotic host by quorum-sensing bacteria is documented (Givskov et al. 1996). The seaweed *Delisea pulchra* produces halogenated furanones, molecules that are structurally related to AHL autoinducers. The furanone of *D. pulchra* has the ability to inhibit a social motility phenotype called "swarming" in *Serratia liquefaciens* and other bacterial species. Swarming motility allows bacteria to move over and colonize a surface. Swarming in *S. liquefaciens* is controlled by an AHL quorum-sensing system (Eberl et al. 1996). The halogenated furanone produced by the eukaryotic host specifically binds with high affinity to the *Vibrio fischeri* LuxR protein and presumably to the S. liquefaciens LuxR homologue to inhibit motility (Manefield et al. 1999). Although *D. pulchra* and *S. liquefaciens* do not encounter each other in nature, the ability of *D. pulchra* to inhibit swarming is likely a general host defense mechanism that prevents colonization of the plant surface by bacteria (Givskov et al. 1997). While the details and in vivo significance of eukaryotic interference with bacterial quorum sensing are yet to be defined, it remains an intriguing eukaryotic defense mechanism that may have long-range implications in antimicrobial therapy. Likewise, competing bacterial populations might also inhibit each other's quorum sensing by developing autoinducer antagonists. To date, there is only one documented example of such a process between populations of coexisting bacteria (Dong et al. 2000). It has recently been shown that AiiA, an enzyme produced by *B. subtilis*, inactivates the *E. carotovora* AHL quorum-sensing signal, thereby attenuating the virulence of *E. carotovora*. The AiiA enzyme is similar to members of a zinc-binding metallohydrolase family of proteins. Much emphasis is now being placed on identifying such antagonistic eukaryotic-bacterial and bacterial-bacterial interactions and the molecules that control these interactions.

Conclusions

Quorum-sensing systems have been widely adapted for a variety of uses by bacteria. However, in every case, quorum sensing confers on bacteria the ability to communicate and further to alter gene expression in response to the presence of other bacteria. This ability allows a population of small organisms to behave

as a multicellular unit and to gain power and reap benefits that would otherwise be exclusive to eukaryotes. The study of quorum sensing is in its infancy. We need to learn more about how quorum sensing is used by bacteria to communicate both within and between species and how it is used by bacteria to act synergistically and to overcome competitors. We need to understand the variety of signals produced and how information contained within those signals is transduced and integrated to control an elaborate series of responses. Further, the knowledge we gain from studies of quorum sensing can be used as the basis for the design of novel antibacterial therapies. This is especially important at a time when new antibacterial pharmaceuticals are required to combat the ever-increasing problem of multidrug resistance in bacteria.

Finally, the phenomenon of quorum sensing and how bacteria talk to each other is a fascinating one, and its study could reveal fundamental principles about cell-cell communication and information flow. Additionally, if antibiotics can be designed that specifically counteract quorum sensing, these fundamental quorum-sensing studies could prove to have enormous practical application.

References

Ahmer BMM, Van Reeuwijk J, Timmers CD, Valentine PJ, Heffron F (1998) *Salmonella typhimurium* encodes an sdiA homolog, a putative quorum sensor of the luxR family, that regulates genes on the virulence plasmid. J Bacteriol 180:1185–1193

Atkinson S, Throup JP, Stewart GSAB, Williams P (1999) A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. Mol Microbiol 33:1267–1277

Bainton NJ, Bycroft BW, Chhabra SR, Stead P, Gledhill L, Hill PJ, Rees CED, Winson MK, Salmond GPC, Stewart GSAB, Williams P (1992) A general role for the lux autoinducer in bacterial cell signalling: control of antibiotic biosynthesis in Erwinia. Gene 116:87–91

Bassler BL (1999a) A multichannel two-component signaling relay controls quorum sensing in *Vibrio harveyi*. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 259–276

Bassler BL (1999b) How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr Opin Microbiol 2:582–587

Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Mol Microbiol 9:773–786

Bassler BL, Wright M, Silverman MR (1994a) Multiple signalling systems controlling gene expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. Mol Microbiol 13:273–286

Bassler BL, Wright M, Silverman M (1994b) Sequence and function of luxO, a negative regulator of luminescence in *Vibrio harveyi*. Mol Microbiol 12:403–412

Bassler BL, Greenberg EP, Stevens AM (1997) Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. J Bacteriol 179:4043–4045

Beck von Bodman S, Farrand SK (1995) Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an n-acylhomoserine lactone autoinducer. J Bacteriol 177:5000–5008

Cao J-G, Meighen EA (1989) Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. J Biol Chem 264:21670–21676

Chernin LS, Winson MK, Thompson JM, Haran S, Bycroft BW, Chet I, Williams P, Stewart GSAB (1998) Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by quorum sensing. J Bacteriol 180:4435–4441

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

Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298

Davis JM, Mayor J, Plamann L (1995) A missense mutation in rpoD results in an A-signalling defect in *Myxococcus xanthus*. Mol Microbiol 18:943–952

De Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849

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

Dworkin M (1973) Cell-cell interactions in the myxobacteria. Symp Soc Gen Microbiol 23:125–147

Dworkin M, Kaiser D (1985) Cell interactions in myxobacterial growth and development. Science 230:18–24

Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ (1981) Structural identification of autoinducer of *Photobacterium fischeri*. Biochemistry 20:2444–2449

Eberl L, Winson MK, Sternberg C, Stewart GSAB, Christiansen G, Chhabra SR, Bycroft B, Williams P, Molin S, Givskov M (1996) Involvement of n-acyl-l-homoserine lactone autoinducers in controlling the multicellular behavior of *Serratia liquefaciens*. Mol Microbiol 20:127–136

Engebrecht J, Silverman M (1984) Identification of genes and gene products necessary for bacterial bioluminescence. Proc Natl Acad Sci 81:4154–4158

Engebrecht J, Silverman M (1987) Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. Nucleic Acids Res 15:10455–10467

Engebrecht J, Nealson K, Silverman M (1983) Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. Cell 32:773–781

Evans K, Passador L, Ramakrishnan S, Tsang E, Nezezon J, Poole K (1998) Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 180:5443–5447

Flavier AB, Ganova-Raeva LM, Schell MA, Denny TP (1997) Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. J Bacteriol 179:7089–7097

Freeman JA, Bassler BL (1999a) A genetic analysis of the function of luxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. Mol Microbiol 31:665–677

Freeman JA, Bassler BL (1999b) Sequence and function of luxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. J Bacteriol 181:899–906

Freeman JA, Lilley BN, Bassler BL (2000) A genetic analysis of the functions of luxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. Mol Microbiol 35:139–149

Fuqua C, Eberhard A (1999) Signal generation in autoinduction systems: synthesis of acylated homoserine lactones by luxI-type proteins. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 211–230

Fuqua C, Winans SC, Greenberg EP (1996) Census and consensus in bacterial ecosystems: the luxR-luxI family of quorum-sensing transcriptional regulators. Annu Rev Microbiol 50:727–751

Garcia-Lara J, Shang LH, Rothfield LI (1996) An extracellular factor regulates expression of sdiA, a transcriptional activator of cell division genes in *Escherichia coli*. J Bacteriol 178:2742–2748

Gilson L, Kuo A, Dunlap PV (1995) AinS and a new family of autoinducer synthesis proteins. J Bacteriol 177:6946–6951

Givskov M, De Nys R, Manefield M, Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178:6618–6622

Givskov M, Eberl L, Molin S (1997) Control of exoenzyme production, motility and cell differentiation in *Serratia liquefaciens*. FEMS Microbiol Lett 148:115–122

Gorski L, Gronewold T, Kaiser D (2000) A sigma(54) activator protein necessary for spore differentiation within the fruiting body of *Myxococcus xanthus*. J Bacteriol 182:2438–2444

Gray KM, Passador L, Iglewski BH, Greenberg EP (1994) Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. J Bacteriol 176:3076–3080

Gray KM, Pearson JP, Downie JA, Boboye BEA, Greenberg EP (1996) Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum*: autoinduction of a stationary phase and rhizosphere-expressed genes. J Bacteriol 178:372–376

Hagen DC, Bretcher AP, Kaiser D (1978) Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev Biol 64:284–296

Hanzelka BL, Greenberg EP (1996) Quorum sensing in *Vibrio fischeri*: evidence that s-adenosylmethionine is the amino acid substrate for autoinducer synthesis. J Bacteriol 178:5291–5294

Havarstein LS, Morrison DA (1999) Quorum sensing and peptide pheromones in streptococcal competence for genetic transformation. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 9–26

Jones S, Yu B, Bainton NJ, Birdsall M, Bycroft BW, Chhabra SR, Cox AJR, Golby P, Reeves PJ, Stephens S, Winson MK, Salmond GPC, Stewart GSAB, Williams P (1993) The lux autoinducer regulates the production of exoenzyme virulence determinants in *Erwinina carotovora* and *Pseudomonas aeruginosa*. EMBO J 12:2477–2482

Kaplan HB, Greenberg EP (1985) Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. J Bacteriol 163:1210–1214

Kaplan HB, Kupsa A, Kaiser D (1991) Suppressors that permit A-signal-independent developmental gene expression in *Myxococcus xanthus*. J Bacteriol 173:1460–1470

Kim SY, Lee SE, Kim YR, Kim JH, Ryu PY, Chung SS, Rhee JH (2000) Virulence regulatory role of luxS quorum sensing system in *Vibrio vulnificus*. In: Abstract, ASM general meeting, Los Angeles, CA.Abstract B-248

Kleerebezem M, Quadri LEN, Kuipers OP, de Vos WM (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 25:895–904

Kuo A, Blough NV, Dunlap PV (1994) Multiple n-acyl-l-homoserine lactone autoinducers of luminescence in the marine symbiotic bacterium *Vibrio fischeri*. J Bacteriol 176:7558–7565

Kupsa A, Plamann L, Kaiser D (1992a) A-signalling and the cell density requirement for *Myxococcus xanthus* development. J Bacteriol 174:7360–7369

Kupsa A, Plamann L, Kaiser D (1992b) Identification of heat-stable A-factor from *Myxococcus xanthus*. J Bacteriol 174:3319–3326

LaRossa R, Kuner J, Hagen D, Manoil C, Kaiser D (1983) Developmental cell interactions of *Myxococcus xanthus*: analysis of mutants. J Bacteriol 153:1394–1404

Latifi A, Foglino M, Tanaka K, Williams P, Luzdunski A (1996) A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR to expression of the stationary-phase sigma factor rpoS. Mol Microbiol 21:1137–1146

Lazazzera BA, Grossman AD (1998) The ins and outs of peptide signaling. Trends Microbiol 6:288–294

Lazazzera BA, Palmer T, Quisel J, Grossman AD (1999) Cell-density control of gene expression and development in *Bacillus subtilis*. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 27–46

Lee K-H, Ruby EG (1994) Effect of the squid host on the abundance and distribution of symbiotic *Vibrio fischeri* in nature. Appl Environ Microbiol 60:1565–1571

Lewenza S, Conway B, Greenberg EP, Sokol PA (1999) Quorum sensing in *Burkholderia cepacia*: identification of the luxRI homologs CepRI. J Bacteriol 181:748–756

Lithgow JK, Wilkinson A, Hardman A, Rodelas B, Wisniewski-Dye F, Williams P, Downie JA (2000) The regulatory locus cinRI in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. Mol Microbiol 37:81–97

Luo Z, Qin Y, Farrand S (2000) The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interacting with the C-terminal region of the quorum-sensing activator. J Biol Chem 275:7713–7722

Manefield M, de Nys R, Kumar N, Read R, Givskov M, Steinberg P, Kjelleberg S (1999) Evidence that halogated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. Microbiology 145:283–291

Martin M, Showalter R, Silverman M (1989) Identification of a locus controlling expression of luminescence genes in *Vibrio harveyi*. J Bacteriol 171: 2406–2414

Mayville P, Ji G, Beavis R, Yang H, Goger M, Novick RP, Muir TW (1999) Structure-activity analysis of synthetic autoinducing thiolactone peptides from S*taphylococcus aureus* responsible for virulence. Proc Natl Acad Sci 96:1218–1223

McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, Daykin M, Lamb JH, Swift S, Bycroft BW, Stewart GSAB, Williams P (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of n-acylhomoserine lactones. Microbiology 143:3703–3711

McKnight SL, Iglewski BH, Pesci EC (2000) The Pseudomonas quinolone signal regulates Rhl quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 182:2702–2708

Milton DL, Hardman A, Camara M, Chhabra SR, Bycroft BW, Stewart GSAB, Williams P (1997) Quorum sensing in *Vibrio anguillarum*: characterization of the vanI/vanR locus and identification of the autoinducer n-(3-oxodecanoyl)-l-homoserine lactone. J Bacteriol 179:3004–3012

Miyamoto CM, Lin YH, Meighen EA (2000) Control of bioluminescence in *Vibrio fischeri* by the luxO signal response regulator. Mol Microbiol 36:594–607

More MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC (1996) Enzymatic synthesis of a quorum sensing autoinducer through use of defined substrates. Science 272:1655–1658

Morfeldt E, Tegmark K, Arvidson S (1996) Transcriptional control of the agr-dependent virulence gene regulator, RNAIII, in *Staphylococcus aureus*. Mol Microbiol 21:1227–1237

Nasser W, Bouillant ML, Salmond G, Reverchon S (1998) Characterization of the *Erwinia chrysanthemi* ExpI-ExpR locus directing the synthesis of two n-acyl-homoserine lactone signal molecules. Mol Microbiol 29:1391–1405

Novick RP (1999) Regulation of pathogenicity in *Staphylococcus aureus* by a peptide-based density-sensing system. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 129–146

Ochsner UA, Reiser J (1995) Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. Proc Natl Acad Sci 92:6424–6428

Parkinson JS (1995) Genetic approaches for signaling pathways and proteins. In: Hoch JA, Silhavy TJ (eds) Two-component signal transduction. ASM Press, Washington, DC, pp 9–24

Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP (1999) Acyl homoserine-lactone quorum-sensing signal generation. Proc Natl Acad Sci 96:4360–4365

Passador L, Cook JM, Gambello MJ, Rust L, Iglewski BH (1993) Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science 260:1127–1130

Pearson JP, Gray KM, Passador L, Tucker KD, Eberhard A, Iglewski BH, Greenberg EP (1994) Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. Proc Natl Acad Sci 91:197–201

Pearson JP, Passador L, Iglewski BH, Greenberg EP (1995) A second n-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc Natl Acad Sci 92:1490–1494

Pesci EC, Iglewski BH (1997) The chain of command in *Pseudomonas* quorum sensing. Trends Microbiol 2:132–134

Pesci EC, Iglewski BH (1999) Quorum sensing in *Pseudomonas aeruginosa*. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 147–155

Pesci EC, Milbank JBJ, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc Natl Acad Sci 96:11229–11234

Pierson LS III, Keppenne VD, Wood DW (1994) Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by phzR in response to cell density. J Bacteriol 176:3966–3974

Pierson LS III, Wood DW, Beck von Bodman S (1999) Quorum sensing in plant-associated bacteria. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 101–115

Piper KR, Beck von Bodman S, Farrand SK (1993) Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. Nature 362:448–450

Pirhonen M, Flego D, Heikinheimo R, Palva ET (1993) A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. EMBO J 12:2467–2476

