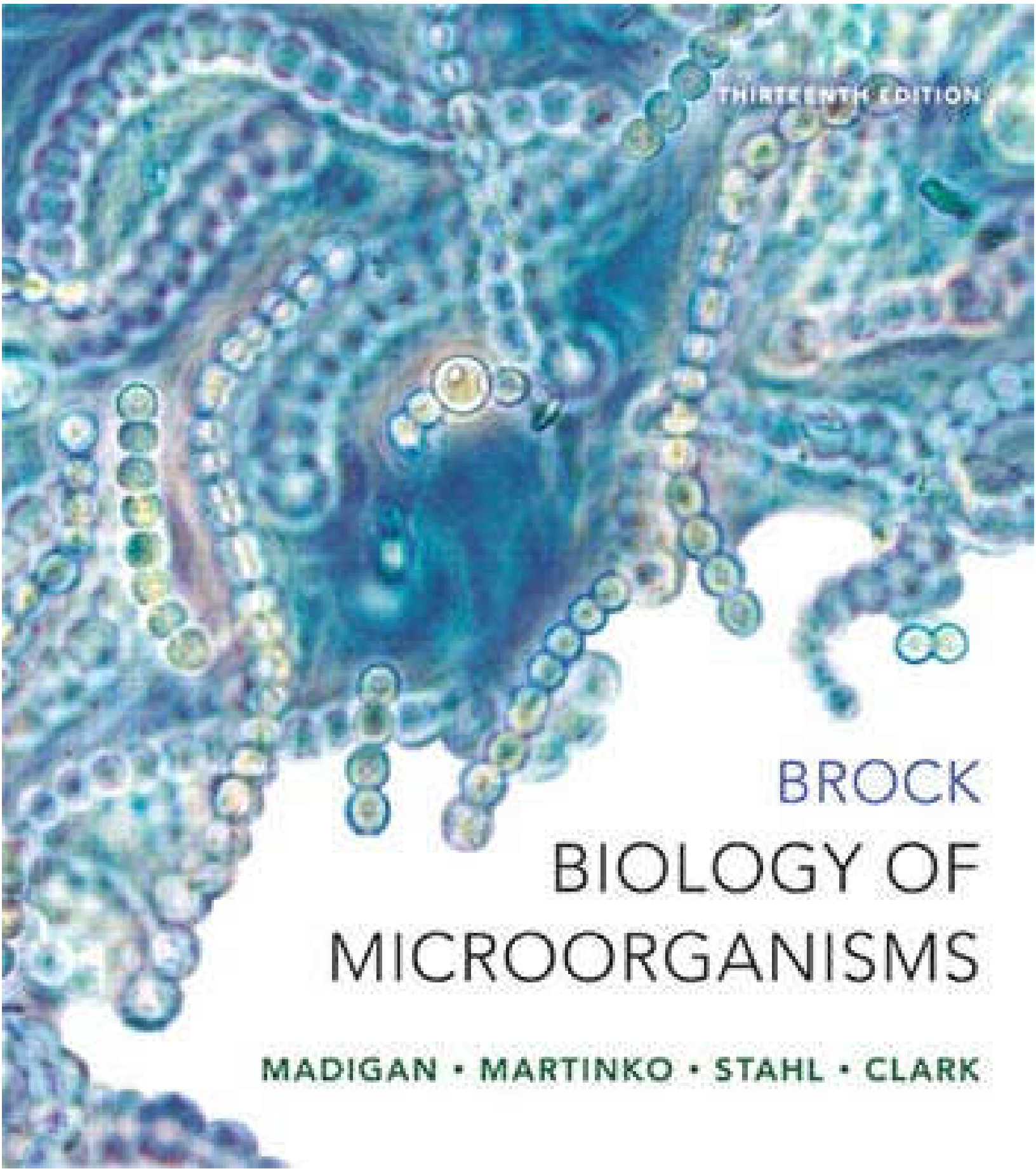
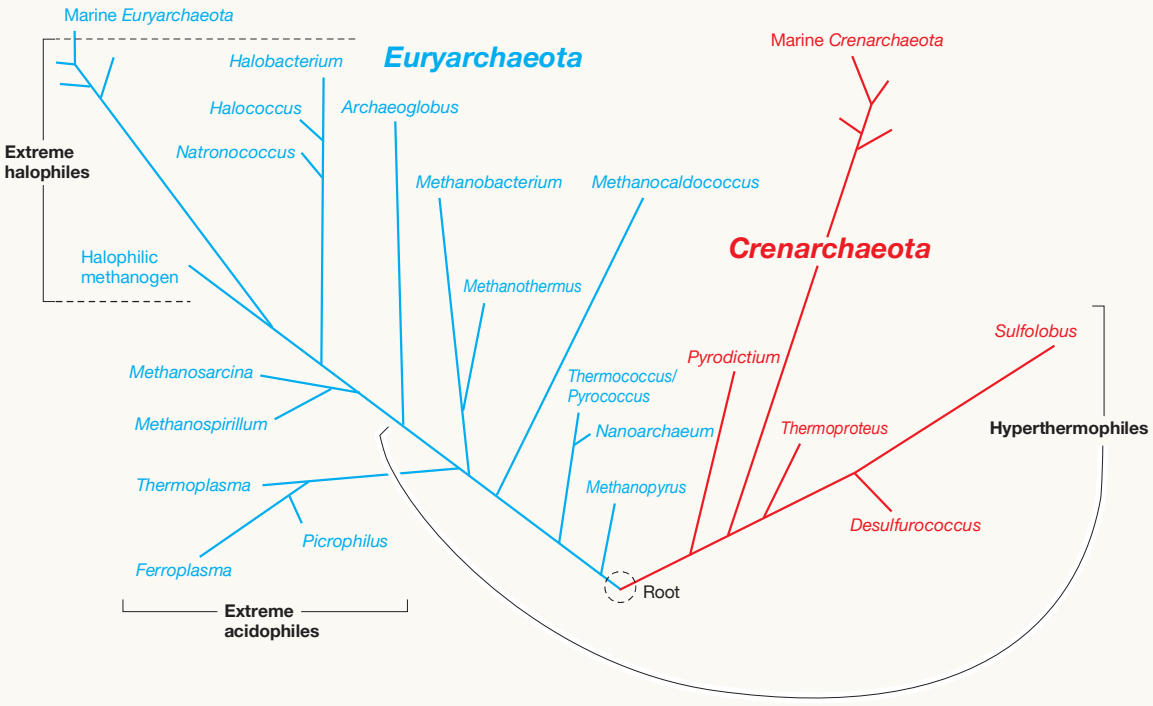


