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CONTRIBUTORS

_Birgit Adam_
Max Planck Institute for Marine Microbiology,
Celsiusstrasse 1, 28359 Bremen, Germany

_Daniel H. Buckley_
Department of Crop and Soil Sciences,
Cornell University, Ithaca, NY 14853

_Trevor C. Charles_
Department of Biology, University of Waterloo, 200 University
Avenue West, Waterloo, Ontario, N2L 3G1, Canada

_Yin Chen_
School of Life Sciences, University of Warwick,
Coventry, CV4 7AL, United Kingdom

_Ralf Conrad_
Max-Planck-Institute for Terrestrial Microbiology,
Karl-von-Frisch-Str., 35043 Marburg, Germany

_R. P. Evershed_
Organic Geochemistry Unit, Bristol Biogeochemistry
Research Centre, School of Chemistry, University of Bristol,
Cantock's Close, Bristol BS8 1TS, United Kingdom

_Michael W. Friedrich_
Faculty of Biology/Chemistry, University of
Bremen, D-28359 Bremen, Germany

_Maria-Luisa Gutierrez-Zamora_
Centre for Marine BioInnovation, University of
New South Wales, Sydney 2035, Australia
CONTRIBUTORS

Nico Jehmlich
Interfaculty Institute for Genetics and Functional
Genomics, University of Greifswald, Friedrich-Ludwig-
Jahn-Strasse 15a, D-17487 Greifswald, Germany

Sasha N. Jenkins
School of Earth and Environment, Faculty of Natural and Agricultural
Sciences, The University of Western Australia, Crawley, WA 6009, Australia

Marcel M. M. Kuypers
Max Planck Institute for Marine Microbiology,
Celsiusstrasse 1, 28359 Bremen, Germany

Yahai Lu
College of Resources and Environmental Sciences, China
Agricultural University, Beijing 100193, China

Eugene L. Madsen
Department of Microbiology, Wing Hall, Cornell
University, Ithaca, NY 14853–8101

Mike Manefield
Centre for Marine BioInnovation, University of
New South Wales, Sydney 2035, Australia

P. J. Maxfield
Organic Geochemistry Unit, Bristol Biogeochemistry
Research Centre, School of Chemistry, University of Bristol,
Cantock’s Close, Bristol BS8 1TS, United Kingdom

Hélène Moussard
School of Life Sciences, University of Warwick,
Coventry, CV4 7AL, United Kingdom

J. Colin Murrell
School of Life Sciences, University of Warwick,
Coventry, CV4 7AL, United Kingdom

Niculina Musat
Max Planck Institute for Marine Microbiology,
Celsiusstrasse 1, 28359 Bremen, Germany

Kenneth Nealson
University of Southern California, Los Angeles, CA 90089

Josh D. Neufeld
Department of Biology, University of Waterloo, 200 University
Avenue West, Waterloo, Ontario, N2L 3G1, Canada
Anthony G. O’Donnell
Institute of Agriculture, Faculty of Natural and Agricultural Sciences,
The University of Western Australia, Crawley, WA 6009, Australia

Lee J. Pinnell
Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, N2L 3G1, Canada

Daniel S. Read
Centre for Ecology and Hydrology, Benson Lane, Wallingford, OX10 8BB, United Kingdom

Hans-Hermann Richnow
Helmholtz—Centre for Environmental Research–UFZ, Department of Isotope Biogeochemistry, Permoserstrasse 15, D-04318 Leipzig, Germany

Frank Schmidt
Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Friedrich-Ludwig-Jahn-Strasse 15a, D-17487 Greifswald, Germany

Egbert Schwartz
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640

Jana Seifert
Helmholtz—Centre for Environmental Research–UFZ, Department of Proteomics, Permoserstrasse 15, D-04318 Leipzig, Germany

Thomas J. Smith
Biomedical Research Centre, Sheffield Hallam University, Sheffield, S1 1WB, United Kingdom

Martin Taubert
Helmholtz—Centre for Environmental Research–UFZ, Department of Proteomics, Permoserstrasse 15, D-04318 Leipzig, Germany

Koen Venema
Department of BioSciences, TNO Quality of Life, Zeist, The Netherlands