Plamann L, Kuspa A, Kaiser D (1992) Proteins that rescue A-signal-defective mutants of *Myxococcus xanthus*. J Bacteriol 174:3311–3318

Plamann L, Davis JM, Cantwell B, Mayor J (1994) Evidence that asgB encodes a DNA-binding protein essential for growth and development of *Myxococcus xanthus*. J Bacteriol 176:2013–2020

Plamann L, Li Y, Cantwell B, Mayor J (1995) The *Myxococcus xanthus* asgA gene encodes a novel signal transduction protein required for multicellular development. J Bacteriol 177:2014–2020

Puskas A, Greenberg EP, Kaplan S, Schaefer AL (1997) A quorum-sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. J Bacteriol 179:7530–7537

Reverchon S, Bouillant ML, Salmond G, Nasser W (1998) Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*. Mol Microbiol 29:1407–1418

Rodelas B, Lithgow JK, Wisniewski-Dye F, Hardman A, Wilkinson A, Economou A, Williams P, Downie JA (1999) Analysis of quorum-sensing-dependent control of rhizosphere-expressed (rhi) genes in *Rhizobium leguminosarum* bv. viciae. J Bacteriol 181:3816–3823

Rosemeyer V, Michiels J, Verreth C, Vanderleyden J (1998) LuxI-and luxR-homologous genes of Rhizobium etli CNPAF512 contribute to synthesis of autoinducer molecules and nodulation of *Phaseolus vulgaris*. J Bacteriol 180:815–821

Ruby EG, McFall-Ngai MJ (1992) A squid that glows in the night: development of an animal-bacterial mutualism. J Bacteriol 174:4865–4870

Salmond GPC, Bycroft BW, Stewart GSAB, Williams P (1995) The bacterial 'enigma': cracking the code of cell-cell communication. Mol Microbiol 16:615–624

Showalter R, Martin MO, Silverman MR (1990) Cloning and nucleotide sequence of luxR, a regulatory gene controlling bioluminescence in *Vibrio*. J Bacteriol 172:2946–2954

Sitnikov DM, Schineller JB, Baldwin TO (1996) Control of cell division in *Escherichia coli*: regulation of transcription of ftsQA involves both rpoS and sdiA-mediated autoinduction. Proc Natl Acad Sci 93:336–341

Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB (1999) Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic Escherichia coli. Proc Natl Acad Sci 96:15196–15201

Stevens AM, Greenberg EP (1999) Transcriptional activation by luxR. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 231–242

Stock JB, Ninfa AJ, Stock AM (1989) Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol Rev 53:450–490

Surette MG, Bassler BL (1998) Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. Proc Natl Acad Sci 95:7046–7050

Surette MG, Miller MB, Bassler BL (1999) Quorum sensing in *Escherichia coli, Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc Natl Acad Sci 96: 1639–1644

Swift S, Winson MK, Chan PF, Bainton NJ, Birdsall M, Reeves PJ, Rees CED, Chhabra SR, Hill PJ, Throup JP, Bycroft BW, Salmond GPC, Williams P, Stewart GSAB (1993) A novel strategy for the isolation of luxI homologues: evidence for the widespread distribution of a luxR:luxI superfamily in enteric bacteria. Mol Microbiol 10:511–520

Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, Chhabra SR, Williams P, Macintyre S, Stewart GSAB (1997) Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the luxRI homologs AhyRI and AsaRI and their cognate n-acylhomoserine lactone signal molecules. J Bacteriol 179:5271–5281

Swift S, Lynch MJ, Fish L, Kirke DF, Tomas JM, Stewart GSAB, Williams P (1999) Quorum sensing-dependent regulation and blockade of exoprotease production in *Aeromonas hydrophila*. Infect Immun 67:5192–5199

Throup JP, Camara M, Briggs GS, Winson MK, Chhabra SR, Bycroft BW, Williams P, Stewart GSAB (1995) Characterisation of the yenI/yenR locus from *Yersinia enterocolitica* mediating the synthesis of two n-acylhomoserine lactone signal molecules. Mol Microbiol 17:345–356

Tortosa P, Dubnau D (1999) Competence for transformation: a matter of taste. Curr Opin Microbiol 2:588–592

Val DL, Cronan JE Jr (1998) In vivo evidence that s-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the luxI family of autoinducer synthases. J Bacteriol 180:2644–2651

Visick KL, McFall-Ngai MJ (2000) An exclusive contract: specificity in the *Vibrio fischeri*-Euprymna scolopes partnership. J Bacteriol 182:1779–1787

Williams P, Bainton NJ, Swift S, Chhabra SR, Winson MK, Stewart GSAB, Salmond GPC, Bycroft BW (1992) Small molecule-mediated density-dependent control of gene expression in prokaryotes: bioluminescence and the biosynthesis of carbapenem antibiotics. FEMS Microbiol Lett 100:161–168

Winans SC, Zhu J, More MI (1999) Cell density-dependent gene expression by *Agrobacterium tumefaciens* during colonization of crown gall tumors. In: Dunny GM, Winans SC (eds) Cell-cell signaling in bacteria. ASM Press, Washington, DC, pp 117–128

Winson MK, Camara M, Latifi A, Foglino M, Chhabra SR, Daykin M, Bally M, Chapon V, Salmond GPC, Bycroft BW, Lazdunski A, Stewart GSAB, Williams P (1995) Multiple n-acyl-l-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. Proc Natl Acad Sci 92:9427–9431

Withers HL, Nordstrom K (1998) Quorum-sensing acts at initiation of chromosomal replication in *Escherichia coli*. Proc Natl Acad Sci 95:15694–15699

Wood DW, Gong F, Daykin MM, Williams P, Pierson LS III (1997) N-acyl-homoserine lactone-mediated regulation of phenazine gene expression by *Pseudomonas aureofaciens* 30-84 in the wheat rhizosphere. J Bacteriol 179:7663–7670

Xu D, Yang C, Kaplan HB (1998) *Myxococcus xanthus* sasN encodes a regulator that prevents developmental gene expression during growth. J Bacteriol 180:6215–6223

Yang C, Kaplan HB (1997) *Myxococcus xanthus* sasS encodes a sensor histidine kinase required for early developmental gene expression. J Bacteriol 179:7759–7767

Zhang L, Murphy PJ, Kerr A, Tate ME (1993) Agrobacterium conjugation and gene regulation by n-acyl-l-homoserine lactones. Nature 362:446–448

Zhu J, Beaber JW, More MI, Fuqua C, Eberhard A, Winans SC (1998) Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of *Agrobacterium tumefaciens*. J Bacteriol 180:5398–5405

23 Cell-Cell Interactions

Dale Kaiser
Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA

What Is Swarming?

As defined by Harshey (Harshey and Matsuyama 1994), "Swarming (with flagella) is a coordinated multicellular activity during which swarmer cells maintain lengthwise contact with each other. Although individual swarmer cells move rapidly back and forth within the swarming bacterial mass, the colony as a whole migrates outward. This manner of surface translocation is generally not observed in isolated cells." Swarming involves interactions between cells that may be touching each other. As will be seen below, Harshey's definition is useful for swarming with motility engines other than flagella, such as type IV pili that are found in gliding bacteria such as the myxobacteria (Kaiser 2007). The similarities between swarming with flagella and with type IV pili encourage a search for general patterns of swarm cell movement that is independent of the engine employed. Henrichsen's earlier definition of swarming (Henrichsen 1972), which restricted it to rotating flagella, was based on a misunderstanding of gliding motility.

Swarming with Flagella and Chemotaxis

To swarm with flagella requires some of an organism's chemosensory genes, but apparently they are not used to ensure movement up a gradient of attractant. Instead, swarming appears to use a subset of *che* genes to regulate flagellar structural gene expression and to ensure hyper-flagellation. None of the cell-surface chemotaxis receptors are required for swarming. Without them, how could a gradient of attractant be sensed? Moreover, Wolfe and Berg (Wolfe and Berg 1989) found that their "gutted" mutant of *Escherichia coli*, which lacked most of the chemotaxis receptors still swarmed, albeit at a very low rate. They observed that by adding CheY alone, they restored tumbling and coordinately increased the rate of swarm expansion, although the final rate was less than wild type with a full set of *che* genes. Harshey and Matsuyama (1994) observed that most receptor-deficient mutants of *E. coli* and *Salmonella typhimurium* were able to swarm on complex medium (semi-)solidified with Eiken agar, while their *che*A, *che*B, *che*R, *che*W, *che*Y, and *che*Z mutants that were defective in the chemotactic signaling pathway failed to swarm. *Bacillus cereus* (Senesi et al. 2002) requires fliY (equivalent to fliN in gram-negative bacteria) that encodes the C-ring of the flagellum. The C-ring also includes fliM and fliG proteins, all parts of the flagellar switch complex that produces hyper-flagellation for swarming. *Rhodospirillum centenum* (Berleman and Bauer 2005) has three *che* operons, but only the che_2 operon is needed for swarming. MCP_2, which is cytosolic by amino acid sequence and therefore not membrane localized, is not needed for swarming, but $CheW_2$, $CheB_2$, $CheR_2$, and $CheY_2$ are needed for swarming. *R. centenum* swarms on agar-solidified media, and it will hyper-flagellate in liquid medium, if the medium is viscous. Berleman concludes from his experiments that regulation of hyper-flagellation in *R. centenum* is posttranscriptional. In *Bacillus subtilis che*V, *che*W, and *che*B mutants are Che^- but swarm normally (Kearns and Losick 2003). However, *che*VW, *che*A, and *che*Y mutants are both Che^- and $Swarm^-$. These observations are consistent with the proposition that some *che* genes are required to trigger the differentiation of hyper-flagellated swarm cells, while the running and tumbling behavior of swimming chemotaxis is not necessary for swarming.

Triggers for Swarm Differentiation

Swarm cells are longer than swimming cells and they are hyper-flagellated. In accord with a posttranscriptional mechanism, the triggers appear to be physical rather than chemical in formation

E. Rosenberg et al. (eds.), *The Prokaryotes – Prokaryotic Communities and Ecophysiology*, DOI 10.1007/978-3-642-30123-0_10,

of the lateral flagella of *Vibrio parahaemolyticus*. For example, increasing the viscosity of the medium by adding branched polymers is a trigger where the nutrient content of the medium had little effect (Belas et al. 1986). Viscosity does matter: Polyvinylpyrrolidone with MW = 360,000 triggers while MW = 40,000 does not. Lateral flagella were also induced when cells were agglutinated with antibody to the cell surface, which also tethered the cells (McCarter et al. 1988). High viscosity also triggers hyper-flagellation in *Serratia marcesans* (Alberti and Harshey 1990) and in *Proteus mirabilis* (Allison et al. 1993). An important condition discovered in *S. typhimurium* is a certain degree of wetness in the thin film of liquid on the swarm agar surface. Surface wetness appears to be detected via the inhibition of flagellar rotation when there is too little liquid. Wang (Wang et al. 2005) found that swarming cells upregulate a set of "late" flagellar genes including the genes that encode flagellin, the genes that encode motor force-generating elements, *mot*A and *mot*B, and the genes that encode components of the chemotaxis signaling pathway. The same set of genes are downregulated when chemotaxis-defective mutants are transferred from liquid medium to swarm plates, confirming the correlation. Moreover, non-swarming mutant colonies are dry, not moist, while the swarming wild type is moist.

How does the rotation of many flagella on elongated cells lead them to move, and a swarm to expand? The question has recently been investigated by visualizing cell bodies by phase-contrast microscopy and labeled flagellar filaments by fluorescence microscopy (Turner et al. 2010). Most of the time cells were found to be driven forward by a bundle of co-rotating flagella formed at the cell's trailing end similar to the bundle used for swimming. However, only the flagella that belonged to an individual cell were included in the bundle; rarely did flagellar filaments from different cells form common bundles (Turner et al. 2010). This implies that adjacent swarm cells need not be rotating their flagella in the same direction for them to swarm together. Also, unlike the runs and tumbles of swimming cells, swarming cells were seen to back up without changing the cell's orientation. Those cell bodies reversed direction by a simple, new maneuver: They moved back through the middle of the bundle of flagella as each flagellum underwent a series of curly polymorphic transformations (Turner et al. 2010). This maneuver suggests that most of the cell's flagella were changing their direction of rotation at the same time. Otherwise swarm cells either stalled or reoriented when they collided with one another. Reorientation led to the formation of dynamic packs of cells whose individual speed and direction were strongly correlated (Darnton et al. 2010). Those correlations recall the organization of single layered rafts in *B. subtilis* (Kearns 2010) and multilayered fingers of rafted cells in *B. cereus* (Senesi et al. 2002).

Reorientation of the hyper-flagellated swarm cells takes place within a thin layer of fluid in which the cells swarm, and that thin layer has been shown to be essential for the swarming of *S. typhimurium*. Wang et al. (Wang et al. 2005) were able to rescue the swarming of a *che*Y mutant that failed to differentiate hyper-flagellated swarm cells and formed dry colonies by misting those colonies with a small amount of water, strongly suggesting physical, not biochemical constraints. Rotating the flagella on cells that are stuck to the substratum causes the fluid to flow within the layer. The flow trajectories around individual cells were traced by following streams of micron-sized bubbles (Wu et al. 2011). The flow was found to be unidirectional over distances as much as 0.5 mm, at speeds of 10 μm/s, which is about three times the rate of swarm spreading (Wu et al. 2011). Meanwhile, the upper surface of an *E. coli* swarm was found to be stationary (Zhang et al. 2010). Such flow would pump fluid outward from the leading edge of the swarm, facilitating the outward migration of cells. The flow patterns are general; they were observed with *E. coli*, *B. subtilis*, and *Serratia marcescens* (Wu et al. 2011).

Swarming Without Flagella

Many bacteria swarm using flagella, but bacteria that lack flagella can also swarm using other kinds of motility engines that translocate cells over surfaces. *Synechocystis*, for example, flourishes in mats where the light intensity fluctuates during the day and it glides over the mat using its retractile type IV pili apparently to seek optimal light for its photosynthesis (Bhaya 2004). *Cytophaga hutchinsonii* glides rapidly over the surface of cellulose fibers as it digests the cellulose for carbon and energy. That movement, which requires direct contact with the cellulose, uses a novel mechanism because *C. hutchinsonii* lacks genes encoding any established gliding engine (Xie et al. 2007). Moreover many, if not all species of myxobacteria, have type IV pili and a second engine for A-motility that are able to cooperate with each other in support of swarming (Ronning and Nierman 2008). Significantly, myxobacterial swarming is accurately described as "a coordinated multicellular activity during which swarmer cells maintain lengthwise contact with each other. Although individual swarmer cells move rapidly back and forth within the swarming bacterial mass," the swarm, which is not a colony at all, because the cells are constantly moving, expands outward (Harshey 1994).

