

A Proposal for the creation of a textbook on Microbial Diversity

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Synopsis

The ASM recommends that undergraduate curricula for Microbiology majors contain a required senior-level core course on Microbial Diversity. Unlike the other recommended core courses, however, there is no appropriate textbook for such a class. I propose, therefore, to create a textbook to fill this void.

History

I have been teaching Microbial Diversity for the past 12 years as part of our undergraduate Microbiology curriculum. From the very beginning, my students and I have wished in vain for an appropriate textbook. As a result, I began early on to post increasingly detailed lecture notes on a course web site (this was viewed as 'innovative' at the time!). This web site soon became sufficiently detailed for me to offer the lecture portion of my class on-line for those students that could not participate in the traditional lecture. This site is accessible to anyone, and I have heard from a number of people that they're using the site for their own courses on Microbial Diversity. During the past year or so, I have thought a lot about what a good Microbial Diversity textbook would be like, and so I was excited when Greg Payne approached me with the prospect of creating one with the ASM Press.

Why is this book needed?

The ASM recommends that all undergraduate Microbiology majors take a senior-level core course in Microbial Diversity. The reality, however, is that most Microbiology programs lack this course. The reason for this is the synergistic combination of 1) the uncommonness of faculty with backgrounds in microbial diversity, and 2) the absence of an appropriate textbook.

There are four existing books that could potentially be used in a Microbial Diversity course of the sort envisioned by the ASM; it is unfortunate that none of these are designed, or are adequate, for this purpose.

Microbial Diversity: Form and Function in Prokaryotes, by Oladele Ogunseitan (Blackwell Publishing) is probably the closest there is to an appropriate textbook. This is "the competition"; for instructors planning to teach Microbial Diversity right now this is the only textbook available. It is the only one of these books that is not a collection of chapters written by a variety of authors. Despite this, however, most instructors teaching Microbial Diversity have not adopted this book, preferring to go without a textbook. I can't speak for the others, but I haven't used this book in my own Microbial Diversity course for main two reasons: (1) it is written from a biogeochemical process, rather than phylogenetic/organismal, perspective, and (2) I like to use primary research articles in senior-level classes, which this book does not do. This would be a good textbook for a course in Environmental Microbiology, but doesn't seem to fill the need for a textbook for a broad microbial diversity course. Assuming a ca. 2010 release date for the book being proposed here, *Ogunseitan* would be in need of an updated edition if it is to remain competitive.

Diversity of Microbial Life, edited by Jim Staley and Anna-Louis Reysenbach (Wiley), is probably the closest of these books to being useful as a primary text for a Microbial Diversity course, even more so than *Ogunseitan*, despite the fact that this is not a textbook. The chapters, written by a wide range of experts in the field, are not integrated in any meaningful way, but are a bit more general and approachable to a microbiology audience. The book is more oriented towards the organismal (rather than 'process') side of microbial ecology than any of the other books, and so is a better fit to the ASM recommendations for the content of a Microbial Diversity course. If I were teaching a graduate-level Microbial Diversity course, this is the book I'd use (supplemented, of course, with primary research pa-

Microbial Diversity textbook proposal

pers). However, I have not found it useful for students at the undergraduate level, nor is it intended for this purpose.

Microbial Diversity and Bioprospecting, edited by Alan Bull (ASM Press) is collection of 44 chapters, 6 by the editor, collected into 7 broad topics. This is a great resource, one that I use a great deal; I especially appreciate the coherence Alan has created in the book. But the target audience is senior graduate students and above; this book is neither designed for, nor useful as, a textbook. Notice that the ASM eStore does *not* include this book in their textbook category.

Prokaryotic Diversity: Mechanisms and Significance, edited by Niall Logan, Hilary Lappin-Scott and Petra Oyston (Cambridge University Press) is a Symposium Proceedings, from the 66th (2006) Symposium of the Society for General Microbiology (at the University of Warwick). It's 13 chapters are each written by specialists on 13 independent facets of microbial diversity; these are reviews of specific aspects of diversity intended for a sophisticated audience. There's a lot here an instructor could incorporate into a class on Microbial Diversity course, but this book is not a textbook, nor something for the students to use directly.