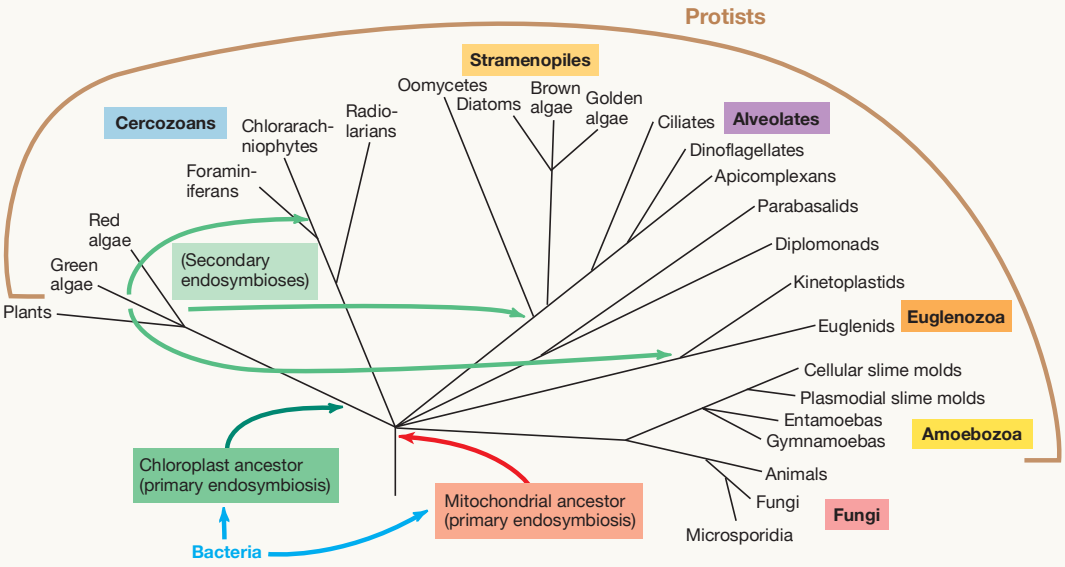
BROCK BIOLOGY OF MICROORGANISMS

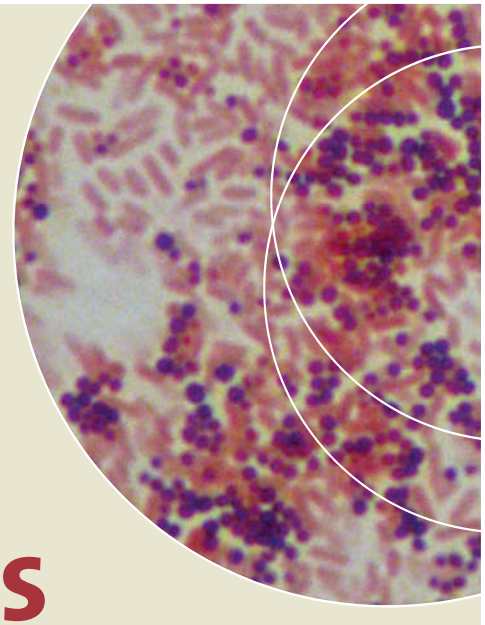
MADIGAN • MARTINKO • STAHL • CLARK

The Phylogeny of *Archaea*



The Phylogeny of *Eukarya*





Brock

Biology of Microorganisms

Thirteenth Edition

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Southern Illinois University Carbondale

John M. Martinko
Southern Illinois University Carbondale

David A. Stahl
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Benjamin Cummings

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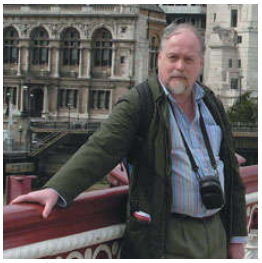
About the Authors

Michael T. Madigan received his B.S. in Biology and Education from Wisconsin State University–Stevens Point (1971) and his M.S. (1974) and Ph.D. (1976) in Bacteriology from the University of Wisconsin–Madison. His graduate research was on the hot spring bacterium *Chloroflexus* in the laboratory of Thomas Brock. Following a three-year postdoctoral in the Department of Microbiology, Indiana University, Mike moved to Southern Illinois University–Carbondale, where he has been a professor of microbiology for 32 years. He has coauthored *Biology of Microorganisms* since the fourth edition (1984) and teaches courses in introductory microbiology, bacterial diversity, and diagnostic and applied microbiology. In 1988 Mike was selected as the Outstanding Teacher in the College of Science and in 1993, the Outstanding Researcher. In 2001 he received the SIUC Outstanding Scholar Award. In 2003 he received the Carski Award for Distinguished Undergraduate Teaching from the American Society for Microbiology (ASM), and he is an elected Fellow of the American Academy of Microbiology. Mike's research is focused on bacteria that inhabit extreme environments, and for the past 12 years he has studied the microbiology of permanently ice-covered lakes in the McMurdo Dry Valleys, Antarctica. In addition to his research papers, he has edited a major treatise on phototrophic bacteria and served for over a decade as chief editor of the journal *Archives of Microbiology*. He currently serves on the editorial board of the journals *Environmental Microbiology* and *Antonie van Leeuwenhoek*. Mike's nonscientific interests include forestry, reading, and caring for his dogs and horses. He lives beside a peaceful and quiet lake with his wife, Nancy, five shelter dogs (Gaino, Snuffy, Pepto, Peanut, and Merry), and four horses (Springer, Feivel, Gwen, and Festus).