Myxobacteria as Models of Swarm Motility

Myxobacteria have adopted multicellularity as their grand strategy for survival. They feed on particulate organic matter in soil including colonies of other bacteria. When food is abundant, they feed, grow, and swarm. When their food supply begins to decline, they stop growing and construct fruiting bodies (Reichenbach 1993). Each mature fruiting body contains about 100,000 cells differentiated as asexual spores. Sporulation within a fruiting body is thought to improve their long-term survival by enhancing spore dispersal and, when the spores have germinated, by providing a high cell density for swarming and cooperative feeding (Reichenbach 1984). *M. xanthus* swarming generates multilayered mounds that appear to be patterns for its spherical fruiting bodies (Kaiser and Warrick 2011). A remarkable set

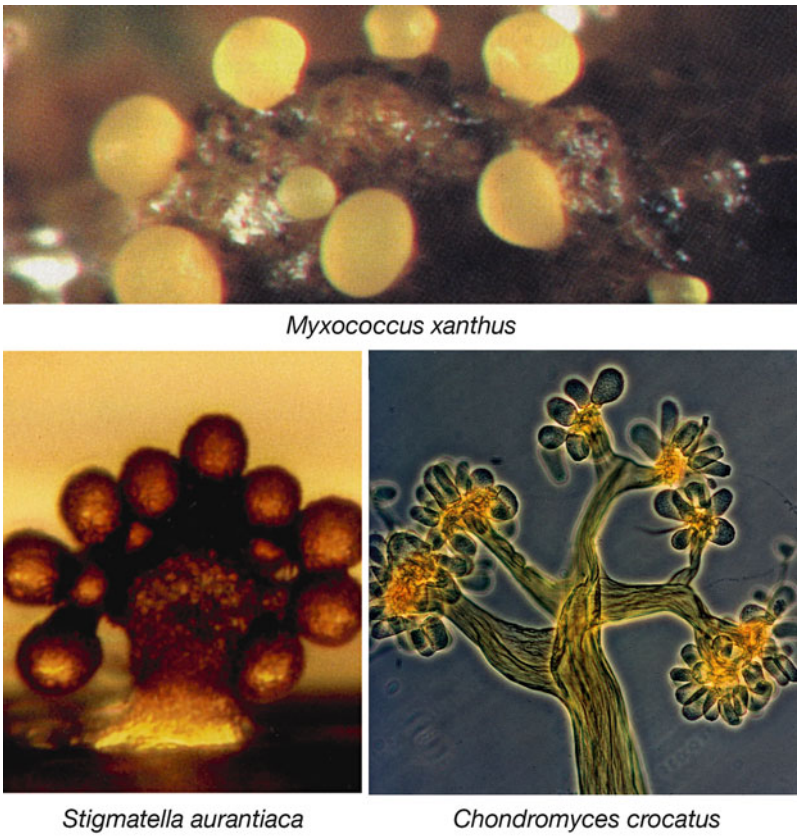


Fig. 23.1
Myxobacteria build multicellular fruiting bodies. *Top*, Eight fruiting bodies of *Myxococcus xanthus* on the same dung pellet are shown to illustrate their uniformity (Vos and Velicer 2006). *Bottom* from *left* to *right*: *Stigmatella aurantiaca*, *Chondromyces crocatus*, courtesy Hans Reichenbach (Reprinted with permission from Kaiser 2006)

of time-lapse movies (Reichenbach 1966; Reichenbach et al. 1965a, b, 1975/1976) show in great detail how several different species swarm and how they form their fruiting bodies.

The evolutionary origin of the myxobacteria, which are monophyletic, is found within the delta subgroup of proteobacteria, according to the sequence of their 16S ribosomal RNA (Sproer et al. 1999). Among the delta-proteobacteria, only the myxobacteria have the capacity to swarm and to develop multicellular fruiting bodies whose form is species specific. Five different species are illustrated in *Fig. 23.1*. The best-studied myxobacterium, *Myxococcus xanthus*, shares the ability to lyse and digest other bacteria with another delta-proteobacterium, *Bdellovibrio bacteriovorus* (Evans et al. 2008). Predatory feeding seems to have evolved within the delta-proteobacteria. Myxobacteria are abundant in cultivated top-soils around the earth because a wide variety of Gram- and Gram+ bacteria that are potential prey are found in association with plant roots. For predation, *M. xanthus* is seen to surround a prey colony, as diagrammed in steps D–F of *Fig. 23.2*, to make direct contact with the prey cell (Berleman et al. 2006, 2008) and to lyse the prey envelope. Finally, it appears that *M. xanthus* extracts the cytoplasmic proteins from prey cells, digests them to peptides and amino acids before consuming them for carbon, nitrogen, phosphorous, and energy. *M. xanthus* has eight Che clusters of chemosensory proteins scattered about its genome (Kirby et al. 2008). One, the Che3 system, was shown in 2003 to regulate gene expression, not motility (Kirby and Zusman 2003). Thus, *M. xanthus* is another example of bacteria that swarm and possess chemotaxis-like genes that are used to regulate gene expression rather than chemotaxis. Gene knockout experiments and measurements of the swarm expansion rate have shown that three chemosensory-like Frz proteins in *M. xanthus*, FrzCD, FrzF, and FrzE interact to form a pacemaker (Kaiser and Warrick 2011). By creating a negative feedback loop, the levels of Me-FrzCD and FrzE ~ P are led to oscillate with the same fixed period but out of phase with each other as first proposed by Igoshin (Igoshin et al. 2004). The period of oscillation is tuned by the methylation level of FrzCD and the phosphorylation level of FrzE. For swarming, this Frz oscillator drives MglAB to oscillate between its GTP and GDP-bound states. MglA is a small Ras-like protein with a G-loop. Mutations within the G-loop prevent swarming (Fremgen et al. 2010; Stephens et al. 1989), strongly suggesting that MglA switches between GDP-bound and GTP-bound states (Bourne et al. 1990). Leonardy et al. have provided biochemical evidence that MglB is the cognate GAP protein for MglA

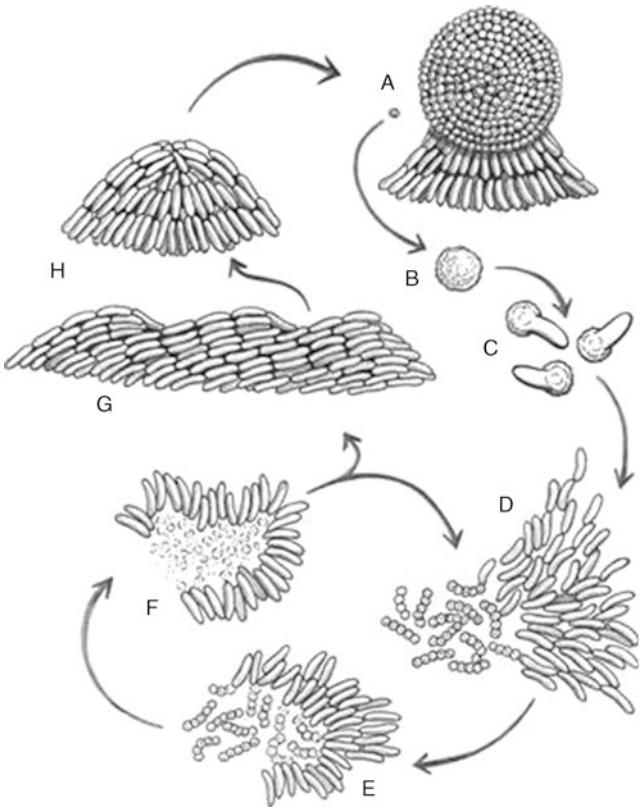


Fig. 23.2
The lifecycle of *M. xanthus*. A swarm (a group of moving and interacting cells) can have either of two fates depending on their environment. The fruiting body (A) is a spherical structure of approximately 1×10^5 cells that have become stress-resistant spores (B). The fruiting body is small (1/10 mm high), sticky, and its spores are tightly packed. When a fruiting body receives nutrients, the individual spores germinate (C) and thousands of *M. xanthus* cells emerge together as an "instant" swarm (D). When prey is available (micrococci in the figure), the swarm becomes a predatory collective that surrounds the prey. Swarm cells feed by contacting, lysing, and consuming the prey bacteria (E–F). Fruiting body development is advantageous given the collective hunting behavior. Nutrient-poor conditions elicit a unified starvation stress response. That response initiates a self-organized program that changes cell movement behavior, leading to aggregation. The movement behaviors include wave formation (G), and streaming into mounded aggregates (H), which become spherical (A). Spores differentiate within mounded and spherical aggregates (Reprinted with permission from Goldman et al. 2006)

that stimulates its GTPase activity (Leonardy et al. 2010). The regulatory circuit switches both the A- and the S-engines to their opposite cell ends and is illustrated in ❯ *Fig. 23.3*. That switch seems to have been designed for two equally polar engines.

Myxobacterial Swarming

M. xanthus and all myxobacteria that have been studied require a solid surface on which to build an expanding swarm. They

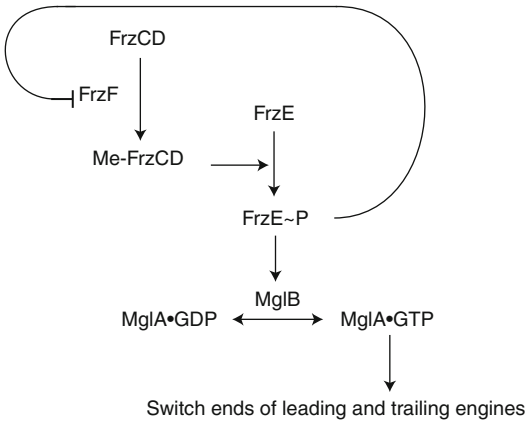


Fig. 23.3
Feedback-induced oscillator that drives the MglAB reversal switch as demonstrated by the phenotypes of gene deletion mutants. *Arrows* indicate reactions; –| FrzF indicates that the action of the FrzF methyltransferase is inhibited by FrzE ~ P. FrzCD and FrzE constitute a two-component system. A high level of FrzE ~ P activates the formation of MglA•GTP which causes both the A- and the S-engines to switch from one cell end to the other (Reprinted with permission from Kaiser and Warrick 2011)

cannot swarm in liquid culture just as they cannot deploy their gliding engines in liquid. They do swarm rapidly on the surface of agar that is covered with a very thin film of liquid. The solid surface provides a foundation on which permanent, albeit constantly moving, multicellular structures can be built. Ninety percent of the swarm expansion rate arises from the self-powered movement of individual cells. This is related to the fact that an *mglA* single mutant or any double mutant with defects in both of its gliding engines is unable to swarm (Kroos et al. 1988), but is able to grow at the same rate in shaken liquid culture as the wild type. Because moving cells pile on top of each other in a swarm, they are competing with one another for oxygen from the atmosphere and nutrient from the agar beneath them. By constantly moving and circulating they mitigate that competition. An annulus of exponentially growing cells is found at the edge of an expanding swarm (Kaiser and Warrick 2011). The growth rate of swarm cells in the annulus is the same as that in shaken liquid culture. Thus, swarming seems to have evolved under selection for rapid growth when cells compete for nutrients. That evolution will naturally have taken place with a swarm feeding on prey bacteria, long before their cultivation in laboratories (Beebe 1941).

The Pacemaker

Wu discovered that periodic reversals are essential for a swarm to expand its diameter on agar (Wu et al. 2009). This requirement explains why individual, rod-shaped swarm cells, always moving in the direction of their long axis, surprisingly allot the same amount of time to moving forward as to moving backward.

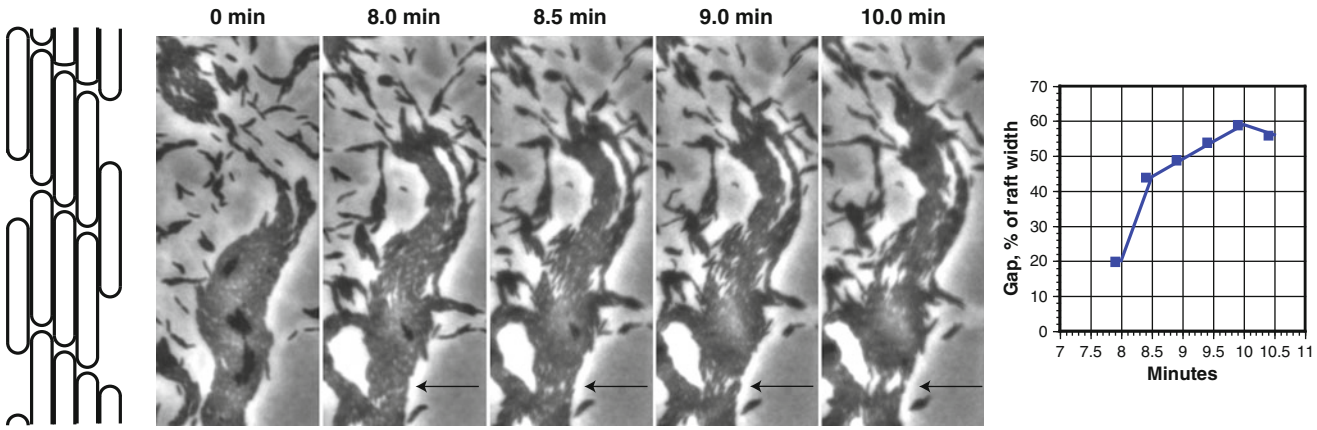


Fig. 23.4

Opening of a cell-free gap within a raft of cells, reflecting the asynchronous reversal of adjacent cells. The first panel is a sketch of the cell arrangement in a raft, emphasizing the long, side-by-side courses of cells whose rounded ends are out of register from one course to the next. Then, running from left to right are snapshots of the 0.0-min movie frame, the 8.0-min frame, the 8.5-min frame, the 9.0-min frame, and the 10.0-min frame; the sequence shows the enlargement of a gap. A kinetic curve of gap width as a function of time is presented in the last panel. Location of the growing gap is indicated by the *arrow* in the 9.0-min frame; the indicated gap, which has a fringe of cell ends above it and below it, is found at the corresponding Y coordinate in each of the snapshots. The width of the gap was measured along the line at that coordinate with a millimeter scale (From Kaiser and Warrick 2012)

Apparently, the purpose of swarm cell movement is simply to circulate the cells for better access to oxygen and to nutrient. This cannot be achieved by chemotaxis toward oxygen because that would lead them to abandon the swarm. Apparently, it can be achieved by connecting three of its chemosensory proteins into a negative feedback loop that oscillates with a fixed period of 8–9 min. As shown in *Fig. 23.3*, those oscillations drive a G-protein switch—consisting of the Mgl A and B proteins—to alternate between the G-protein's GDP- and GTP-bound states (Bourne et al. 1990, 1991) with the 8–9 min period, changing each cell's polarity. Because both the A- and the S-engines are located at the two poles of the rod, the cell moves first in one direction, stops, then moves in the opposite direction for an equal time on the average. Most important, the forward then back pattern of cell movement facilitates the building of organized multicellular structures.

Swarms Build Two Kinds of Large Multicellular Structures

Rafts, planar rectangular arrays of cells with their long axes parallel, have from 50 to 500 cells. Raft cells adhere to each other by means of their polysaccharide capsules that completely cover each cell, protecting them from lysis from without (Cuthbertson et al. 2009). The plasticity of their capsular polysaccharide allows pairs of aligned cells to move either in the same or in opposite direction with no apparent preference. Rafts are observed to grow laterally by addition of individual cells to either their left or right margins. At the same time, rafts are also observed to shrink by loss of one or more cells from either margin. After a while, growth and shrinkage of the width balance each other, and the raft defined by its leading and trailing edges appears to flow in some particular direction. Although individual cells can be resolved at the left and right edges of a moving raft, cells in the raft's interior are not resolved from each other. However, the ends of individual cells can be resolved and enumerated in the fringes located on either end of a gap, such as those in *Fig. 23.4*. Nevertheless, only half of the individual cells in the raft are moving in the direction that the raft appears to be moving, while the remaining half are moving in the opposite direction. Considering a single file of cells that belong to the same course in the raft, the counter-movement is not apparent because the tiny gap, only one cell wide, between the back end of the counter-moving cell and the back end of the cell ahead that belongs to the same course is too narrow to be resolved with a 20X phase-contrast objective lens, as suggested in the first panel of *Fig. 23.4b*. For this reason, the raft appears gray in *Figs. 23.4* and *23.5*, whereas side-by-side clusters of single cells are black in the same figure (Giglio et al. 2011).