Carsten Vogt
Helmholtz—Centre for Environmental Research–UFZ, Department of Isotope Biogeochemistry, Permoserstrasse 15, D-04318 Leipzig, Germany

Martin von Bergen
Helmholtz—Centre for Environmental Research–UFZ, Department of Proteomics, Permoserstrasse 15, D-04318 Leipzig, Germany
CONTRIBUTORS

Michael Wagner
Department of Microbial Ecology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Andrew S. Whiteley
Centre for Ecology and Hydrology, Benson Lane, Wallingford, OX10 8BB, United Kingdom
Since the discovery of bacteria by Antonie van Leeuwenhoek over 300 years ago, microbiologists have striven to identify the many thousands of bacteria that exist in the environment. Since the morphological features of most bacteria are similar, it has been difficult to differentiate between them by using conventional microscopy techniques. Therefore, until the advent of molecular biology techniques, microbiologists have relied on the enrichment, isolation, and characterization of microbes, using strategies pioneered by Biejerinck and Winogradski in the early part of the 20th century, to be able to determine what role different microbes play in the environment. Environmental microbiology was revolutionized through the pioneering work of Woese (Woese and Fox, 1977), who developed a taxonomic framework with which to relate the identity of microbes to each other. This tree of life is based on small subunit ribosomal (rRNA) sequences of different organisms. The polymerase chain reaction (PCR) facilitated the direct isolation of 16S rRNA gene sequences from environmental samples (Pace et al., 1985), and through the work of Pace and others, a molecular view of microbial diversity in the biosphere generally emerged in the 1990s (Head et al., 1998). As more and more 16S rRNA gene sequences were retrieved from many different environments, and these were then matched against 16S rRNA sequences from extant bacteria, i.e., those that had been previously isolated and cultivated in the laboratory, the understanding emerged that environmental microbiologists had only cultivated a small fraction of the total microbial diversity in the biosphere (Ward et al., 1990). We do not subscribe to the notion that the majority of the microorganisms present in the environment are “unculturable,” but suggest that microbiologists have not yet had the time nor the techniques to be able to isolate into pure culture a significant fraction of the total microbial population. There are good examples in recent years of many more novel microbes being grown under laboratory conditions, both in axenic culture or as coculture, revealing many new biological processes that have been around for many thousands (millions?) of years. Anaerobic methane and ammonia oxidation are good examples of this (Strous et al., 1999; Raghoebarsing et al., 2006).

From molecular biology-based studies, principally by retrieving 16S rRNA gene sequences from the environment, it becomes obvious that microbes inhabit virtually all ecological niches on Earth. A major challenge for microbial ecologists has been to try to determine what is the function of many of the thousands of
different microbes on Earth; i.e., who eats what, where, when, and how? “Linking the identification of uncultivated microbes with their potential to metabolize compounds in the environment represents the Holy Grail of scientific discovery for microbial ecologists” (Neufeld et al., 2007). In this book, we hope to inform readers of the many techniques and approaches that are now available with which to start to answer these fundamental questions.

The American Society for Microbiology (ASM) General Meeting in New Orleans in May, 2004, provided a forum to bring together a number of researchers who were starting to develop stable isotope probing (SIP) techniques for use in microbial ecology. We were approached by Greg Payne, Senior Acquisitions Editor for ASM, with an invitation to consider an edited volume on SIP techniques. At that time there were only a handful of laboratories developing and starting to use these techniques, and only around 30 publications in the ISI Web of Knowledge database were identified with the search tag “stable isotope probing.” Therefore, we put off the idea for a few years until the field developed and the techniques “proved themselves” to the environmental microbiology community. Of course, stable isotopes had been successfully used in microbiology for process-based studies many years prior to this (e.g., Meselson and Stahl, 1958; Kaplan and Rittenberg, 1964). However, the first use of stable isotopes for tracing process through metabolic labeling of biomarkers in a strict environmental microbiology context is attributed to Boschker et al. (1998). In their pioneering work, described in the journal *Nature*, they directly linked identity and function within complex microbial populations by labeling the polar lipid-derived fatty acids (PLFAs) of sulfate-reducing bacteria with $^{13}$C. Several other papers from this group followed, again describing the use of $^{13}$C to label the PLFAs of microbes in situ (e.g., see Boschker et al., 1999; Nold et al., 1999; Middelburg et al., 2000).