John M. Martinko received his B.S. in Biology from Cleveland State University. He then worked at Case Western Reserve University, conducting research on the serology and epidemiology of *Streptococcus pyogenes*. His doctoral work at the State University of New York–Buffalo investigated antibody specificity and antibody idiotypes. As a postdoctoral fellow, he worked at Albert Einstein College of Medicine in New York on the structure of major histocompatibility complex proteins. Since 1981, he has been in the Department of Microbiology at Southern Illinois University–Carbondale where he was Associate Professor and Chair, and Director of the Molecular Biology, Microbiology, and Biochemistry Graduate Program. He retired in 2009, but remains active in the department as a researcher and teacher. His research investigates structural changes in major histocompatibility proteins. He teaches an advanced course in immunology and presents immunology and host defense lectures to medical students. He also chairs the Institutional Animal Care and Use Committee at SIUC. He has been active in educational outreach programs for pre-university students and teachers. For his educational efforts, he won the 2007 SIUC Outstanding Teaching Award. He is also an avid golfer and cyclist. John lives in Carbondale with his wife Judy, a high school science teacher.

David A. Stahl received his B.S. degree in Microbiology from the University of Washington–Seattle, later completing graduate studies in microbial phylogeny and evolution with Carl Woese in the Department of Microbiology at the University of Illinois–Champaign-Urbana. Subsequent work as a postdoctoral fellow with Norman Pace, then at the National Jewish Hospital in Colorado, focused on early applications of 16S rRNA-based sequence analysis to the study of natural microbial communities. In 1984 Dave joined the faculty at the University of Illinois–Champaign-Urbana, holding appointments in Veterinary Medicine, Microbiology, and Civil Engineering. In 1994 he moved to the Department of Civil Engineering at Northwestern University, and in 2000 returned to his alma mater, the University of Washington–Seattle, as a professor in the Departments of Civil and Environmental Engineering and Microbiology. Dave is known for his work in microbial evolution, ecology, and systematics, and received the 1999 Bergey Award and the 2006 Procter & Gamble Award in Applied and Environmental Microbiology from the ASM; he is also an elected Fellow of the American Academy of Microbiology. His main research interests are the biogeochemistry of nitrogen and sulfur compounds and the microbial communities that sustain these nutrient cycles. His laboratory was first to culture ammonia-oxidizing *Archaea*, a group now believed to be the main mediators of this key process in the nitrogen cycle. He has taught several courses in environmental microbiology, is one of the co-founding editors of the journal *Environmental Microbiology,* and has served on many advisory committees. Outside teaching and the lab, Dave enjoys hiking, bicycling, spending time with family, reading a good science fiction book, and, with his wife Lin, renovating an old farmhouse on Bainbridge Island, Washington.



David P. Clark grew up in Croydon, a London suburb. He won a scholarship to Christ's College, Cambridge, where he received his B.A. degree in Natural Sciences in 1973. In 1977 he received his Ph.D. from Bristol University, Department of Bacteriology, for work on the effect of cell envelope composition on the entry of antibiotics into *Escherichia coli*. He then left England on a postdoctoral studying the genetics of lipid metabolism in the laboratory of John Cronan at Yale University. A year later he moved with the same laboratory to the University of Illinois at Urbana-Champaign. David joined the Department of Microbiology at Southern Illinois University–Carbondale in 1981. His research has focused on the growth of bacteria by fermentation under anaerobic conditions. He has published numerous research papers and graduated over 20 Masters and Doctoral students. In 1989 he won the SIUC College of Science Outstanding Researcher Award. In 1991 he was the Royal Society Guest Research Fellow at the Department of Molecular Biology and Biotechnology, Sheffield University, England. In addition to *Brock Biology of Microorganisms*, David is the author of four other science books: *Molecular Biology Made Simple and Fun,* now in its fourth edition; *Molecular Biology: Understanding the Genetic Revolution*; *Biotechnology: Applying the Genetic Revolution*; and *Germs, Genes, & Civilization: How Epidemics Shaped Who We Are Today.* David is unmarried and lives with two cats, Little George, who is orange and very nosey, and Mr. Ralph, who is mostly black and eats cardboard.

Dedications

Michael T. Madigan dedicates this book to the memory of his children who rest on Boot Hill: Andy, Marcy, Willie, Plum, Teal, and Sugar. Whether in good times or bad, they always greeted him with tails a waggin'.

John M. Martinko dedicates this book to his daughters Sarah, Helen, and Martha, and to his wife Judy. Thanks for all of your support!

David A. Stahl dedicates this book to his wife, Lin. My love, and one that helps me keep the important things in perspective.

David P. Clark dedicates this book to his father, Leslie, who set him the example of reading as many books as possible.

Preface

The authors and Benjamin Cummings Publishers proudly present the 13th edition of *Brock Biology of Microorganisms* (*BBOM* 13/e). This book is truly a milestone in the annals of microbiology textbooks. *Brock Biology of Microorganisms*, and its predecessor, *Biology of Microorganisms*, has introduced the field of microbiology to students for 41 years, more than any other textbook of microbiology. Nevertheless, although this book goes back over four decades, its two main objectives have remained firm since the first edition was published in 1970: (1) to present the principles of microbiology in a clear and engaging fashion, and (2) to provide the classroom tools necessary for delivering outstanding microbiology courses. The 13th edition of *BBOM* fulfills these objectives in new and exciting ways.

Veteran textbook authors Madigan, Martinko, and Clark welcome our new coauthor, Dave Stahl, to this edition of *BBOM*. Dave is one of the world's foremost experts in microbial ecology and has masterfully crafted an exciting new view of the ecology material in *BBOM*, including a new chapter devoted entirely to microbial symbioses, a first for any textbook of microbiology. Users will find that the themes of ecology and evolution that have permeated this book since its inception reach new heights in the 13th edition. These fundamental themes also underlie the remaining content of the book—the basic principles of microbiology, the molecular biology and genetics that support microbiology today, the huge diversity of metabolisms and organisms, and the medical and immunological facets of microbiology. It is our belief that outstanding content coupled with outstanding presentation have come together to make *BBOM* 13/e the most comprehensive and effective textbook of microbiology available today.