Multilayered Mounds

The second kind of multicellular structures found at the swarm edge are the mounds with multiple layers; the layers are nested on top of each other. Like the rafts, which are also in that figure, the mound shown in *Fig. 23.5* is a dynamic structure with its cells constantly in motion. Each time the pacemaker (*Fig. 23.3*) calls for a reversal of gliding direction, cells are seen to move within the same layer, and to move up or down from one layer to the next. Mounds are thought to be built from the bottom up by adding layer upon layer, in the same way that rafts have gained pieces of their 2nd layer.

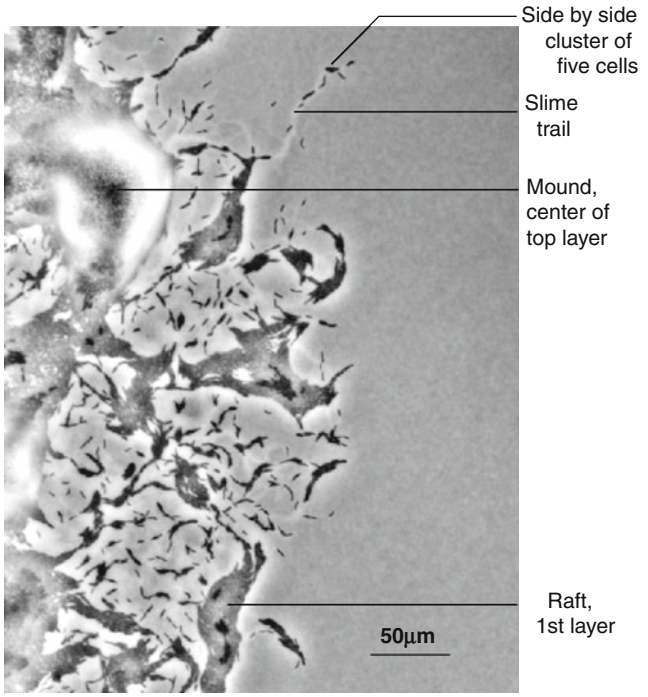


Fig. 23.5
Distribution of cells at the edge of a DK1622 swarm on 1% agar. The swarm is expanding in the radial direction which is to the right in this image of a small section of the swarm. (Scale bar, 50 μ). Photographed with a 20X phase-contrast objective. A single cell on a slime trail, a side-by-side cluster of cells, a multicellular mound with five layers, and a large multicellular raft are identified (This figure is the first frame of supplemental movie S1 in Wu et al. 2009)

The mound shown in *Fig. 23.5* has five layers that are readily distinguished in oblique light (Kaiser and Warrick 2012). Sixty-five minutes after the movie's start, the top (5th) layer of the mound appears to explode because all of its cells drop down to the periphery of the 4th layer with a speed three-times that of cells that are gliding over the agar substratum, as demonstrated in *Fig. 23.6*. Recently, it has been shown (Kaiser and Warrick 2012) that the "explosion" results from the synchronous reversal of all the cells in layer 5 and their simultaneous descent to the periphery of the 4th layer. Moreover, once the 5th layer cells have been synchronized, all the pacemakers in all the cells of the mound have taken the same phase, and the mound remains synchronized. As shown in *Fig. 23.6*, the synchronization is accurate to at least 1 part in 16, which is the best that can be observed when making exposures for a time-lapse movie once every 30 s. Moreover, the structural accuracy with which the mound is rebuilt is equally high as shown by measurements of 5th layer area, position, and time. The first explosion shown in *Fig. 23.6* is followed 14 min later by reassembly of a new 5th layer that has about the same number of cells as the original, which is estimated from the area covered by the layer of cells. Even though rafts and individual cells outside the mound had been in active motion, the center of the new 5th layer is located at the same x, y coordinates within the swarm as the first. Then, 15.7 min later, the reassembled 5th layer explodes yet again and for the last time since the movie ends before another 14 min would have been recorded. Both an hour's delay before the first synchronized reversal and repeated explosions thereafter offer strong evidence for the existence of a contact signal between pairs of aligned cells as described below.

Evidence for Signaling Between Pairs of Adjacent, Aligned Cells in Rafts

Rafts are rectangular assemblies of many cells that have their long axes roughly parallel to one another. Moreover, the ends of adjacent cells within a raft are staggered like adjacent courses in a masonry wall, as suggested in the first frame of *Fig. 23.4*, the sketch of an idealized raft. Nevertheless, individual raft cells reverse their gliding direction regularly (Kaiser and Warrick 2012). As mentioned, a raft expands its width when a cell that happens to be near the raft and to be moving in the same direction associates with the left or the right side of a raft. And a roughly equal number of cells dissociate from the sides of a raft, whenever the phase of the leaving cells' pacemakers differs from that of their former neighbor so that one reverses before the other. One consequence of such growth in width is that empty spaces between cell ends accumulate in the body of raft. These initially microscopic spaces reveal themselves when they are consolidated into a wide cell-free gap, *Fig. 23.4* and see Kaiser and Warrick (2012). Those spaces are revealed by the general mottled gray color in the phase-contrast microscope instead of a solid black color like single cells or clusters of a few cells evident in *Fig. 23.5*. Gray coloration also implies that adjacent raft cells are reversing independently of each other. Gap consolidation reaches its maximum at 10 min, which corresponds to completion of a single reversal period of the pacemaker. This behavior would be expected because each cell has its own pacemaker, and because reversal of any pair of cells would be expected to occur independently. Nevertheless, a low but significant correlation between pacemaker phases has been detected in the cells of patches of the raft's 2nd layer evident in *Fig. 23.5*: Roughly ½ the patch cells left the patch by reversing during the final 30 s of a 6-min sampling period, suggesting that those cells had recently begun to bring the phases of their pacemakers together.

Spreading the Signal Within a Mound

At the start (movie time 0), all five layers of the mound were in place and all of the cells were reversing regularly, with the pacemaker's 8–9-min period. Moreover, cells in the mound apparently had been transposing from one layer to the subjacent layer each time a cell was triggered by its pacemaker to reverse. Translations within the same layer as well as layer transpositions would bring cells into direct contact with new partners for

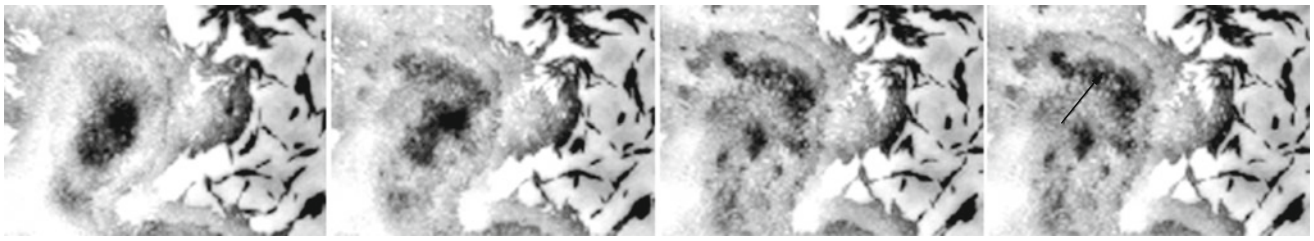


Fig. 23.6

Three consecutive frames from the movie of the mound indicated in *Fig. 23.5*, showing the explosion of its top layer. Photos were taken every 30 s for 2.5 h. The images shown were exposed at 62.5, 63.0, and 63.5 min. The *arrow* in the fourth frame shows the lateral displacement of cells from the center of the top layer at 62.5 min to the periphery of the next layer down at 63.5 min, from which the speed of fragmentation was calculated

contact signaling. Significantly, cells in the topmost layer of the mound are expected to have been signaling to their neighbors longer than any other cell in the mound. Consequently if, as proposed, a synchronizing signal is transmitted by cell-cell contact, synchrony should appear in the topmost before any other layer. It follows that phase correlations in mounds and in the 2nd layers of a raft could arise from a signal that is passed between pairs of adjacent cells when they make transient contacts with each other. After contact, both cells would move on to interact with other cells and spread the synchrony, as described (Strogatz 2003). Even though swarming cells seem to move continuously, they often stop, albeit for less than a minute (Yu and Kaiser 2007), when they reverse following their pacemakers' instructions (Kaiser and Warrick 2011). Whenever two adjacent rod-cells in a raft stop at the same time, they would be able to form a bridging structure for signal transfer. Similar signaling interactions would be expected to occur in each layer of a multilayered mound because the cells are densely packed. Thus, in mounds, signaling between pairs of cells could bring them to the same phase of their pacemaker's cycle as observed (Kaiser and Warrick 2012).

Noting that two different motility engines switch their polarity coordinately when the MglA-G protein switch binds GTP (*Fig. 23.3*), we must consider the mechanics of the S- and the A-engines to see how they are polarized and how they are reversed.

S-Motility, Structure, and Assembly of the Type IV Pili

Several type IV pili (Tfp) extend forward only from the leading end of each cell; the trailing end or the sides have none. Many Tfp proteins are common to *M. xanthus*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Synechocystis* PCC6803. The most highly conserved proteins (*M. xanthus* designations), PilA, PilB, PilC, PilD, PilM, PilN, PilO, PilP, PilQ, and PilT, are listed with their molecular function in *Table 23.1*. More details can be found in reviews (Mattick 2002; Nudleman and Kaiser 2004; Pelicic 2008). Type IV pilins in these organisms, encoded by their *pilA* gene(s), conserve only their amino terminal region of about 60 amino acids. Conservation of the PilA amino terminal sequence reflects the sequence requirements of PilD peptidase around its cleavage site (Strom et al. 1993) as well as the ability of the amino terminal region to form an α-helix that is capable of regularly staggered coiled-coiling with copies of itself. A structure was inferred from a 2.6 Å resolution X-ray crystal structure of *N. gonorrhoeae* pilin dimers, a mapping of surface-exposed peptides, and cryo-electron microscopy (Craig et al. 2006). Because individual pili are 3–10-μm long, while the rise per monomer is of the order of 1 nm, a single pilus fiber polymerizes many thousands of pilin monomers (Hansen and Forest 2006). The ß-strands of one pilin monomer in the helical filament interact with the ß-strands of the next monomer, lending tensile strength to the fiber. Flexible α-helices in the core of the pilus fiber allow the fiber to bend, twist, and thereby associate with other pilus fibers. Despite its small diameter (6 nm), the fiber can withstand tension stresses in excess of 100 pN (Maier et al. 2002). The absence of any channel in the center of the pilus fiber implies that the fiber is polymerized from its base in the inner membrane, where the PilB and the PilT hexamers are located. The hexamers catalyze extension and retraction, respectively (*Table 23.1*).

M. xanthus cells, lacking A-motility and separated by more than a pilus length (several micrometers), are rarely if ever observed to move by pilus retraction (Kaiser and Crosby 1983). It is as if pili retract only if their tips have firmly attached to something (fibrils apparently) on a group of cells ahead of them (Kaiser 1979; Merz et al. 2000; Semmler et al. 1999). Li et al. have argued that binding is less strict (Li et al. 2003), and experiments are called for to settle this issue. Fibrils, consisting of almost equal amounts of protein and polysaccharide that contains galactose, glucosamine, glucose, rhamnose, and xylose (Behmlander and Dworkin 1994; Dworkin 1999), make up a linked meshwork of elastic strands, some 30 nm in diameter, that bundle cells close together. Typical meshworks can be seen in scanning electron micrographs (Kearns and Shimkets 2001). Fibril-deficient mutant cells are found to accept fibrils from an extracellular polysaccharide fraction of normal

Table 23.1
S-motility genes

Protein	Function	Cellular localization	Clustered[a]	Conserved in five species[b]
PilA	Pilin, monomer unit of the pilus filament	Assembled into the pilus fiber Stored in the Inner Membrane	+	+
PilB	Pilus extension	Inner membrane	+	+
PilT	Pilus retraction	Inner membrane	+	+
PilC	Unknown	Inner membrane	+	+
PilD	PilA leader peptidase	Inner membrane	+	+
PilG	ABC Transporter	Periplasm	+	
PilH	ABC Transporter	Periplasm	+	
PilI	ABC Transporter	Periplasm	+	
PilM	ATPase	Inner membrane	+	+
PilN	Unknown	Periplasm	+	+
PilO	Unknown	Periplasm	+	+
PilP	Unknown	Anchored in outer membrane	+	+
PilQ	Secretin	Outer membrane	+	+
PilR	Regulates transcription of pilA		+	–
PilS	Two-component sensor for pilR		+	–
PilR1	Transcriptional regulator		+	–
PilS1	Two-component sensor for pilR1		+	–
Tgl	Secretin assembly factor	Outer membrane	–	–

[a]A set of 16 contiguous genes in *M. xanthus*
[b]*M. xanthus*, *P. aeruginosa*, *N. gonorrhoeae*, *N. meningitidis* and *Synechocystis* PCC 6803 (Nudleman and Kaiser 2004)

cells (Lu et al. 2005), gaining thereby the ability to bind the tips of pili. These rescue experiments suggest that pilus tips bind fibrils tightly while the strands themselves are not attached to the cells. Additional support for tip binding to fibrils is found in the mutants that retain pilus fibers, but lack S-motility: the *pilT* mutants, the *dif* or *dsp* (dispersed growth) mutants, and certain lipopolysaccharide-defective mutants. Cultures of *pilT* mutants contain many clumps of cells that are held together by their large numbers of unretracted pili. Cultures of the *dsp* and *dif* mutants grow dispersed in agitated liquid culture and have normal numbers of pili but fail to clump. The *dif/dsp* mutants are fibril deficient (Lu et al. 2005) because they downregulate fibril production. It appears that fibrils (and perhaps O-antigen) regulate the number of pili that cells assemble, as if *M. xanthus* cells are trying to strike a balance between the two functionally related proteins that are normally made in very large amounts. Pilus structure, tip-binding specificity, and the hyperpiliation of *pilT* mutants suggest that *M. xanthus* type IV pili work as follows: A relatively stiff pilus extends ahead of the cell, it adheres to fibrils on cells located near the pilus tip, the pilus retracts, and it pulls the piliated cell forward toward the clump of cells ahead. Pilin monomers, released by retraction, are stored in the inner membrane for reuse (Skerker and Berg 2001). Evidently, once an attachment has been made, the connection to fibrils on the target cell can withstand 100 pN of tension without rupturing. The cell group to which the pilus has attached moves but slightly because the group, having more cells, adheres with greater strength to the substrate than the single piliated cell which has contributed much ATP to retract the pilus.