I am not aware of any existing course on Microbial Diversity that uses these or any other specialized text as a primary resource for the students; these course seem to rely instead on material gathered *ad hoc* by the instructor. (Some use *Brock* for general information, not a bad choice.) Instructors with a strong background in general microbial diversity can, and do, create exciting, useful courses this way, but at the expense of a great deal of effort. Those with more specialized backgrounds might find developing such a course an overwhelmingly daunting task in the absence of a good textbook. I believe a good textbook, pitched at the senior undergraduate level (see below) and with an organismal/phylogenetic perspective (also below), would be welcomed by the community, be widely adopted, and result in an increase in the number of Microbial Diversity courses offered.

Target audience

The audience for this textbook (and the associated course) would be primarily senior undergraduate students majoring in Microbiology or a related field such as Genetics or Biology. The presumption would be that these students have already had a general microbiology course, genetics, and either biochemistry or microbial physiology. The course could also include strong junior students with the appropriate background (my existing course is typically 1/3rd juniors) and occasional graduate students either in other programs, or incoming graduate students with non-microbiology backgrounds. The textbook could be used by either colleges or universities in the US or other English-speaking nations.

Scope of the book

The proposed textbook would be based largely on my existing senior-level lecture/lab course on Microbial Diversity. The lecture portion of the course meets 3 times weekly (50 minutes each time) for a 15-week semester, twice for traditional lectures and once for an informal discussion session. Each of the Chapters proposed would be designed to be covered in one of the traditional lecture periods, to fill a traditional semester.

The textbook would be divided into three sections. The first section would begin with some preliminaries; defining the scope of the book/course, defining the perspective, providing a historical context, etc. The main part of this section would be a practical guide to molecular phylogenetic analysis, focusing on how to create and interpret phylogenetic trees, and a overview or survey of "the Tree of Life".

The second section would be a tour through each of the major phylogenetic groups of Bacteria and Archaea (microbial eukaryotes and viruses would also be covered briefly), discussing the general properties (morphology, phenotype, metabolism, habitat, life cycle, etc) of the organisms in each group, describing in more detail some specific representatives. One or two specific topics raised by these organisms would be discussed, perhaps in the form of self-contained "Box"es. For example, a discussion of the spirochaetes might raise the more general topic of motility, or a discussion of *Aquifex* and relatives would raise the problem of life at high temperatures.

The third section would be more conceptual and experimentally defined, making heavy use of the primary research literature. This section would discuss, by example, how molecular phylogenetic analysis is used by microbiologists beyond the basic identification of cultivated species covered in section I; from identification of unknown and potentially uncultivable organisms, to molecular surveys of populations, and linking processes with specific organisms. This would lead in the final section to a discussion of various conceptual aspects of microbial complexity, from genomics and cell biology. The unifying theme to this section would be the under-appreciated complexity of bacterial and archaeal life, including internal organization, compartmentalization, chromosome structure, symbiosis, communication, and multicellularity. These would be compared to those of eukaryotes, in terms of the differences, variation, and most importantly, similarities.