What's New in the 13th Edition?

In terms of content and pedagogy, instructors who have used *BBOM* previously will find the 13th edition to be the same old friend they remember; that is, a book loaded with accurate, up-to-the-minute content that is impeccably organized and visually enticing. The 36 chapters in *BBOM* 13/e are organized into modules by numbered head, which allows instructors to fine-tune course content to the needs of their students. In addition, study aids and review tools are an integral part of the text. Our new MiniQuiz feature, which debuts in the 13th edition, is designed to quiz students' comprehension as they work their way through each chapter. Also new to this edition is the end-of-chapter review tool called "Big Ideas." These capsule summaries pull together the key concepts from each numbered section in a wrap-up style that is certain to be a big hit with students, especially the night before examinations! Our end-of-chapter key terms list, two detailed appendices, a comprehensive glossary, and a thorough index complete the hard copy learning package. Many additional learning resources are available online (see below).

In terms of presentation, *BBOM* 13/e will easily draw in and engage the reader. The book has been designed in a beautiful yet simple fashion that gives the art and pedagogical elements the breathing room they need to be effective and the authors the freedom to present concepts in a more visually appealing way. Supporting the narrative are spectacular illustrations, with every piece of art rendered in a refreshing new style. Moreover, the art complements, and in many cases integrates, the hundreds of photos in *BBOM*, many of which are new to the 13th edition. And, as users of *BBOM* have come to expect, our distinctive illustrations remain the most accurate and consistent of those in any microbiology textbook today.

The authors are keenly aware that it is easy to keep piling on new material and fattening up a textbook. In response to this trend, *BBOM* 13/e went on a diet. With careful attention to content and presentation, *BBOM* 13/e is actually a shorter book than *BBOM* 12/e. The authors have carefully considered every topic to ensure that content at any point in the book is a reflection of both what the student already knows and what the student needs to know in a world where microbiology has become the most exciting and relevant of the biological sciences. The result is a more streamlined and exciting treatment of microbiology that both students and instructors will appreciate.

Revision Highlights:

Chapter 1

- Find new coverage on the evolution and major habitats of microorganisms—Earth's most pervasive and extensive biomass.
- A more visually compelling presentation of the impacts of microorganisms on humans better emphasizes the importance of microorganisms for the maintenance of all life on Earth.

Chapter 2

- New coverage of cell biology and the nature of the chromosome in prokaryotic and eukaryotic cells is complemented by a visually engaging overview of the microbial world.

Chapter 3

- The cell chemistry chapter that previously held this position is now available online (www.microbiologyplace.com). The new Chapter 3 explores cell structure and function with strong new visuals to carry the text and new coverage of the lipids and cell walls of *Bacteria* and *Archaea*.

Chapter 4

- Find updated coverage of catabolic principles along with an overview of essential anabolic reactions.
- Newly rendered and more instructive art makes mastering key metabolic pathways and bioenergetic principles a more visual experience.

Chapter 5

- Updated coverage of the events in cell division and their relation to medical microbiology connects basic science to applications.
- Newly rendered art throughout makes the important concepts of cell division and population growth more vivid, engaging, and interactive.

Chapter 6

- The concise primer on molecular biology that every student needs to know is updated and now includes an overview of the structures of nucleic acids and proteins and the nature of chromosomes and plasmids.

Chapter 7

- Find new coverage of the latest discoveries in the molecular biology of *Archaea* and comparisons with related molecular processes in *Bacteria*.
- A new section highlights the emerging area of regulation by microRNA in eukaryotes.

Chapter 8

- Review major updates on the regulation of gene expression—one of the hottest areas in microbiology today—including expanded coverage of cell sensing capacities and signal transduction.
- Enjoy the new Microbial Sidebar featuring CRISPR, the newly discovered form of RNA-based regulation used by *Bacteria* and *Archaea* to ward off viral attack.

Chapter 9

- Major updates of the principles of virology are complemented with an overview of viral diversity.
- New art reinforces the relevance and importance of viruses as agents of genetic exchange.

Chapter 10

- The fundamental principles of microbial genetics are updated and supplemented with new coverage that compares and contrasts bacterial and archaeal genetics.

Chapter 11

- Find "one-stop shopping" for coverage of molecular biological methods, including cloning and genetic manipulations, as a prelude to the genomics discussion in the next chapter.
- Enjoy the colorful new Microbial Sidebar on new fluorescent labeling methods that can differentiate even very closely related bacteria.

Chapter 12

- Extensive updates on microbial genomics and transcriptomics will be found along with new coverage of the emerging related areas of metabolomics and interactomics.
- Readers will marvel at the diversity of prokaryotic genomes in the new Microbial Sidebar "Record-Holding Bacterial Genomes."

Chapter 13

- The two chapters covering metabolic diversity have been revised and moved up to Chapters 13 and 14 to precede rather than follow coverage of microbial diversity, better linking these two important and often related areas.
- This chapter is loaded with reworked art and text that highlight the unity and diversity of the bioenergetics underlying phototrophic and chemolithotrophic metabolisms.

Chapter 14

- Restyled and impeccably consistent art showcases the comparative biochemistry of the aerobic and anaerobic catabolism of carbon compounds.

Chapter 15

- This retooled chapter combines the essentials of industrial microbiology and biotechnology, including the production of biofuels and emerging green microbial technologies.

Chapter 16

- Find new coverage of the origin of life and how the evolutionary process works in microorganisms.
- Microbial phylogenies from small subunit ribosomal RNA gene analyses are compared with those from multiple-gene and full genomic analyses.

Chapters 17–19

- Coverage of the diversity of *Bacteria* and *Archaea* better emphasizes phylogeny with increased focus on phyla of particular importance to plants and animals and to the health of our planet.
- Spectacular photomicrographs and electron micrographs carry the reader through prokaryotic diversity.