Of the more than 15 different proteins that constitute a type IV pilus, some are found in the cytoplasmic membrane, others reach across the periplasm, some may be anchored to the peptidoglycan. Still others pass through the outer membrane into the extracellular space, as diagrammed in *Fig. 23.7*. The helical pilus fiber passes through the doughnut-shaped assembled PilQ secretin embedded in the outer membrane (*Fig. 23.7*). The assembled complex of PilQ is a 12-14mer with a 60 Å diameter hole in the center, through which the pilus fiber can pass (Bitter et al. 1998; Collins et al. 2001, 2004; Frye et al. 2006). Without a pilus fiber, the 60 Å hole in the PilQ multimer is closed. When a pilus fiber, growing up from its base in the inner membrane, pushes on the multimer, it opens, allowing the pilus to slip through. Thus, the assembled PilQ acts like a sealed bushing to surround the pilus as it elongates or retracts. Meanwhile periplasmic proteins do not leak out. When PilQ protein is extracted from whole cells by exposing them to a hot detergent solution, roughly half of the PilQ protein is extracted as 98 kDa monomers; the other half is extracted as high molecular weight, detergent-resistant multimers (Nudleman et al. 2006).

Nudleman et al. found that PilQ multimer assembly requires Tgl that is found as a 17 kDa outer membrane lipoprotein in *M. xanthus* (*Table 23.1*). Tgl is necessary for the production of

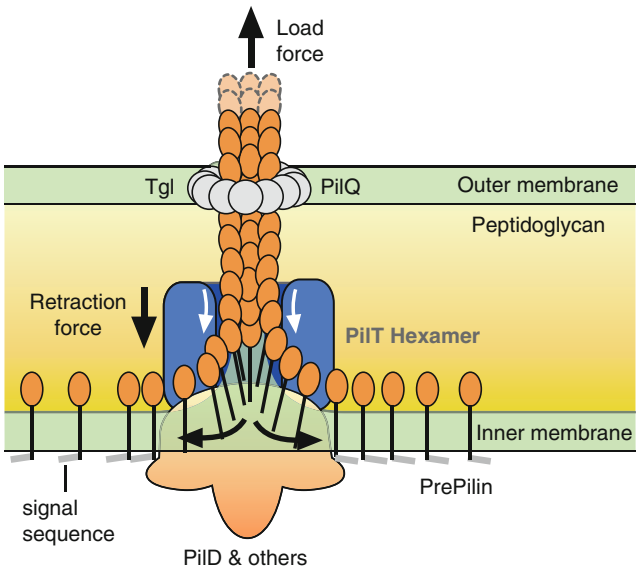


Fig. 23.7

Cartoon interpretation of type IV pilus retraction. The pilin monomer is dissolved in the inner membrane bilayer with its hydrophilic head in the periplasm. In conjunction with the assembly proteins, PilD, pilF, PilG, PilB, the pilin signal sequence is cleaved and the pilus extended, driven possibly by PilB, which is an ATPase. After extension is completed, and possibly following a signal from the pilus tip, retraction commences, driven by PilT. The PilT motor is a hexameric ATPase in the AAA family of motor proteins that lies in the periplasmic space between the inner and outer membranes, associated with the inner membrane and the PilB protein. The structure of PilT hexamer crystals has been solved by Satyshur et al. (2007) (Reprinted with permission from Kaiser 2000 Current Biology 10:R777–R780)

pili in *M. xanthus*, and it is the only required pilus-associated protein whose gene resides outside the large *pil* gene cluster found there (Rodriguez-Soto and Kaiser 1997a, b). The primary translation product of the *tgl* gene has a signal peptidase II recognition sequence for outer membrane lipoproteins, and processed Tgl protein is found in the outer membrane (Simunovic et al. 2003). Wei et al. (2011) have recently shown that a signal peptidase II recognition sequence is, in fact, sufficient to localize a lipoprotein to the outer membrane of *M. xanthus*. Beyond the signal peptidase recognition site, the Tgl amino acid sequence comprises six tetratrico peptide repeats (TPRs). TPR sequences are found in many proteins that assist the assembly of multi-protein complexes, and each TPR has the capacity to form an α-helix (D'Andrea and Regan 2003). Perhaps the six TPR helices interdigitate within two adjacent PilQ monomers, bonding them so well that the assembled multimer resists dissociation in heated detergent. However, the integrity of such PilQ oligomers would depend upon the molecular integrity of the Tgl links. A PilQ-linking role of Tgl is clearly shown by the observation that when Tgl is transferred by contact from one cell to another, the recipient assembles its PilQ monomers and is able to move using S-motility (Nudleman et al. 2005). All of the PilQ molecules at both poles are found within a single, large, polar condensate in the outer membrane (Nudleman et al. 2006). Each pole has approximately half of the PilQ from whole cells (Nudleman et al. 2006). Nevertheless, the PilQ condensations at opposite poles of the same cell are strikingly different. The condensate at the pole without pili has unassembled 98 kDa monomers that are simply clustered. The other condensation includes several open PilQ channels, each of which surrounds a single pilus fiber; those channels are detergent-resistant PilQ multimers and they include Tgl.

PilT, an AAA ATPase, is the motor for pilus retraction; it develops more than 110 pN of tension (Clausen et al. 2009; Maier et al. 2002). The structure of crystalline hexamers of PilT has been solved by x-ray diffraction (Satyshur et al. 2007). The structure implies that PilT changes its conformation during the process of retraction. *M. xanthus* pili are thought to be extended by PilB, another AAA ATPase that is also found in the inner membrane (*Table 23.1*). It is suggested that *M. xanthus* pili switch between a phase of extension and a separate phase of retraction. In this way the *M. xanthus* pilus will have a defined duty cycle. A cycle would be initiated by a signal from the pacemaker to reverse polarity and to assemble PilQ, then PilB would be able to extend a new pilus until its tip makes a firm attachment to fibrils. After attachment, PilT would rapidly and forcefully retract the pilus, and thereby terminate the cycle. This duty cycle can be integrated within the cell's cycle of replication, whether the cell is growing and swarming or is preparing for fruiting body development, as described below. The non-piliated pole has a pilus-like organelle lacking a pilus fiber. It includes PilB, PilM, PilN, PilO, and PilT proteins that localize to the inner membrane and to the periplasm. A precedent for such polar differentiation is that PilB and PilT are bipolarly localized in *Pseudomonas aeruginosa* while the PilU protein and the assembled pili are strictly unipolar (Chiang et al. 2005). In *M. xanthus*, such an organelle without a pilus fiber would, nevertheless, be passing through the peptidoglycan meshwork, because PilQ is found in the outer membrane while PilB and PilT are in the inner membrane. The organelle would thus pierce, with an opportunity to join to, the rigid sacculus (Bui et al. 2009), which the PilB and PilT motors could use as a fulcrum against which to push or pull. Because the organelle pierces the rigid peptidoglycan meshwork, its position is fixed within the cell, and the associated PilQ monomers would not be expected to diffuse laterally in the membrane away from their polar condensate. Both Tgl and PilA can be added at the time and place of PilQ and pilus fiber assembly. The precursor organelle, capped with a patch of unassembled PilQ, resides at the non-piliated pole, waiting for the polarity reversal signal and Tgl to induce assembly of the PilQ monomers prior to elongating a new pilus filament.

A-Motility

In 2002, Wolgemuth et al. showed how slime secretion through nozzles found at the trailing end of each cell could produce a gliding force directed along the cell's long axis. Point mutations

in several genes that encode a series of sugar-specific glycosyl transferases were found to decrease A-motility specifically (Yu and Kaiser 2007). Moreover, *M. xanthus* has homologs of the *E. coli* genes that encode capsular polysaccharides (Cuthbertson et al. 2009). Despite the cogency of the polar slime secretion mechanism and the precedent set for it by cyanobacteria (Hoiczyk 2000; Hoiczyk and Baumeister 1998), Mignot et al. claimed to have a new motor for A-motility that employed focal adhesion complexes distributed along the sides of cells (Mignot et al. 2005, 2007). Their reasoning included the assumption that AglZ protein, an essential part of the focal adhesion complex, was a motor protein. However, in 2009, evidence was presented that AglZ protein was not a motor when several *aglZ frzCD* double knockout mutants and an *aglZ pilA frzCD* triple knockout mutant were found to have fully active A-motility for swarming and for development (Mauriello et al. 2009). This implies that focal adhesions are serving some function other than providing a motor for A-motility. It could be providing the reversal synchronizing signal proposed above and thought to issue from the side of a cell.

Early evidence for propulsion by polar slime secretion rested upon observing ribbons of secreted polysaccharide gel emerging from the trailing end of each cell (Wolgemuth et al. 2002). Also, several hundred nozzles were found to be clustered at both poles of each cell. However, 1/5 of all nozzles were found scattered about the sides of cells, and they are thought to secrete polysaccharide slime the cell uses as its water-binding capsule that covers the entire surface of the cell and protects it from lysis. Evidently, the capsule is made of the same polysaccharide as the propulsive slime, and both bind extracellular water. Many electron micrographs and light micrographs show polysaccharide slime extrusion from one cell pole only in wild type cells (A^+S^+) and in A^+S^- mutants (Wolgemuth et al. 2002; Yu and Kaiser 2007). Nozzles at the leading end of the cell appear to be inactive, and they are not extruding slime. Consequently, the gliding force is unidirectional and directed toward the pole. Force is generated when some of the water is stripped from the polysaccharide synthesized in the wet cytoplasm as it is passed through the inner membrane. Finally, the (partially) dried polysaccharide, now having the capacity to bind environmental water, is secreted through one of the nozzles and forms a gel as water from outside the cell binds the extruded sugar residues (Wolgemuth et al. 2002).

Motility phenotypes of 50 A-motility mutants of *M. xanthus* are listed in ❯ *Table 23.2*. Point mutants with a Pgl phenotype move more slowly than the wild type because they pause frequently (Yu and Kaiser 2007). The *pgl* mutations are found in several different sugar transferase genes that are structurally and functionally similar to the *E. coli* genes that encode group 1 and group 4 capsular polysaccharides (Whitfield 2006). They provide a direct biochemical link between A-motility and slime secretion, while elasticotaxis provides the genetic link specifically to A-motility (as opposed to S-motility), and to slime secretion (Fontes and Kaiser 1999; Kaiser 2009). Several A-motility genes identified by mariner transposon insertion could be involved in polysaccharide synthesis or transport (Youderian et al. 2003). Homologs of all 50 genes are found in *Stigmatella aurantiaca* as well; it is a close relative of *M. xanthus*.

Motility Effectors of MglAB

Proteins that mediate the coordinated polarity reversal of both the A- and the S-engines are considered here. Those proteins, called effectors, are taken to be triggered by the MglAB switch, as indicated in ❯ *Fig. 23.3*. Reversal involves effectors of MglAB specific for A-motility and other effector molecules specific for S-motility. S-motility effectors convert the fiber-less, pilus-like organelles described above, whose PilQ is clustered but unassembled, into an active pilus-extending organelle located at the leading pole of the cell and capable of searching for and binding to fibrils. Similarly, effectors of A-motility convert the inactive nozzles of the sort that are found at the leading end of a cell into nozzles that, located at a new lagging pole, actively secrete polysaccharide slime. When a cell divides, the newly created ends of both daughter cells have both A- and S-engines. However, only one of the two engines is complete and active, and the job of designating which engine to complete falls to the molecules we are calling effectors. On the one hand, the process of switching engines from one pole to the other is "all or none": At any particular cell pole, all the active engines are either for A-motility (slime secretion) or for S-motility (type IV pili), and mixtures of different active engines have never been reported. The excluded engines are always present in their inactive, precursor forms. Exclusion is enforced by the specificity of the effector proteins, and those proteins are taken to be regulated by the MglAB switch as a result of protein phosphorylation and/or effector assembly. On the other hand, the high speed of engine assembly is set by the (genetically determined) concentration of the effector. The RomR effector, for example, which is essential for A-motility, is a phosphorylated response regulator. (Its phosphorylation might be catalyzed by MglAB•GTP, but that remains to be shown.) RomR protein forms clusters at both cell poles, but the cluster at the lagging pole of the cell is larger than the cluster at the leading pole (Leonardy et al. 2007). Moreover, the larger cluster of RomR switches from one pole to the other in parallel with the cell's reversal of gliding direction (Leonardy et al. 2007). Thus, at every instant, the larger cluster is found at the cell pole that is believed to be actively secreting slime; somehow that difference in the RomR ~ P cluster size locks the A-engines on and the S-engines off at the lagging pole. The dynamic re-localization of RomR was shown to depend on the Frz system as well as on the Mgl switch (Leonardy et al. 2007), as implied by the circuit of ❯ *Fig. 23.3*.

CglB, an outer membrane lipoprotein that is essential for A-motility (Hodgkin and Kaiser 1977, 1979), behaves like an effector of A-motility. Yu observed that mutants lacking CglB protein simultaneously secrete slime from both its ends, indicating that CglB is essential for activating slime secretion from nozzles found only at the trailing pole (Yu and Kaiser 2007).

Table 23.2
A-motility genes

Gene Name	Location	Phenotype of knockout mutant	Related gene function(s)	References
mglA	MXAN1925	Nonmotile (A^-S^-)	Ras-like small GTPase	Yu and Kaiser (2007)
aglAR	MXAN2541	No A-motility	Unknown	Yu and Kaiser (2007)
aglCR	MXAN7296	No A-motility	Unknown	Yu and Kaiser (2007)
aglR	MXAN6862	No A-motility	MotA/TolQ/ExbB proton channel	Youderian et al. (2003)
aglS	MXAN6860	No A-motility	TolR/ExbD?	Youderian et al. (2003)
aglT	MXAN4869	No A-motility	*N*-acetylglucosaminyltransferase	Youderian et al. (2003)
aglU	MXAN3008	No A-motility	WD-repeat lipoprotein, acylaminoacyl-peptidase	Yu and Kaiser (2007)
aglV	MXAN5754	No A-motility	TolR/ExbD	Youderian et al. (2003)
aglW	MXAN5756	No A-motility	TolB	Youderian et al. (2003)
aglX	MXAN5753	No A-motility	TolQ biopolymer transport	Youderian et al. (2003)
aglZ	MXAN3536	Conditional A-motile	Receiver domain, coiled-coil, assembly protein	Mauriello et al. (2009)
cglB	MXAN3060	Compl[a] A-motility	Outer membrane lipoprotein, stimulatable	Rodriguez-Soto and Kaiser (1997a, b)
cglC	MXAN2538	Compl[a] A-motility	Outer membrane lipoprotein, stimulatable	Hodgkin and Kaiser (1977)
cglD	MXAN0962	Compl[a] A-motility	Outer membrane lipoprotein, stimulatable	Hodgkin and Kaiser (1977)
cglE	MXAN4866	Compl[a] A-motility	Outer membrane lipoprotein, stimulatable	Hodgkin and Kaiser (1977)
cglF	MXAN4868	Compl[a] A-motility	Outer membrane lipoprotein, stimulatable	Hodgkin and Kaiser (1977)
agmA	MXAN3886	No A-motility	*N*-acetylmuramoyl-L-alanine amidase	Youderian et al. (2003)
agmB; hrpB	MXAN3055	No A-motility	ATP-dependent RNA helicase	Youderian et al. (2003)
agmD; trpS	MXAN3842	No A-motility	trpS tRNA synthetase	Youderian et al. (2003)
agmE	MXAN0635	No A-motility	ParA family; Soj/Par	Youderian et al. (2003)
agmG	MXAN6519	No A-motility	Site-specific recombinase	Youderian et al. (2003)
agmH	MXAN4638	No A-motility	Lysophospholipase	Youderian et al. (2003)
agmI	MXAN3502	No A-motility	Unknown	Youderian et al. (2003)
agmK	MXAN4863	No A-motility	TPR repeat protein	Youderian et al. (2003)
agmL	MXAN3537	No A-motility	Isocitrate dehydrogenase	Youderian et al. (2003)
agmM	MXAN5820	No A-motility	Metalloprotease	Youderian et al. (2003)
agmN	MXAN1673	No A-motility	Unknown	Youderian et al. (2003)
agmR	MXAN5818	No A-motility	Ion transporting ATPase	Youderian et al. (2003)
agmT	MXAN6607	Partial A-motile	Periplasmic solute-binding protein	Yu and Kaiser (2007), Youderian et al. (2003)
agmU	MXAN4870	No A-motility	TPR repeat protein	Youderian et al. (2003)
agmV	MXAN4866	No A-motility	Unknown	Youderian et al. (2003)
agmW	?	No A-motility	Carboxy-terminal protease	Youderian et al. (2003)
agmX	MXAN4862	No A-motility	DnaJ-like, outer membrane lipoprotein	Youderian et al. (2003)
agmZ	MXAN2991	No A-motility	Unknown	Youderian et al. (2003)
agnB	MXAN6403	No A-motility	ABC transporter permease protein	Yu and Kaiser (2007)
mglB	MXAN1926	Partial A-motile	GAP for MglA	Leonardy et al. (2010)
pglH	MXAN2050	Partial A-motile	TPR, CheY-like receiver, and a DNA-binding domain	Yu and Kaiser (2007)
pglJ	MXAN2919	Partial A-motile	Polysaccharide polymerase	Yu and Kaiser (2007)
pglB	MXAN2921	Partial A-motile	Glycosyl transferase, RfaG	Yu and Kaiser (2007)