It was in 2000, with the first report of the use of $^{13}$C isotopes to label the DNA of methylotrophic bacteria directly in environmental samples, that the term “stable isotope probing” was used (Radajewski et al., 2000). This earliest “flavor” of SIP was described as DNA-SIP. Soon after followed RNA-SIP (Manefield et al., 2002), which provided another novel means of linking the phylogeny of members of a microbial community to their function. Since the ASM meeting in 2004, the technique of protein-SIP (Jehmlich et al., 2008) has been developed, together with examples of the use of $^{15}$N (first described by Cadisch et al., 2005) and $^{18}$O (first described by Schwartz, 2007) as alternative stable isotopes with which to label biomarkers and thus identify microbes that are active in the environment.

In 2009, we felt that SIP techniques had been sufficiently tried and tested and well established as part of the microbial ecologist’s “toolbox” to consider the production of this book. Indeed, a survey of the ISI Web of Knowledge database from 2000 to the time of writing (March 2010) indicates that using the search term stable isotope probing there are over 212 publications recorded which have been cited around 3,300 times (nearly 800 citations in 2009). Google Scholar lists about 110,000 hits with the same search term. Since the early days of SIP, when simple $^{13}$C-labeled compounds such as methane, methanol, and acetate were used to label specific biomarkers of bacteria in the environment, a number
of more complex carbon sources have been used in a variety of different contexts. For example, SIP has been used to identify microbes involved in bioremediation processes; to begin to understand the interactions between microbes and plants; to follow the flow of carbon through microbial food webs and trophic interactions; and to examine the function of gut microflora. SIP has also been used in gene mining and has been coupled with metagenomics not just to rescue gene fragments but to label and access the genomes of specific microbes carrying out key metabolic functions in the environment. We have been very fortunate in persuading many of the key practitioners in their fields to contribute chapters covering all of these topics, giving significant insights into the methodology and applications of these various SIP techniques.

Of course, SIP techniques are not the only tools available for microbial ecologists, and the past decade has seen significant developments in a number of other exciting techniques in environmental microbiology. Such techniques include methods for labeling and identifying microbes in situ using radioisotopes, and therefore we have also included elegant technologies such as $^{14}$C-RNA-SIP, FISH-MAR (fluorescence in situ hybridization coupled to microautoradiography), and isotope arrays in this book. Single-cell microbiology techniques exploiting stable isotopes, including Raman-FISH (Raman spectroscopy coupled to fluorescence in situ hybridization) and nanoSIMS (mass spectrometry of secondary ions) coupled to in situ hybridization, are also covered by contributions from world leaders in these technologies.

We are very grateful to all authors for their scholarly and insightful contributions to this book on stable isotope probing and related techniques. In hindsight, we set them a particularly interesting submission deadline spanning the Christmas of 2009, and all authors pretty much delivered as they said they would. We thank you all for the speedy submissions and hope we didn’t ruin the holiday celebrations too much! We would also like to specifically thank Greg Payne at ASM and the ASM production team who made the commissioning and editing process extremely smooth and simple. Overall, the quality of the authors and the ASM team had us asking each other several times the question, “Shouldn’t this be harder?” Finally, we would like to thank Ken Nealson for accepting the invitation to write a “crystal ball” forward-looking view—these are always extremely difficult to do. But, as ever, Ken’s opinions are highly relevant, very thought provoking, and tinged with a good dose of humor!

Finally, we hope this volume will serve as an overview for current practitioners and stimulate new investigators and questions within microbial ecology. It is clear from the chapters that the field is developing rapidly, from newer technologies through ever-widening portfolios of applications and ultimately the generation of new and fundamental microbial-based questions. It is still astounding to think that all this has happened in less than a decade. We look forward to the possibilities of the next 10 years and hope we have done the current progress justice. As ever, any errors or omissions are entirely down to us.

J. Colin Murrell
Andrew S. Whiteley
April, 2010
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