Table of Contents

- Section I - Molecular phylogenetics
 - Chapter 1. Prelude
 - What is microbial Diversity?
 - The fundamental similarity of all living things
 - Chapter 2. Context & historical baggage
 - The false prokaryote/eukaryote dichotomy
 - Taxonomy vs phylogenetics
 - The evolution of evolutionary thought
 - Chapter 3. Where does the information come from?
 - Molecular clocks
 - the ssu-rRNA
 - collecting data for phylogenetic analysis
 - Chapter 4. Constructing a phylogenetic tree
 - Sequence alignment
 - tree construction
 - how to read a phylogenetic tree
 - Example analysis
 - Chapter 5. Tree construction alternatives
 - protein-based trees
 - alternative substitution models
 - alternative treeing algorithms
 - bootstrap analysis
 - Chapter 6. Other methods for assessing phylogeny
 - DNA:DNA hybridization
 - FAME/MIDI
 - serology
 - RFLP/ribotyping
 - phenotype
- Section II - The Microbial World
 - Chapter 7. The Tree of Life
 - Overview of the Tree of Life
 - Rooting the universal tree
 - The issue of horizontal transfer
 - Chapter 8. Primitive thermophiles
 - Aquifex and relatives
 - Thermotoga and relatives
 - Life at high temperatures
 - Chapter 9. Green photosynthesizers
 - Green non-sulfur Bacteria
 - Chlorobium & relatives
 - Cyanobacteria
 - bacterial photosynthesis
 - Chapter 10. Proteobacteria
 - Purple Bacteria & relatives
 - Electron transport and the concept of proteobacteria

- Chapter 11. Gram-positive Bacteria & relatives
 - Firmicutes
 - Actinobacteria
 - Bacterial development
 - Secondary metabolism
- Chapter 12. Spirochaetes & Bacteroids
 - Spirochaetes
 - Bacteroids
 - Motility
- Chapter 13. unnamed
 - Deinococcus & Thermus
 - Chlamydia & relatives
 - Planctomycetes
 - Compartmentalization in Bacteria
- Chapter 14. Bacterial groups with few or no cultivated members
 - How do we know about these organisms?
 - Survey of these groups
 - How much of the microbial world do we know about?
- Chapter 15. The Archaea
 - General properties of the Archaea
 - Crenarchaea
 - Euryarchaea
 - A 'missing link' between bacteria and eukaryotes?
- Chapter 16. Eukaryotes as microbes
 - Protists
 - Fungi
 - Microscopic animals
 - Microscopic plants
- Chapter 17. Viruses & Prions
 - The origins of viruses
 - Prions
- Section III - Concepts in Microbial Diversity
 - Chapter 18. Molecular microbial ecology : Sequence-based approaches
 - The identification of uncultivated organisms
 - The pink filaments of Octopus Spring
 - PCR-based surveys of microbial populations
 - Bacterial and Archaeal populations in Obsidian Pool, or...
 - Surveying the normal human gut flora
 - Fluorescent in situ hybridization
 - Full-cycle analysis of Chloroflexi in wastewater treatment
 - Chapter 19. Molecular microbial ecology : Population-based approaches
 - Denaturing gradient gel electrophoresis
 - Filamentous communities in an alkaline hot spring?
 - Terminal RFLP
 - Survey of bacteria in periodontal disease, or...
 - Microbial community analysis of the large intestine of ... Manatees
 - Chapter 20. Linking phylotype and phenotype
 - The genomic approach
 - SAR11 and the discovery of proteorhodopsin
 - Stable isotope probing
 - The original rRNA-based SIP paper
 - Chapter 21. Genomics
 - How to sequence a microbial genome
 - The Thermotoga genome
 - Comparative genomics
 - Reductive evolution in Chlamydiae (?)
 - Metagenomics
 - The Sargasso Sea survey

Microbial Diversity textbook proposal

- Chapter 22. Bacterial cell biology
 - The cytoskeletons
(no paper identified)
 - Cell division
 - Caulobacter life-cycle
 - Chromosome structure
 - Mitosis in Caulobacter
- Chapter 23. Symbiosis
 - Microbe:microbe symbiosis
 - Nanoarchaeum
 - Chlorobium
 - Microbe:eukaryote symbiosis
 - Arthromitis (or Buchnera?)
 - another recent example?
- Chapter 24. Microbial multicellularity
 - Communication
 - Vibrio fisheri quorum sensing
 - The Myxococcus C and A signals
 - Bacterial multicellularity
 - Programmed cell death in Streptomyces
- Chapter 25. Conclusion
 - the phylogenetic perspective

Other resources that could be provided

The textbook would include a series of “Questions for thought” at the end of each chapter. Some of these questions would be answered in subsequent chapters, others the student might be encouraged to investigate themselves, but mostly these would be questions without specific hard answers, questions designed to help students think about what they’ve learned in the chapter, and integrate it into their experience.