Table 23.2 (continued)

Gene Name	Location	Phenotype of knockout mutant	Related gene function(s)	References
pglK	MXAN4148	Partial A-motile	Transmembrane protein of unknown function	Yu and Kaiser (2007)
pglF	MXAN4616	Partial A-motile	Glycosyl transferase	Yu and Kaiser (2007)
pglN	MXAN4710	Partial A-motile	ADP-heptose synthase, sugar kinase/ transferase	Yu and Kaiser (2007)
pglI	MXAN4867	Partial A-motile	Unknown	Yu and Kaiser (2007)
pglC	MXAN5319	Partial A-motile	TPR repeat	Yu and Kaiser (2007)
aspT	MXAN5382	Partial A-motile	tRNA-Asp	Yu and Kaiser (2007)
pglE	MXAN5585	Partial A-motile	4-amino-4-deoxy-L-arabinose transferase	Yu and Kaiser (2007)
pglD	MXAN6501	Partial A-motile	GDP-mannose pyrophosphorylase	Yu and Kaiser (2007)
pglM	MXAN7160	Partial A-motile	Alanine racemase	Yu and Kaiser (2007)
pglA	MXAN7252	Partial A-motile	Exopolysaccharide synthesis, ExoD-like	Yu and Kaiser (2007)

Genes listed in table, except *agm*N, have orthologs in *S. aurantiaca* (Ronning and Nierman 2008)
[a]Compl. Complementable by cell end-to-end contact (Hodgkin and Kaiser 1977)

Clusters of CglB protein were shown to assemble at the trailing pole from monomers found in the outer membrane (Nudleman et al. 2005). In addition, a mutant that lacks CglB protein can be stimulated by end-to-end contact with any cglB$^+$ strain to activate polar clustering at the lagging pole (Nudleman et al. 2005).

Tgl, a lipoprotein assembly factor for the PilQ secretin, is one effector of S-motility, whose role in pilus assembly has been clearly demonstrated (Nudleman et al. 2006). Tgl localizes to one pole of a cell; only to the leading pole, the one with pili (Kaiser 1979). Pole specificity differentiates Tgl from all other Pil proteins in *M. xanthus*, which are normally found at both poles (*Table 23.1*). FrzS, which specifically regulates S-motility, is thought to be an effector of S-motility because it responds to the Mgl switch (Ward et al. 2000). FrzS relocates from one cell pole to the other in apparent synchrony with RomR (Leonardy et al. 2007). However, neither the target nor the regulatory activity of FrzS has been reported.

Fruiting Body Development in *M. xanthus*

When growth begins to outrun the food supply, a swarm begins to allocate those few resources that remain. Strict allocation is necessary because the cells are constantly circulating to access oxygen for efficient aerobic ATP production (Kaiser and Warrick 2011). Cell behavior changes in response to starvation: The swarm stops expanding outward as it feeds and retreats by migrating inward to build elevated, multicellular fruiting bodies that display their stress-resistant spores by elevating them. In addition to allocating ATP for cell movement, ATP must be allocated to DNA replication so that each spore will contain two complete copies of the genome (Tzeng and Singer 2005). *M. xanthus* has a single origin of bidirectional replication near the *dnaA* locus on its circular genome (Goldman et al. 2006). Using flow cytometry, Tzeng and Singer showed that a population of exponentially growing *M. xanthus* cells contain 1–2 copies of the chromosome, indicating a single DNA replication initiation event per cell cycle (Tzeng and Singer 2005). Tzeng and Singer found that as cells deplete their nutrient sources and approach the stationary phase, no new rounds of DNA replication are initiated. Singer et al. showed that the accumulation of (p)ppGpp was both necessary and sufficient to trigger fruiting body development (Singer and Kaiser 1995). *B. subtilis* and *E. coli* arrest DNA synthesis immediately with their stringent responses. By contrast, *M. xanthus* chromosome replication continues even as development is induced by their stringent response; continued DNA synthesis ensures that each myxospore contains two complete copies of the genome (Tzeng et al. 2006). Evidently, *M. xanthus* has a different survival strategy for its sporulation than bacilli that may be related to the threat posed by long exposure of myxospores in the top soil to bright sunlight. A more detailed description of starvation and sporulation can be found in Diodati et al. (2008).

In addition, more ATP must be set aside for protein synthesis on ribosomes. More than 30 new proteins must be made as spore-filled fruiting bodies are under construction (Dahl et al. 2007; Inouye et al. 1979). Consequently, the swarm must initiate its program for fruiting body development before any nutrient essential for protein synthesis has been eliminated. This explains why growth-limiting concentrations of any amino acid, of usable carbon sources, or of phosphate induce *M. xanthus* to initiate fruiting body development (Manoil and Kaiser 1980a, b). By contrast, neither the lack of oxygen nor the lack of purines or of pyrimidines, which *M. xanthus* scavenges by digesting its prey's nucleic acids, will induce development (Kimsey and Kaiser 1991). These observations point to deficiencies of any amino-acylated tRNA leading the swarm to initiate fruiting body development. In *M. xanthus* as in many other

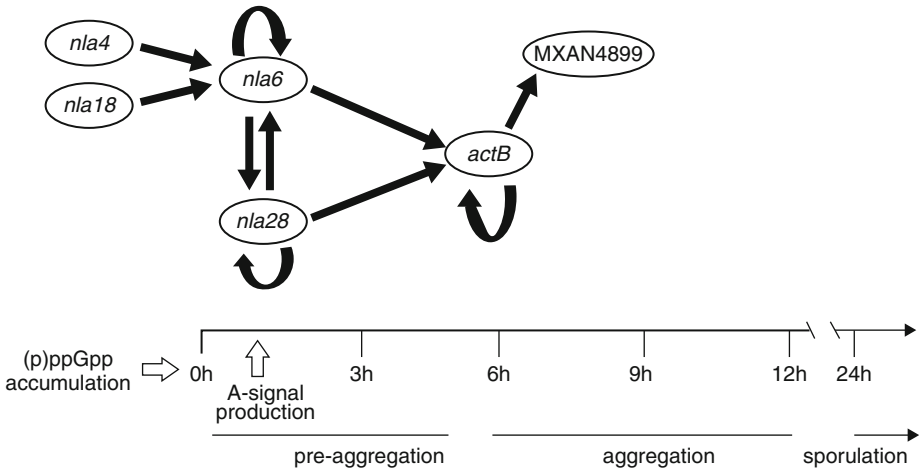


Fig. 23.8

The cascade of enhancer-binding proteins that controls the development of *M. xanthus* fruiting bodies. The *lines* above preaggregation and aggregation indicate the approximate extent of these stages (1–5 h and 6–12 h, respectively), and the *arrow* above sporulation indicates that this stage begins at about 24 h and continues for several days (24–120 h). The *white arrows* indicate that (p)ppGpp accumulation is required to make the transition from growth to development and that A-signal is required in the early part of preaggregation. The products of EBP genes shown here have a specific operational sequence as indicated by their placement above the developmental time line. The *straight black arrows* represent direct transcriptional regulation, and the *curved black arrows* represent auto-regulation (Reprinted with permission from Giglio et al. 2011)

bacteria, the absence or shortage of any one of the charged tRNAs leads a ribosome, sensing with a codon that lacks its cognate amino-acylated tRNA, to synthesize guanosine tetraphosphate (and pentaphosphate), (p)ppGpp, in a reaction catalyzed by the *relA* ppGpp synthase. The rise in (p)ppGpp sets off a stringent response that stops the synthesis of ribosomes (Cashel et al. 1996), enabling thereby the synthesis of new proteins essential for sporulation. Then, instead of a cascade of sigma factors such as that used by *B. subtilis* for sporulation (Errington 2003; Kroos et al. 1999), *M. xanthus* adds a cascade of enhancer-binding proteins (EBPs). The cascade organizes the transition from exponential growth through the staged development of fruiting bodies (Caberoy et al. 2009). A cascade of several EBPs, each with its own metabolic sensor, replaces an early commitment to sporulation found in *B. subtilis* with the possibility of responding to newly found nutrient, post-starvation, by restarting growth. *M. xanthus* appears not to commit to sporulation until it has begun to differentiate spores (Licking et al. 2000).

Though ordinarily considered an alternative sigma factor, sigma-54 is essential for *M. xanthus* growth and development (Keseler and Kaiser 1997). Moreover, the myxobacteria have more EBPs than any other taxonomic group of sequenced bacterial genomes (Goldman et al. 2006; Ronning and Nierman 2008). EBPs are specific transcriptional activators that work in conjunction with sigma-54 RNA polymerase to activate transcription at designated sigma-54 promoters (Caberoy et al. 2009). In response to an activating signal such as phosphorylation by a histidine kinase sensor protein, EBPs use the energy from ATP hydrolysis to form a transcription-competent open promoter complex. The *M. xanthus* EBP cascade is shown in ❯ *Fig. 23.8*. EBPs in the cascade appear to be regulated for reliability (Caberoy et al. 2009): First, the expression of a downstream EBP is activated at the proper time by a preceding EBP in the cascade. Second, Nla4 and Nla18 are important for (p)ppGpp production, Third, Nla6, Nla28, and ActB (Gronewold and Kaiser 2001, 2002) positively regulate their own expression, as indicated by the wide, flat arrows in ❯ *Fig. 23.8*. Since EBPs typically activate gene expression in response to a specific interaction with a signal transduction partner that detects a particular environmental cue (Studholme and Dixon 2003), it is suggested that the cascade's sensor kinases measure the level of metabolites that inform a cell whether those levels render fruiting body development an outcome to be sought, despite the cell death that accompanies development. Early detection of approaching starvation seems to be limiting spore formation because no more than 1% of the cells initiating fruiting body development ever become spores. After (p)ppGpp formation, ❯ *Fig. 23.8* shows that Nla6 and Nla28 help to manage the subsequent preaggregation stage, and the ActB and MXAN4899 regulate gene expression during the aggregation of fruiting bodies.

In parallel with the starvation-signal-induced cascade of EBPs, the transition from growth to development is guided by a diffusible cell-to-cell signal, the A-signal (open arrow on the time line, ❯ *Fig. 23.8*). A-signal molecules, purified from medium conditioned by developing cells, is a set of amino acids and peptides containing those amino acids (Kuspa et al. 1986, 1992a). Each developing *Myxococcus* cell releases a small quantity of A-signal about 2 h into development.

Consequently, the extracellular concentration of A-signal is directly proportional to the density of *M. xanthus* cells that are beginning to develop (Kaplan and Plamann 1996; Kuspa et al. 1992b). Cells respond to A-signal only if its concentration is above a certain threshold. Apparently, the threshold reflects the number of cells necessary to produce at least one spore-filled cyst. In *M. xanthus*, the cyst is a single fruiting body, and individual fruiting bodies are very similar in diameter and spherical shape, reflecting inheritance of the threshold value (◉ *Fig. 23.1*). The threshold number of cells constitute a quorum, and A-signal can be considered a quorum sensor. (See ◉ Chap. 15, "Bacterial Behavior"). Quorum sensing is also employed by *S. aurantiaca*, a close relative of *M. xanthus* and pictured with it in ◉ *Fig. 23.1*. *S. aurantiaca* has multiple cysts born on a single large stalk; again individual cysts are spherical and are of very similar size, while their number is highly variable. The number of cysts may reflect the number of cells in the aggregation field, which is parceled into a number of separate cysts. In 2008, the genome sequence of *S. aurantiaca* had been assembled but not closed. It was predicted to have 8,586 genes that include homologs to almost all the 7,380 genes of *M. xanthus*, including A-signaling genes, in line with their closely related phylogenies (Sproer et al. 1999). The minor differences observed are in line with a slightly larger genome and the fact that *S. aurantiaca* produces a unique signaling pheromone, stigmalone; it regulates culmination of its more complex fruiting bodies (Plaga et al. 1998). *S. aurantiaca* also has a different set of genes encoding secondary metabolites; synthesis of secondary metabolites account for 17% of the *M. xanthus* genome; finally, the general order of genes appears to be different in *S. aurantiaca* from that in *M. xanthus*.

After aggregation, *M. xanthus* cells express a unique set of A-signal-dependent genes (Kroos and Inouye 2008). Two A-signal-dependent genes are *csgA*, the gene for C-signal, and *fruA*, an important developmental response regulator. As indicated in ◉ *Fig. 23.9*, C-signaling should follow A-signaling, as observed.