Chapter 20

- A heavily revised treatment of the diversity of microbial eukaryotes is supported by many stunning new color photos and photomicrographs.
- Find an increased emphasis on the phylogenetic relationships of eukaryotes and the "bacterial nature" of eukaryotic organelles.

Chapter 21

- Viruses, the most genetically diverse of all microorganisms, come into sharper focus with major updates on their diversity.
- A new section describes viruses in nature and their abundance in aquatic habitats.

Chapter 22

- This chapter features a major new treatment of the latest molecular techniques used in microbial ecology, including CARD-FISH, ARISA, biosensors, NanoSIMS, flow cytometry, and multiple displacement DNA amplification.
- Find exciting new coverage of methods for functional analyses of single cells, including single-cell genomics and single-cell stable isotope analysis, and expanded coverage of methods for analyses of microbial communities, including metagenomics, metatranscriptomics, and metaproteomics.

Chapter 23

- A comparison of the major habitats of *Bacteria* and *Archaea* is supported by spectacular new photos and by art that summarizes the phylogenetic diversity and functional significance of prokaryotes in each habitat.
- Find broad new coverage of the microbial ecology of microbial mat communities and prokaryotes that inhabit the deep subsurface.

Chapter 24

- Revised coverage of the classical nutrient cycles is bolstered by new art, while new coverage highlights the calcium and silica cycles and how these affect CO_2 sequestration and global climate.
- Improved integration of biodegradation and bioremediation shows how natural microbial processes can be exploited for the benefit of humankind.

Chapter 25

- This new chapter focuses entirely on microbial symbioses, including bacterial–bacterial symbioses and symbioses between bacteria and their plant, mammal, or invertebrate hosts. Find coverage here of all of the established as well as more recently discovered symbioses, including the human gut and how its microbiome may control obesity, the rumen of animals important to agriculture, the hindgut of termites, the light organ of the squid, the symbioses between hydrothermal vent animals and chemolithotrophic bacteria, the essential bacterial symbioses of insects, medicinal leeches, reef-building corals, and more, all supported by spectacular new color photos and art.
- Learn how insects have shaped the genomes of their bacterial endosymbionts.
- Marvel at the new Microbial Sidebar that tells the intriguing story of the attine ants and their fungal gardens.

Chapter 26

- Key updates will be found on microbial drug resistance and are supported by new art that reveals the frightening reality that several human pathogens are resistant to all known antimicrobial drugs.

Chapter 27

- Extensively reworked sections on the normal microbial flora of humans include new coverage of the human microbiome and a molecular snapshot of the skin microflora.
- Find revised coverage of the principles of virulence and pathogenicity that connect infection and disease.

Chapter 28

- Here we present the perfect overview of immunology for instructors who wish to cover only the fundamental concepts and how the immune system resists the onslaught of infectious disease.
- Find late-breaking practical information on the immune response, including vaccines and immune allergies.

Chapter 29

- Built on the shoulders of the previous chapter, here is a more detailed probe of the mechanisms of immunity with emphasis on the molecular and cellular interactions that control innate and adaptive immunity.

Chapter 30

- This short chapter presents an exclusively molecular picture of immunology, including receptor–ligand interactions (the "triggers" of the immune response), along with genetics of the key proteins that drive adaptive immunity.

Chapter 31

- Find revised and expanded coverage of molecular analyses in clinical microbiology, including new enzyme immunoassays, reverse transcriptase PCR, and real-time PCR.

Chapter 32

- Review major updates of the principles of disease tracking, using 2009 pandemic H1N1 influenza as a model for how newly emerging infectious diseases are tracked.
- Find updated coverage throughout, especially of the HIV/AIDS pandemic.

Chapter 33

- Read all about the origins and history of pandemic H1N1 influenza and how the H1N1 virus is related to strains of influenza that already existed in animal populations.
- Hot new coverage of immunization strategies for HIV/AIDS.

Chapter 34

- Follow the emergence, rapid dispersal, and eventual entrenchment of West Nile virus as an endemic disease in North America.
- Expanded coverage of malaria—the deadliest human disease of all time—includes the promise of new antiparasitic drugs and disease prevention methods.

Chapter 35

- Find updates of water microbiology, including new rapid methods for detecting specific indicator organisms.

Chapter 36

- Explore new methods of food processing, including aseptic and high-pressure methods that can dramatically extend the shelf-life and safety of perishable foods and drinks.

Cutting Edge Coverage Includes the Most Current Presentation of Microbial Ecology

The 13th edition enhances the themes of ecology and evolution throughout, and is the only book on the market to include specialized coverage of archael and eukaryotic molecular biology. The book represents the most current research in the field, with special attention paid to the microbial ecology chapters:

Chapter 22, Methods in Microbial Ecology, is heavily updated to present the latest molecular techniques used in microbial ecology, including CARD-FISH, ARISA, biosensors, NanoSIMS, flow cytometry, and multiple displacement DNA amplification. It also includes exciting new coverage of methods for functional analyses of single cells, including single-cell genomics and single-cell stable isotope analysis, and expanded coverage of methods for analyses of microbial communities, including metagenomics, metatranscriptomics, and metaproteomics.

Chapter 23, Major Microbial Habitats and Diversity, compares the major habitats of *Bacteria* and *Archaea* and is supported by spectacular new photos and art that summarize the phylogenetic diversity and functional significance of prokaryotes in each habitat.

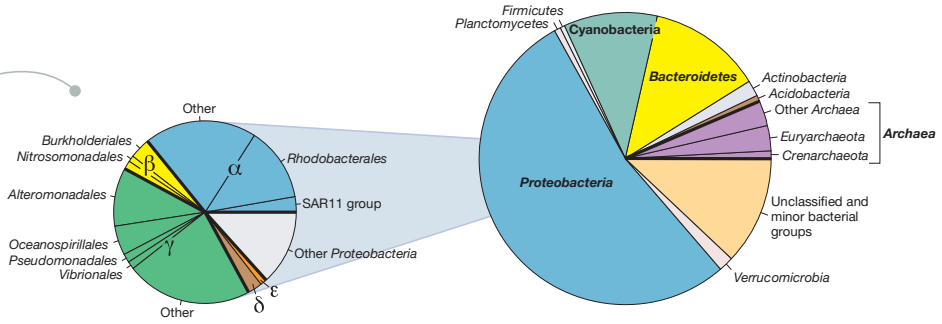


Figure 23.24 **Ocean prokaryotic diversity.** The results are pooled analyses of 25,975 sequences from several studies of the 16S rRNA gene content of pelagic ocean waters. Many of these groups are covered in Chapters 17 and 18 (*Bacteria*) or 19 (*Archaea*). For *Proteobacteria*, major subgroups are indicated. Note the high proportion of cyanobacterial and *Gammaproteobacteria* sequences. Data assembled and analyzed by Nicolas Pinel.