Section I, in Molecular phylogenetic analysis, would largely be approached from the problem-solving perspective, and so students would benefit from problem sets focused on sequence alignment and tree construction. These problem sets could be used as either quizzes or homework by the instructor, or the answers could be given as an appendix at the end of the book. In addition, we could provide the framework for a student project in which the students would be given some 16S rRNA sequence (in the class I teach, the students generate these sequences in lab from their own isolates), which they would analyze phylogenetically using on-line public tools, and by aligning their sequence to a database and generating trees themselves.

In addition, a test question bank could be drawn from 12 years of my exams, provided to instructors electronically or in an instructors CD.

Emphasis and perspective

The perspective in this textbook would be phylogenetic and organismal, from the Carl Woese school. This is in sharp contrast to the competing *Ogunseit* textbook, with its biogeochemical perspective. It is also in contrast to an alternative physiological/metabolic perspective. These *will* be discussed in specific instances, e.g. photosynthesis, methanogenesis, sulfur metabolism, carbon fixation, in the relevant sections. However, most students should get more detailed descriptions of basic physiology and metabolism in a Microbial Physiology course, and so these will be discussed in this textbook primarily in terms of “the big picture”, and from the perspective of their variations. The focus in this textbook will remain evolutionary diversity, i.e. the phylogenetic perspective.

About the author

I attended Ball State University starting in 1976 as a biology major with chemistry and anthropology minors. A single lecture on microbial diversity in a general microbiology class sparked my interest in

microbiology, leading to undergraduate research examining *Beggiatoa* in a southern Indiana sulfur spring. After obtaining an M.S. degree in Microbiology in 1982 from Miami University, I joined the Molecular Cellular and Developmental Biology Program at The Ohio State University to work on the molecular biology of methanogenic Archaea with Prof. John Reeve, and received my Ph.D. in 1988. I then spent 5 years at Indiana University as a postdoc in Prof. Norm Pace's lab on the comparative analysis of the structure of a bacterial ribozyme, RNase P. In 1994, I joined the Department of Microbiology at North Carolina State University as an Assistant Professor, where my research focuses on RNase P in Archaea and RNA informatics. I am currently an Associate Professor in the Department of Microbiology, an associate faculty member of the Department of Biochemistry, a member of the Genomics Graduate Program, the Biotechnology Graduate Training Program, and the CBI/RNA Biology Program. In 2005, I was awarded both the NCSU Outstanding Teacher Award and the Alumni Outstanding Teacher Award.

Footnote - an alternative organization of the material

The most straightforward approach to organizing the information in sections II and III of the proposed textbook would be to start with the survey of phylogenetic groups and follow this with the concept chapters, as described. This is the approach I used in my course until fairly recently. However, an alternative, which I have used with great success in recent years in my course, is to intertwine these two sections. This breaks up the lectures so that the attention of the students can be retained, and allows the descriptive information to be linked directly to the conceptual perspectives. This approach could be provided either explicitly, by rearranging the material in the chapters as described below, or with a suggested teaching outline that would provide an instructor a roadmap for this approach.

In this alternative, each chapter might begin with the discussion of a particular phylogenetic group of organisms, with some discussion of general topics raised these organisms, leading into one of the concept elements, exemplified by a review of a scientific paper using that concept experimentally that highlights organisms in the group being discussed. For example: a chapter might start out discussing the *Chlamydiae*, describing the members of the group, their phenotype, pathogenicity, and life-cycle, followed by a discussion of reductive evolution in parasites. It would then shift gears to an introduction to genomics, how genomes are sequenced and what you can learn from a genome, exemplified by a summary of the *Protochlamydia amoebophila* genome and what it teaches us about the origin of obligate pathogens. The order of topics, as would be taught in the course, would be defined by the conceptual thread, building in complexity.

Alternative Draft Table of Contents

Section I - Molecular phylogenetics - see above

Section II. Organisms and Concepts

Chapter 7.