C-Signaling

Starvation and A-signaling initiate the expression of the *csgA* gene that is essential for *M. xanthus* fruiting body morphogenesis and, unlike diffusible small molecules, requires cell movement for its transmission from one cell to another (Kroos et al. 1988). C-signal deficient mutants (*csgA*) were found to grow and swarm normally, but they failed to aggregate or to sporulate (Hagen et al. 1978; Kim and Kaiser 1990c; Shimkets et al. 1983). Active C-signal was found to be a 17 kDa cell-surface-bound protein that communicates when pairs of cells make an end-to-end contact with each other (Kim and Kaiser 1990a; Sager and Kaiser 1994). CsgA protein is 25 kDa and is secreted to the cell surface where it is cleaved to the active 17 kDa signal by a membrane protease (Lobedanz and Søgaard-Andersen 2003; Rolbetski et al. 2008). No receptors for the C-signal have been found on either the upstream or the downstream cell (Søgaard-Andersen 2008), while subsequent fruiting body development and the activation of FruA, a developmentally important response regulator (Ellehauge et al. 1998), clearly demonstrate that C-signaling has been effective. Insofar as C-signal transfer benefits from forceful collisions between pairs of aligned cells actively moving into end-to-end contact by A-motility (Kim and Kaiser 1990b), C-signal transfer closely parallels the stimulation of CglB, as described (Nudleman et al. 2005). CglB is an outer membrane lipoprotein that apparently diffuses rapidly within the outer membrane. The parallels between the C-signal protein, which is also present in the outer membrane in ever-increasing numbers as development proceeds (Gronewold and Kaiser 2001), and CglB suggest that C-signal transfer could occur by a stimulation process. CglB stimulation was shown to result in the equal sharing of outer membrane CglB protein between pairs of colliding cells (Nudleman et al. 2005; Wall and Kaiser 1998; Wall et al. 1998; Wei et al. 2011). If the parallels can be supported experimentally, C-signaling would result in the equal sharing of outer membrane C-signal protein between two signaling cells. Accordingly, both the C-signal receiving and the C-signal transmitting cell would have the same regulatory circuit (◉ *Fig. 23.9*). Extracellular signals activate both the FruA response regulator and the EBP cascade in that circuit.

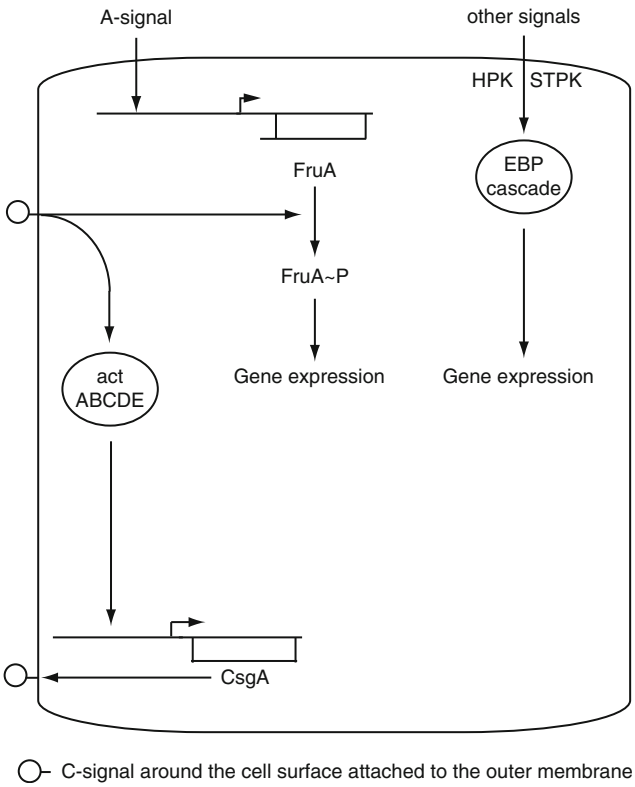


Fig. 23.9
Model showing the positive feedback regulation of cell-surface C-signal protein by act ABCDE. The cascade of enhancer-binding proteins is shown receiving input from outside the cell through histidine protein kinases and serine/threonine protein kinases

Streaming into a Nascent Fruiting Body

At the start of development, there are few C-signal molecules per cell; however, as indicated in ❯ *Fig. 23.9*, expression of the C-signal is increased by a positive feedback loop involving the five proteins of the *act* operon (Gronewold and Kaiser 2001). Jelsbak (Jelsbak and Søgaard-Andersen 1999) has found that cells in an aggregation stream continue to reverse their gliding direction, at the period set by the pacemaker (within measurement error). Whenever a cell reverses in response to its pacemaker, it would come into end-to-end contact with the cell immediately behind it that is still moving toward a nascent fruiting body. That forceful contact, as suggested above, would allow the two cells to exchange the C-signal. Each time C-signal is exchanged between cells in an aggregation stream, the positive feedback loop shown in ❯ *Fig. 23.9* would increase expression of *csgA* and elevate the number of signal molecules on both signaling cells (Gronewold and Kaiser 2001). Spore differentiation is likely to be the final step in fruiting body development because spores have lost their poles and without their polar engines would no longer be able to propel themselves and to raise the level of C-signal further. Consequently, sporulation should be triggered only after cells had been signaling each other long enough for the level of the C-signal to have reached some elevated threshold, to ensure that spores would form inside the nascent fruiting body and not prematurely in an aggregation stream. Threshold effects can be studied using a computational model, and the continuous three-dimensional simulation of Sozinova et al. (2005, 2006) reproduces several experimentally observed stages of fruiting body formation in correct order: asymmetric initial aggregates, linear streams, formation of hemispherical mounds, and finally sporulation within the mounds. Although the circuit of ❯ *Fig. 23.9* models the thresholding of sporulation, it includes only a small fraction of all the genes required for sporulation. Even so, the simple model described in ❯ *Fig. 23.9* can be useful insofar as critical new components can be added to the circuit one by one when they are identified. An expanded model can then be retested by comparison with experiments. That procedure successfully confirmed the pacemaker circuit (❯ *Fig. 23.3*) for swarming driven by growth and clarified the role of reversal in swarm expansion (Wu et al. 2009).

Fruiting Body Morphogenesis

M. xanthus builds its species-specific fruiting bodies using a set of cell movement patterns that are evident in its swarms. The set includes (1) that their polar, slime-secreting A-engines and their polar type IV pili with their suggested duty cycles are required for the constant movement observed; (2) that their cooperative synthesis of fibrils, as described for swarming, is required; (3) that their regular 8–9-min reversal by pacemaker is required; (4) that the ability of cells lying side-by-side to signal each other and synchronize their pacemakers is required; (5) that adjacent layers of cells are likely to be separated by fibrils embedded in a layer of slime that has been secreted from the A-engines. Spherical fruiting body-like aggregates were also observed to form in Welch's movies showing the convergence of three ridges of high cell density, two sets of waves moving in opposite directions and a band of high cell density at the edge of the culture (Kaiser and Welch 2004). Again, there were no indications of chemotaxis. Instead, three ridges of high cell density were seen to converge on round aggregates of constantly moving cells. The round, dynamic aggregates were capable of fusing with each other. Evidently, each *M. xanthus* cell is born with the capacity to build multilayered mounds with others of its kind. For *M. xanthus* that means building mounds having five nested layers by steady-state swarming. It is found that fruiting bodies, having the morphological complexity shown in ❯ *Fig. 23.1*, can be built to inherited specifications without invoking chemotaxis at any stage.

In conclusion, myxobacteria have the unique capacity to build regular structures of hundreds of moving cells using a pacemaker of constant period. Cell behavior is controlled by several different signals passed between cells, each signal evoking a specific response.

References

Alberti L, Harshey RM (1990) Differentiation of *Serratia marcesens* 274 into swimmer and swarmer cells. J Bacteriol 172:4322–4328

Allison C, Lai H-C, Gygi D, Hughes C (1993) Cell differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemoattractant for swarming cells. Mol Microbiol 8:53–60

Beebe JM (1941) Mophology and cytology of *Myxococcus xanthus*. J Bacteriol 42:193–223

Behmlander RM, Dworkin M (1994) Biochemical and structural analyses of the extracellular matrix fibrils of *Myxococcus xanthus*. J Bacteriol 176:6295–6303

Belas G, Simon M, Silverman M (1986) Regulation of lateral flagella gene transcription in *Vibrio parahaemolyticus*. J Bacteriol 167:210–218

Berleman JC, Bauer E (2005) A che-like signal transduction cascade involved in controlling flagella biosynthesis in *Rhodospirillum centenum*. Mol Microbiol 55:1390–1402

Berleman J, Chumley T, Cheung P, Kirby JR (2006) Rippling is a predatory behavior in *Myxococcus xanthus*. J Bacteriol 188:5888–5895

Berleman J, Scott J, Chumley T, Kirby JR (2008) Predataxis behavior in *Myxococcus xanthus*. Proc Natl Acad Sci USA 105:17127–17132

Bhaya D (2004) Light matters: phototaxis and signal transduction in unicellular cyanobacteria. Mol Microbiol 53:745–754

Bitter W, Koster M, Latijnhouwers M, de Cock H, Tommassen J (1998) Formation of oligomeric rings by XcpQ and PilQ, which are involved in protein transport across the outer membrane of *Pseudomonas aeruginosa*. Mol Microbiol 27:209–219

Bourne HR, Sanders DA, McCormick F (1990) The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348:125–132

Bourne HR, Sanders DA, McCormick F (1991) The GTPase superfamily: conserved structure and molecular mechanism. Nature 349:117–127

Bui NK, Gray J, Schwarz H, Schumann P, Blanot D, Vollmer W (2009) The peptidoglycan sacculus of *Myxococcus xanthus* has unusual structural features and is degraded during glycerol-induced myxospore development. J Bacteriol 191:494–505

Caberoy NB, Giglio K, Suen G, Garza AG (2009) A cascade of coregulating enhancer binding proteins initiates and propagates a multicellular developmental program. Proc Natl Acad Sci USA 108:E431–E439

Cashel M, Gentry DR, Hernandez VJ, Vinella D (1996) The stringent response. In: Neidhardt F (ed) *Escherichia coli* and *Salmonella typhimurium* cellular and molecular biology, vol 1. ASM, Washington, DC, pp 1458–1496

Chiang P, Habash M, Burrows LL (2005) Disparate subcellular localization patterns of *Pseudomonas aeruginosa* Type IV pilus ATPases involved in twitching motility. J Bacteriol 187:829–839

Clausen M, Jakovljevic V, Søgaard-Andersen L, Maier B (2009) High-force generation is a conserved property of type IV pilus systems. J Bacteriol 191:4633–4638

Collins RF, Davidsen L, Derrick JP, Ford RC, Tonjum T (2001) Analysis of the PilQ secretin from *Neisseria meningitidis* by transmission electron microscopy reveals a dodecameric quaternary structure. J Bacteriol 183:3825–3832

Collins RF, Frye SA, Kitmitto A, Ford RC, Tønjum T, Derrick JP (2004) Structure of the *Neisseria meningitidis* outer membrane PilQ secretin complex at 12 Å resolution. J Biol Chem 279:39750–39756

Craig L, Volkmann N, Arvai AS, Pique ME, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. Mol Cell 23:651–662

Cuthbertson L, Mainprize I, Naismith J, Whitfield C (2009) Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in Gram-negative bacteria. Microbiol Mol Biol Rev 73:155–177

D'Andrea LD, Regan L (2003) TPR proteins: the versatile helix. Trends Biochem Sci 28:655–662

Dahl JL, Tengra F, Dutton D, Yan J, Andacht T, Coyne L, Windell V, Garza AG (2007) Identification of major sporulation proteins of *Myxococcus xanthus* using a proteome approach. J Bacteriol 189:3187–3197

Darnton N, Turner L, Rojevsky S, Berg HC (2010) Dynamics of bacterial swarming. Biophys J 98:2082–2090

Diodati M, Gill R, Plamann L, Singer M (2008) Initiation and early developmental events. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM Press, Washington DC, pp 43–76

Dworkin M (1999) Fibrils as extracellular appendages of bacteria: their role in contact-mediated cell-cell interactions in *Myxococcus xanthus*. Bioessays 21:590–595

Ellehauge E, Norregaard-Madsen M, Søgaard-Andersen L (1998) The FruA signal transduction protein provides a checkpoint for the temporal coordination of intercellular signals in *Myxococcus xanthus* development. Mol Microbiol 30:807–813

Errington J (2003) Regulation of endospore formation in *Bacillus subtilis*. Nat Rev Microbiol 1:117–126

Evans K, Hobley L, Lambert C, Sockett RE (2008) Bdellovibrio: lone hunter cousin of the pack hunting myxobacteria. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM Press, Washington, DC, pp 351–362

Fontes M, Kaiser D (1999) *Myxococcus* cells respond to elastic forces in their substrate. Proc Natl Acad Sci USA 96:8052–8057

Fremgen S, Burke N, Hartzell P (2010) Effects of site-directed mutagenesis of *mglA* on motility and swarming of *Myxococcus xanthus*. BMC Microbiol 10:295

Frye SA, Assalkhou R, Collins RF, Ford RC, Petersson C, Derrick JP, Tonjum T (2006) Topology of the outer-membrane secretin PilQ from *Neisseria meningitidis*. Microbiology 152:3751–3764

Giglio K, Caberoy NB, Suen G, Kaiser D, Garza AG (2011) A cascade of coregulating enhancer binding proteins initiates and propagates a multicellular developmental program. Proc Natl Acad Sci USA 108: E431–E439

Goldman BS, Nierman WC, Kaiser D, Slater SC, Durkin AS, Eisen JA, Ronning CM, Barbazuk WB, Blanchard M, Field C, Halling C, Hinkle G, Iartchuk O, Kim HS, Mackenzie C, Madupu R, Miller N, Shvartsbeyn A, Sullivan SA, Vaudin M, Wiegand R, Kaplan HB (2006) Evolution of sensory complexity recorded in a myxobacterial genome. Proc Natl Acad Sci USA 103:15200–15205

Gronewold TMA, Kaiser D (2001) The *act* operon controls the level and time of C-signal production for *M. xanthus* development. Mol Microbiol 40: 744–756

Gronewold TMA, Kaiser D (2002) act operon control of developmental gene expression in *Myxococcus xanthus*. J Bacteriol 184:1172–1179

Hagen DC, Bretscher AP, Kaiser D (1978) Synergism between morphogenetic mutants of *Myxococcus xanthus*. Dev Biol 64:284–296

Hansen JK, Forest KT (2006) Type IV pilin structures: insights on shared architecture, fiber assembly, receptor binding and type II secretion. J Mol Microbiol Biotechnol 11:192–207

Harshey RM (1994) Bees aren't the only ones: swarming in Gram-negative bacteria. Mol Microbiol 13:389–394

Harshey RM, Matsuyama T (1994) Dimorphic transition in *E. coli* and *S. typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. Proc Natl Acad Sci USA 91:8631–8634

Henrichsen J (1972) Bacterial surface translocation: a survey and a classification. Bacteriol Rev 36:478–503

Hodgkin J, Kaiser D (1977) Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc Natl Acad Sci USA 74:2938–2942

Hodgkin J, Kaiser D (1979) Genetics of gliding motility in *M. xanthus* (Myxobacterales): genes controlling movement of single cells. Mol Gen Genet 171:167–176

Hoiczyk E (2000) Gliding motility in cyanobacteria: observations and possible explanations. Arch Microbiol 174:11–17

Hoiczyk E, Baumeister W (1998) The junctional pore complex, a prokaryotic secretion organelle, is the molecular motor underlying gliding motility in cyanobacteria. Curr Biol 8:1161–1168

Igoshin O, Goldbetter A, Kaiser D, Oster G (2004) A biochemical oscillator explains the developmental progression of myxobacteria. Proc Natl Acad Sci USA 101:15760–15765

Inouye M, Inouye S, Zusman D (1979) Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis. Dev Biol 68:579–591

Jelsbak L, Søgaard-Andersen L (1999) The cell-surface associated C-signal induces behavioral changes in individual *M. xanthus* cells during fruiting body morphogenesis. Proc Natl Acad Sci USA 96:5031–5036