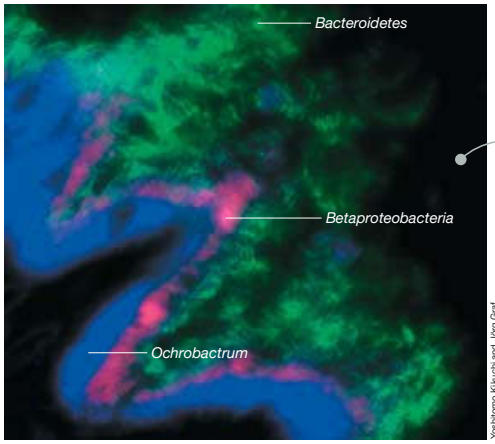


Figure 25.40 **Micrograph of a FISH-stained microbial community in the bladder of *Hirudo verbana*.** A probe (red) targeted at the 16S rRNA of *Betaproteobacteria* and a probe (green) targeted at the 16S rRNA of *Bacteroidetes* reveal distinct layers of different bacteria in the lumen of the bladder. Staining with DAPI (blue), which binds to DNA, reveals the intracellular alphaproteobacterium *Ochrobactrum* and host nuclei.

Chapter 24, Nutrient Cycles, Biodegradation, and Bioremediation. Exciting updates of all the nutrient cycles that form the heart of environmental microbiology and microbial ecology.

Chapter 25, Microbial Symbioses, is a completely new chapter focused entirely on microbial symbioses, including bacterial–bacterial symbioses and symbioses between bacteria and their plant, mammal, or invertebrate hosts. Find coverage here of all the established as well as more recently discovered symbioses—including the human gut and how its microbiome may control obesity, the rumen of animals important to agriculture, the hindgut of termites, the light organ of the squid, the symbioses between hydrothermal vent animals and chemolithotrophic bacteria, and the essential bacterial symbioses of insects, medicinal leeches, reef-building corals, and more.

For a detailed list of chapter-by-chapter updates, see page v of the Preface.

Thoroughly Updated and Revised Art

The art has been revised and updated throughout the book to give students a clear view into the microbial world. Color and style conventions are used consistently to make the art accessible and easy to understand.

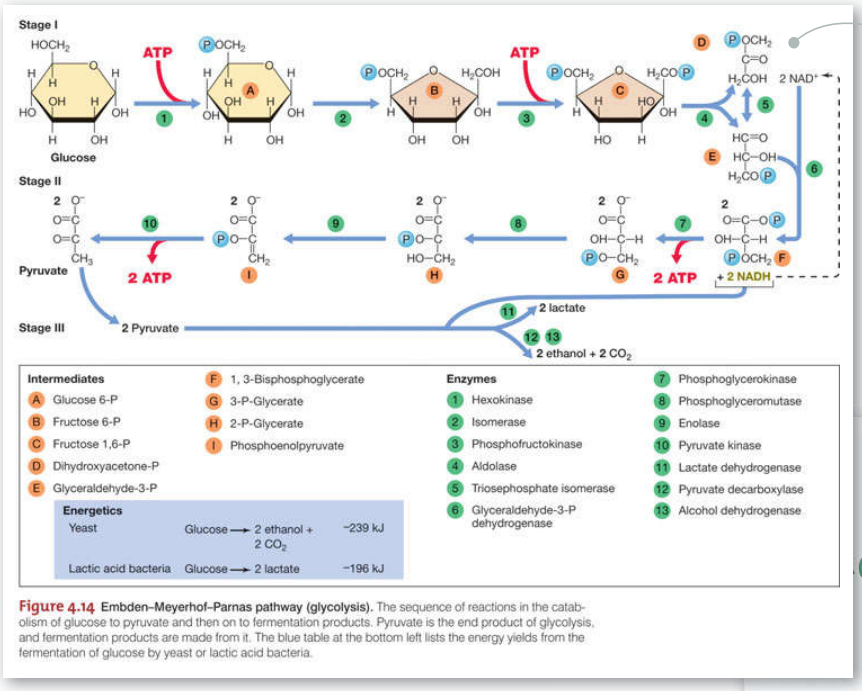


Figure 4.14 **Embden–Meyerhof–Parnas pathway (glycolysis).** The sequence of reactions in the catabolism of glucose to pyruvate and then on to fermentation products. Pyruvate is the end product of glycolysis, and fermentation products are made from it. The blue table at the bottom left lists the energy yields from the fermentation of glucose by yeast or lactic acid bacteria.

Carefully redesigned new art clearly guides students through challenging concepts. The style for metabolic figures and other pathway processes has been simplified, and color-coded steps and chemical structures increase student comprehension.