Organisms. Overview of the Tree of Life
rooting the universal tree
horizontal transfer

Concepts. The molecular phylogenetic approach to microbiology

Chapter 8.

Organisms. Aquifex and relatives

Concepts. Detection and identification of uncultivable organisms
how to read a scientific paper
the hot spring of Yellowstone

Example. The pink filaments of Octopus Spring

Chapter 9.

Organisms. Bacterioids & green sulfur Bacteria

Concepts. PCR-based rRNA surveys of microbial populations
PCR chimeras

Example. Direct survey of the human gut microflora

Chapter 10.

Organisms. Phylogenetic groups with few or no cultivated members

Acidobacteria
Verrucomicrobia

- OP11, etc
Concept. PCR-based rRNA surveys of microbial populations
Example. Compilation of molecular survey data
- Chapter 11.
Organisms. Green non-sulfur Bacteria
Concept. Fluorescent in situ hybridization - phylogenetic probes
Example. Full-cycle analysis of wastewater sludge bulking
- Chapter 12.
Organisms. *Thermus* & *Deinococcus*
Concept. DGGE/TGGE analysis of populations
Example. Filamentous communities in an alkaline hot spring
- Chapter 13.
Organisms. *Spiriochaetes*
bacterial motility
Concept. tRFLP analysis of populations
Example. tRFLP analysis of periodontal disease
- Chapter 14.
Organisms. Gamma proteobacteria
overview of proteobacteria
Concept, Linking genotype and phenotype - the genomic approach
Example. SAR86 and proteorhodopsin
- Chapter 15.
Organisms. Beta proteobacteria
review of electron transport
the concept of proteobacteria
Concept. Linking genotype and phenotype - Stable isotope probing
Example. Original rRNA-based SIP paper
- Chapter 16.
Organisms. *Chlamydia*
reductive evolution in parasites
how to sequence a genome
Concepts. Genome sequencing and comparative genomics
Example. the *Parachlamydia* UWE25 genome
- Chapter 17.
Organisms. *Thermotoga* & relatives
Concepts. Life at high temperatures
Example. the *Thermotoga* genome seurence
horizontal transfer
- Chapter 18.
Organisms. Cyanobacteria
review of bacterial photosynthesis
Concept. metagenomics
Example. The Sargasso Sea metagenome
- Chapter 19.
Organisms. Firmicutes
review of Gram-negative and -positive cell envelops
sigma factor cascades and microbial development
Concept. Microbe:eukaryote symbiosis
Example. Arthromitis = *Bacillus cereus*
- Chapter 20.
Organisms. Crenarchaea
overview of the Archaea
sulfur metabolism
Archaea as reflections of early life on Earth
Concept. Microbe:microbe symbiosis
Example. *Nanoarchaeum equitans*
- Chapter 21.
Organisms. Euryarchaea

Microbial Diversity textbook proposal

- methanogenesis
- Concept. the non-universal code
- Example. selenocysteine and pyrrolysine
- Chapter 22.
 - Organisms. Planctomycetes
 - Concept. Complex cell structure in Bacteria
 - Example. Does Gemmata have a nucleus?
- Chapter 23.
 - Organisms. Alpha proteobacteria
 - Concept. Complex cell biology in Bacteria
 - Example. Mitosis in *Caulobacter*
- Chapter 24.
 - Organisms. Actinobacteria
 - secondary metabolism
 - Concept. Bacterial multicellularity
 - Example. Programmed cell death in *Streptomyces*
- Chapter 25.
 - Organisms. Delta & Epsilon proteobacteria
 - Concept. Bacterial communication
 - Example. Signaling in *Myxococcus*
- Chapter 26.
 - Organisms. Microbial eukaryotes
 - Concept. The origin of eukaryotic complexity
 - Example. Gene expression in Trypanosomes
- Chapter 27.
 - Organisms. Viruses
 - the origins of viruses
 - Concept. Prions
- Section III - Postlude
 - Chapter 28. The phylogenetic perspective