Kaiser AD (1979) Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. Proc Natl Acad Sci USA 76:5952–5956

Kaiser D (2006) A microbial genetic journey. Annu Rev Microbiol 60:1–25

Kaiser D (2007) Bacterial swarming, a re-examination of cell movement patterns. Curr Biol 17:R561–R570

Kaiser D (2009) Are there lateral as well as polar engines for A-motile gliding in myxobacteria? J Bacteriol 191:5336–5341

Kaiser AD, Crosby C (1983) Cell movement and its coordination in swarms of *Myxococcus xanthus*. Cell Motil 3:227–245

Kaiser D, Warrick H (2011) *M. xanthus* swarms are driven by growth and regulated by a pacemaker. J Bacteriol 193:5898–5904

Kaiser D, Warrick H (2012) Focal adhesions as cell-cell signaling channels. Mol Microbiol (In print)

Kaiser D, Welch R (2004) Dynamics of fruiting body morphogenesis. J Bacteriol 186:919–927

Kaplan HB, Plamann L (1996) A *Myxococcus xanthus* cell density-sensing system required for multicellular development. FEMS Microbiol Lett 139:89–95

Kearns DB (2010) A field guide to bacterial swarming motility. Nat Rev Microbiol 8:634–644

Kearns DB, Losick R (2003) Swarming motility in undomesticated *Bacillus subtilis*. Mol Microbiol 49:581–590

Kearns DB, Shimkets LJ (2001) Lipid chemotaxis and signal transduction in *Myxococcus xanthus*. Trends Microbiol 9:126–129

Keseler IM, Kaiser D (1997) Sigma-54, a vital protein for *Myxococcus xanthus*. Proc Natl Acad Sci USA 94:1979–1984

Kim SK, Kaiser D (1990a) Cell alignment required in differentiation of *Myxococcus xanthus*. Science 249:926–928

Kim SK, Kaiser D (1990b) Cell motility is required for the transmission of C-factor, an intercellular signal that coordinates fruiting body morphogenesis of *Myxococcus xanthus*. Genes Dev 4:896–905

Kim SK, Kaiser D (1990c) Purification and properties of *Myxococcus xanthus* C-factor, an intercellular signaling protein. Proc Natl Acad Sci USA 87:3635–3639

Kimsey HH, Kaiser D (1991) Targeted disruption of the *Myxococcus xanthus* orotidine 5'-monophosphate decarboxylase gene: effects on growth and fruiting-body development. J Bacteriol 173:6790–6797

Kirby JR, Zusman DR (2003) Chemosensory regulation of developmental gene expression in *Myxococcus xanthus*. Proc Natl Acad Sci USA 100:2008–2013

Kirby JR, Berleman J, Muller S, Li D, Scott J, Wilson J (2008) Chemosensory signal transduction systems in *Myxococcus xanthus*. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM Press, Washington DC, pp 135–147

Kroos L, Inouye S (2008) Transcriptional regulatory mechanisms during *Myxococcus xanthus* development. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM Press, Washington DC, pp 149–168

Kroos L, Hartzell P, Stephens K, Kaiser D (1988) A link between cell movement and gene expression argues that motility is required for cell-cell signalling during fruiting body development. Genes Dev 2:1677–1685

Kroos L, Zhang B, Ichikawa H, Yu U-TN (1999) Control of sigma factor activity during *Bacillus subtilis* sporulation. Mol Microbiol 31:1285–1294

Kuspa A, Kroos L, Kaiser D (1986) Intercellular signaling is required for developmental gene expression in *Myxococcus xanthus*. Dev Biol 117:267–276

Kuspa A, Plamann L, Kaiser D (1992a) Identification of heat-stable A-factor from *Myxococcus xanthus*. J Bacteriol 174:3319–3326

Kuspa A, Plamann L, Kaiser D (1992b) A-signalling and the cell density requirement for *Myxococcus xanthus* development. J Bacteriol 174:7360–7369

Leonardy S, Freymark G, Hebener S, Ellehauge E, Søgaard-Andersen L (2007) Coupling of protein localization and cell movement by a dynamically localized response regulator in *Myxococcus xanthus*. EMBO J 26:4433–4444

Leonardy S, Miertzschke M, Bulyha I, Sperling E, Wittinghofer A, Søgaard-Andersen L (2010) Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP. EMBO J 29:2276–2289

Li Y, Sun H, Ma X, Lu A, Lux R, Zusman D, Shi W (2003) Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus*. Proc Natl Acad Sci USA 100:5443–5448

Licking E, Gorski L, Kaiser D (2000) A common step for changing the cell shape in fruiting body and starvation-independent sporulation of *Myxococcus xanthus*. J Bacteriol 182:3553–3558

Lobedanz S, Søgaard-Andersen L (2003) Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus*. Genes Dev 17:2151–2161

Lu A, Cho K, Black WP, Duan X-Y, Lux R, Yang Z, Kaplan HB, Zusman D, Shi W (2005) Exopolysaccharide biosynthesis genes required for social motility in *Myxococcus xanthus*. Mol Microbiol 55:206–220

Maier B, Potter L, So M, Seifert HS, Sheetz MP (2002) Single pilus motor forces exceed 100 pN. Proc Natl Acad Sci USA 99:16012–16017

Manoil C, Kaiser D (1980a) Accumulation of guanosine tetraphosphate and guanosine pentaphosphate in *Myxococcus xanthus* during starvation and myxospore formation. J Bacteriol 141:297–304

Manoil C, Kaiser D (1980b) Guanosine pentaphosphate and guanosine tetraphosphate accumulation and induction of *Myxococcus xanthus* fruiting body development. J Bacteriol 141:305–315

Mattick JS (2002) Type IV pili and twitching motility. Annu Rev Microbiol 56:289–314

Mauriello E, Nan B, Zusman D (2009) AglZ regulates adventurous [A-] motility in *Myxococcus xanthus* through its interaction with the cytoplasmic receptor, FrzCD. Mol Microbiol 72:964–977

McCarter L, Hilmen M, Silverman M (1988) Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. Cell 54:345–351

Merz AJ, So M, Sheetz MP (2000) Pilus retraction powers bacterial twitching motility. Nature 407:98–102

Mignot T, Merlie JP, Zusman D (2005) Regulated pole-to-pole oscillations of a bacterial gliding motility protein. Science 310:855–857

Mignot T, Shaevitz J, Hartzell P, Zusman D (2007) Evidence that focal adhesions power bacterial gliding motility. Science 315:853–856

Nudleman E, Kaiser D (2004) Pulling together with type IV pili. J Mol Microbiol Biotechnol 7:52–62

Nudleman E, Wall D, Kaiser D (2005) Cell-to-cell transfer of bacterial outer-membrane lipoproteins. Science 309:125–127

Nudleman E, Wall D, Kaiser D (2006) Polar assembly of the type IV pilus secretin in *Myxococcus xanthus*. Mol Microbiol 60:16–29

Pelicic V (2008) Type IV pili: e pluribus unum? Mol Microbiol 68:827–837

Plaga W, Stamm I, Schairer HU (1998) Intercellular signaling in Stigmatella aurantiaca: purification and characterization of stigmolone, a myxobacterial pheromone. Proc Natl Acad Sci USA 95:11263–11267

Reichenbach H (1966) *Myxococcus* spp. (Myxobacterales) Schwarmentwicklung und Bildung von Protocysten. Institut fur den Wissenschaftlichen Film, Gottingen, Germany

Reichenbach H (1984) In: Rosenberg E (ed) Myxobacteria: a most peculiar group of social prokaryotes. Springer, New York, pp 1–50, p. 34

Reichenbach H (1993) In: Dworkin M, Kaiser D (eds) Biology of the myxobacteria: ecology and taxonomy. ASM, Washington, pp 13–62

Reichenbach H, Heunert HH, Kuczka H (1965a) *Archangium violaceum* (Myxobacterales) - Schwarmentwicklung und Bildung von Protocysten. Film E777. Inst. Wissensch. Film, Gottingen, Ger

Reichenbach H, Heunert HH, Kuczka H (1965b) *Chondromyces apiculatus* (Myxobacterales)-Schwarmentwicklung und Morphogenese. Film E779. Inst. Wissensch. Film, Gottingen, Ger

Reichenbach H, Galle HK, Heunert HH (1975/1976) *Stigmatella aurantiaca* (Myxobacterales). Schwarmentwicklung und Morphogenese. Film E2421. Inst. Wissensch. Film, Gottingen, Ger

Rodriguez-Soto JP, Kaiser D (1997a) Identification and localization of the *tgl* protein, which is required for *Myxococcus xanthus* social motility. J Bacteriol 179:4372–4381

Rodriguez-Soto JP, Kaiser D (1997b) The *tgl* gene: social motility and stimulation in *Myxococcus xanthus*. J Bacteriol 179:4361–4371

Rolbetski A, Ammon M, Jakovljevic V, Konovalova A, Søgaard-Andersen L (2008) Regulated secretion of a protease activity activates intercellular signaling during fruiting body formation in *M. xanthus*. Dev Cell 15:627–634

Ronning CM, Nierman WC (2008) The genomes of *Myxococcus xanthus* and *Stigmatella aurantiaca*. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM, Washington, DC, pp 285–298

Sager B, Kaiser D (1994) Intercellular C-signaling and the traveling waves of *Myxococcus*. Genes Dev 8:2793–2804

Satyshur K, Worzalla G, Meyer L, Heiniger E, Aukema K, Misic A, Forest KT (2007) Crystal structures of the pilus retraction motor PilT suggests large domain movements and subunit cooperation drive motility. Structure 15:363–376

Semmler ABT, Whitchurch CB, Mattick JS (1999) A re-examination of twitching motility in *Pseudomonas aeruginosa*. Microbiology 145:2863–2873

Senesi S, Celandroni F, Salvetti S, Beecher D, Wong A, Ghelardi E (2002) Swarming motility in *Bacillus cereus* and characterization of a *fliY* mutant impaired in swarm cell differentiation. Microbiology 148:1785–1794

Shimkets LJ, Gill RE, Kaiser D (1983) Developmental cell interactions in *Myxococcus xanthus* and the spoC locus. Proc Natl Acad Sci USA 80: 1406–1410

Simunovic V, Gherardini FC, Shimkets LJ (2003) Membrane localization of motility, signaling, and polyketide synthase proteins in *Myxococcus xanthus*. J Bacteriol 185:5066–5075

Singer M, Kaiser D (1995) Ectopic production of guanosine penta- and tetraphosphate can initiate early developmental gene expression in *Myxococcus xanthus*. Genes Dev 9:1633–1644

Skerker J, Berg H (2001) Direct observation of extension and retraction of type IV pili. Proc Natl Acad Sci USA 98:6901–6904

Søgaard-Andersen L (2008) Contact-dependent signaling in *Myxococcus xanthus*: the function of the C-signal in fruiting body morphogenesis. In: Whitworth D (ed) Myxobacteria: multicellularity and differentiation. ASM, Washington, DC, pp 77–91

Sozinova O, Jiang Y, Kaiser D, Alber MS (2005) Three-dimensional model of myxobacterial aggregation by contact-mediated interaction. Proc Natl Acad Sci USA 102:11308–11312

Sozinova O, Jiang Y, Kaiser D, Alber M (2006) A three-dimensional model of myxobacterial fruiting body formation. Proc Natl Acad Sci USA 103: 17255–17259

Sproer C, Reichenbach H, Stackebrandt E (1999) Correlation between morphological and phylogenetic classification of myxobacteria. Int J Syst Bacteriol 49:1255–1262

Stephens K, Hartzell P, Kaiser D (1989) Gliding motility in *Myxococcus xanthus*: the *mgl* locus, its RNA and predicted protein products. J Bacteriol 171: 819–830

Strogatz SH (2003) Sync: the emerging science of spontaneous order. Hyperion, New York

Strom MS, Nunn DN, Lory S (1993) A single bifunctional enzyme, PilD, catalyzes cleavage and N-methylation of proteins belonging to the type IV pilin family. Proc Natl Acad Sci USA 90:2404–2408

Studholme DJ, Dixon R (2003) Minireview: Domain architectures of s^{54}-dependent transcriptional activators. J Bacteriol 185:1757–1767

Turner L, Zhang R, Darnton N, Berg HC (2010) Visualization of flagella during bacterial swarming. J Bacteriol 192:3259–3267

Tzeng L-F, Singer M (2005) DNA replication during sporulation in *Myxococcus xanthus* fruiting bodies. Proc Natl Acad Sci USA 102:14428–14433

Tzeng L-F, Ellis TN, Singer M (2006) DNA replication during aggregation phase is essential for *Myxococcus xanthus* development. J Bacteriol 188:2774–2779

Vos M, Velicer G (2006) Genetic population structure of the soil bacterium *Myxococcus xanthus* at the centimeter scale. Appl Environ Microbiol 72:3615–3625

Wall D, Kaiser D (1998) Alignment enhances the cell-to-cell transfer of pilus phenotype. Proc Natl Acad Sci USA 95:3054–3058

Wall D, Wu SS, Kaiser D (1998) Contact stimulation of Tgl and type IV pili in *Myxococcus xanthus*. J Bacteriol 180:759–761

Wang Q, Suzuki A, Mariconda S, Porwollik S, Harshey RM (2005) Sensing wetness: a new role for the bacterial flagellum. EMBO J 24:2034–2042

Ward MJ, Lew H, Zusman DR (2000) Social motility in *Myxococcus xanthus* requires FrzS, a novel protein with an extensive coiled-coil domain. Mol Microbiol 37:1357–1371

Wei X, Pathak D, Wall D (2011) Heterologous protein transfer within structured myxobacteria biofilms. Mol Microbiol 81:315–326

Whitfield C (2006) Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. Annu Rev Biochem 75:39–68

Wolfe AJ, Berg HC (1989) Migration of bacteria in semisolid agar. Proc Natl Acad Sci USA 86:6973–6977

Wolgemuth C, Hoiczyk E, Kaiser D, Oster G (2002) How myxobacteria glide. Curr Biol 12:369–377

Wu Y, Kaiser D, Jiang Y, Alber M (2009) Periodic reversal of direction allows myxobacteria to swarm. Proc Natl Acad Sci USA 106:1222–1227

Wu Y, Hosu BG, Berg HC (2011) Microbubbles reveal chiral fluid flows in bacterial swarms. Proc Natl Acad Sci USA 108:4147–4151

Xie G, Bruce DC, Challacombe JF, Chertkov O, Detter JC, Gilna P, Han CS, Lucas S, Misra M, Myers GL, Richardson P, Tapia R, Thayer N, Thompson LS, Brettin TS, Henrissat B, Wilson DB, McBride MJ (2007) Genome sequence of the cellulolytic gliding bacterium *Cytophaga hutchinsonii*. Appl Environ Microbiol 73:3536–3546

Youderian P, Burke N, White DJ, Hartzell PL (2003) Identification of genes required for adventurous gliding motility in *Myxococcus xanthus* with the transposable element mariner. Mol Microbiol 49:555–570

Yu R, Kaiser D (2007) Gliding motility and polarized slime secretion. Mol Microbiol 63:454–467

Zhang R, Turner L, Berg HC (2010) The upper surface of an *Escherichia coli* swarm is stationary. Proc Natl Acad Sci USA 107:288–290