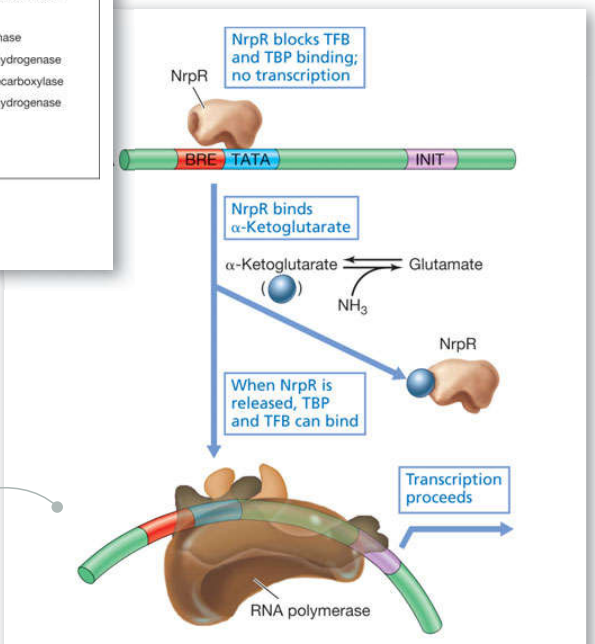


Figure 8.15 **Repression of genes for nitrogen metabolism in *Archaea*.** The NrpR protein of *Methanococcus maripaludis* acts as a repressor. It blocks the binding of the TFB and TBP proteins, which are required for promoter recognition, to the BRE site and TATA box, respectively. If there is a shortage of ammonia, α-ketoglutarate is not converted to glutamate. The α-ketoglutarate accumulates and binds to NrpR, releasing it from the DNA. Now TBP and TFB can bind. This in turn allows RNA polymerase to bind and transcribe the operon.

Dimensionality has been added to some figures, lending more realism and vivacity to the presentation. Figures in which nucleic acids or cells are depicted are now more dimensional to clearly identify key genes and cell structures.

Illustrations and photos are often paired to give an idealized view next to a realistic view and to reinforce the connection between theory and practice.

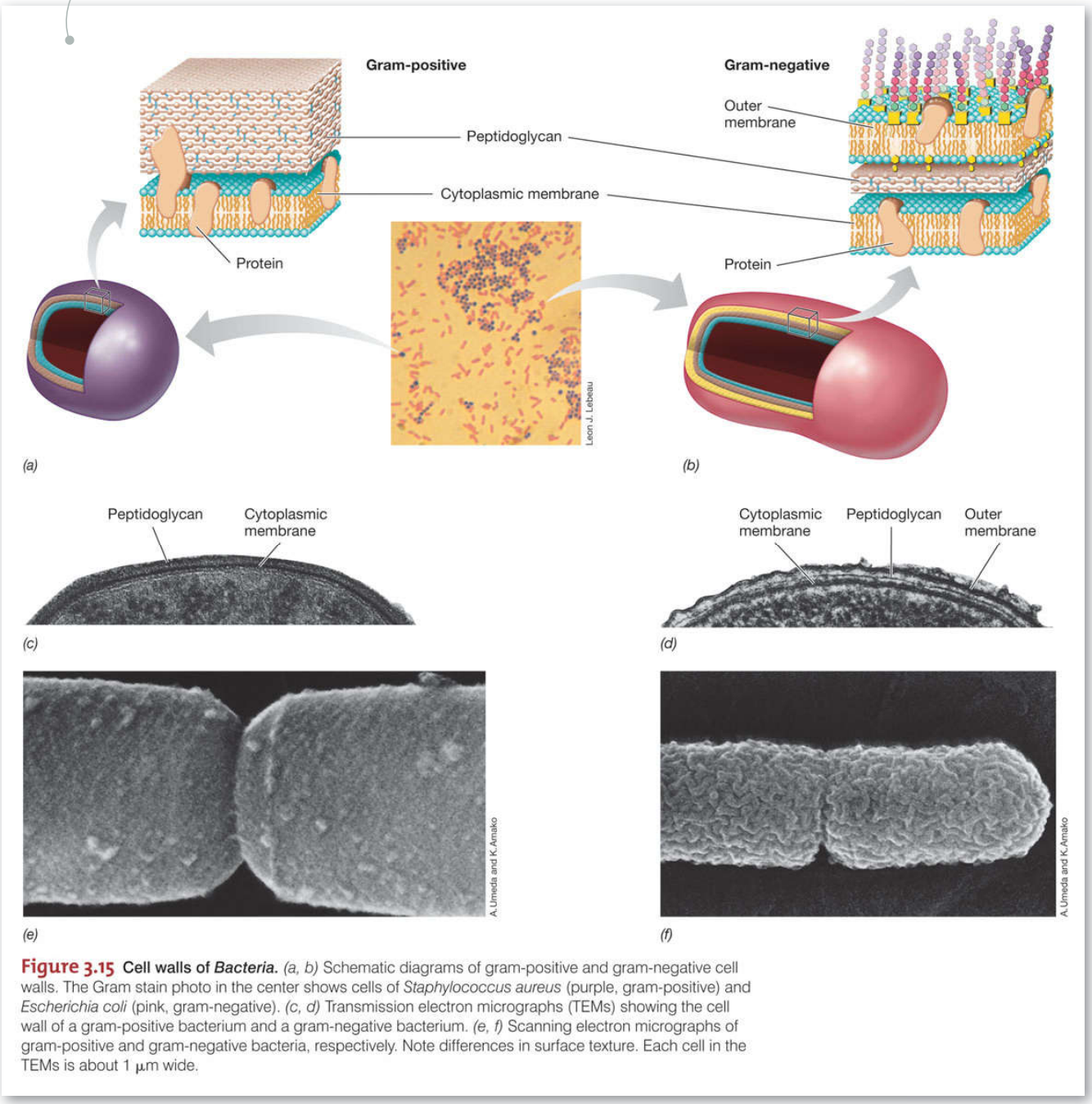


Figure 3.15 Cell walls of *Bacteria*. *(a, b)* Schematic diagrams of gram-positive and gram-negative cell walls. The Gram stain photo in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative). *(c, d)* Transmission electron micrographs (TEMs) showing the cell wall of a gram-positive bacterium and a gram-negative bacterium. *(e, f)* Scanning electron micrographs of gram-positive and gram-negative bacteria, respectively. Note differences in surface texture. Each cell in the TEMs is about 1 μm wide.

Conceptual Framework Helps Students Focus on the Key Concepts

The first twelve chapters cover the principles of microbiology. Basic principles are presented early on and then used as the foundation to tackle the material in greater detail later.

Brief Contents

New chapter on symbiosis ties together the core concepts of the book—health, diversity, and the human ecosystem.

