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-
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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

   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
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 in 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 Ogunsieitan 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 the 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. Bacteriods & 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. Spriochaetes
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 sequence
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 envelopes
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
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