This newly revised chapter is the perfect overview for instructors who wish to cover immunology at a generalized level including the fundamental concepts of how the immune system resists the onslaught of infectious disease. Instructors who like to go into more detail can build on the core principles taught in Chapter 28 by covering Immune Mechanisms (Ch. 29) and Molecular Immunology (Ch. 30).

Information on metabolic diversity precedes the coverage of microbial diversity, better linking these important and often related areas.

The new Big Ideas sections at the end of each chapter focus on the core concepts students need to know.

Big Ideas

2.1
Microscopes are essential for studying microorganisms. Bright-field microscopy, the most common form of microscopy, employs a microscope with a series of lenses to magnify and resolve the image.

2.2
An inherent limitation of bright-field microscopy is the lack of contrast between cells and their surroundings. This problem can be overcome by the use of stains or by alternative forms of light microscopy, such as phase contrast or dark field.

2.3
Differential interference contrast microscopy and confocal scanning laser microscopy allow enhanced three-dimensional imaging or imaging through thick specimens. The atomic force microscope gives a very detailed three-dimensional image of live preparations.

2.4
Electron microscopes have far greater resolving power than do light microscopes, the limits of resolution being about 0.2 nm. The two major forms of electron microscopy are transmission, used primarily to observe internal cell structure, and scanning, used to examine the surface of specimens.

2.7
Comparative rRNA gene sequencing has defined three domains of life: *Bacteria*, *Archaea*, and *Eukarya*. Molecular sequence comparisons have shown that the organelles of *Eukarya* were originally *Bacteria* and have spawned new tools for microbial ecology and clinical microbiology.

2.8
All cells need sources of carbon and energy for growth. Chemoorganotrophs, chemolithotrophs, and phototrophs use organic chemicals, inorganic chemicals, or light, respectively, as their source of energy. Autotrophs use CO_2 as their carbon source, while heterotrophs use organic compounds. Extremophiles thrive under environmental conditions of high pressure or salt, or extremes of temperature or pH.

2.9
Several phyla of *Bacteria* are known, and an enormous diversity of cell morphologies and physiologies are represented. *Proteobacteria* are the largest group of *Bacteria* and contain many well-known bacteria, including *Escherichia coli*. Other major phyla include gram-positive bacteria, cyanobacteria, spirochetes, and green bacteria.

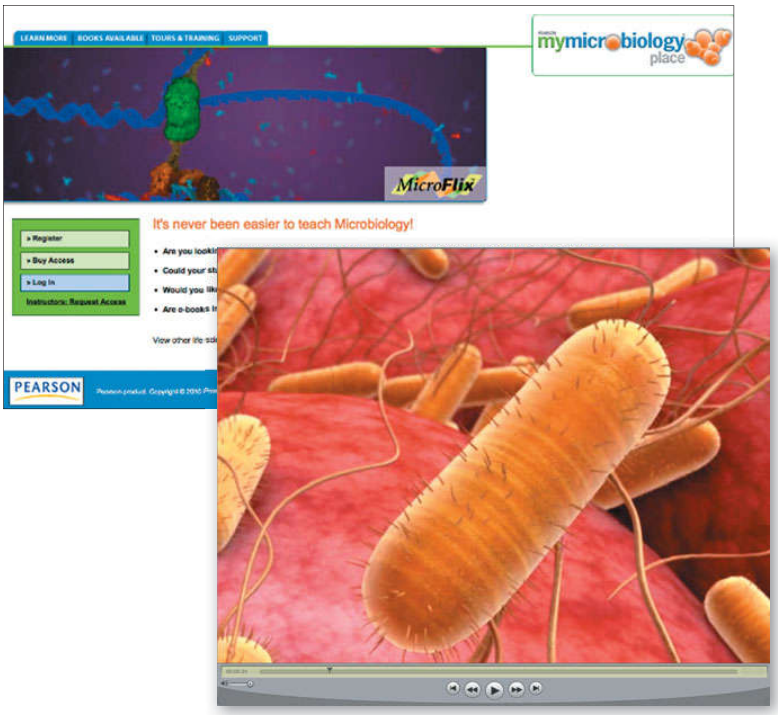
MiniQuiz critical thinking questions integrated throughout the text test student comprehension of core principles from each section.

MiniQuiz

- What are the primary response regulator and the primary sensor kinase for regulating chemotaxis?
- Why is adaptation during chemotaxis important?
- How does the response of the chemotaxis system to an attractant differ from its response to a repellent?

Additional Resources

FOR STUDENTS



The MyMicrobiologyPlace website is rich with media assets to give students extra practice. It includes chapter quizzes, new quantitative questions, animations, and additional tutorials. *www.microbiologyplace.com*

Quantitative Questions

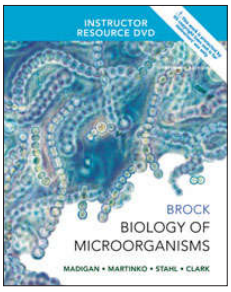
1. **Number of genes in plasmid R100.** The *Escherichia coli* plasmid R100 is a circular molecule of DNA containing 93.4 kbp. The average *E. coli* protein contains 300 amino acids; assume that the same is true for R100 proteins. With this assumption, calculate how many genes are in this plasmid.

2. **Compare DNA polymerases.** *Escherichia coli* contains at least five different DNA polymerases. The three most characterized are DNA Pol I, Pol II, and Pol III. Polymerase I and II replicate DNA at about 20–40 nucleotides/sec whereas Pol III replicates at 250 to 1000 nucleotides/sec. The genome of *E. coli* strain K-12 is 4,639,221 bp. At the higher rates, how long does it take to reproduce the chromosome? How do these numbers agree with the roles of these DNA polymerases?

FOR INSTRUCTORS

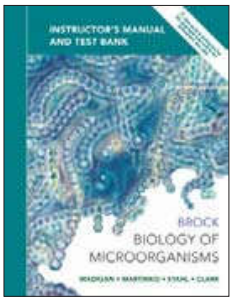
CourseCompass™

CourseCompass includes all of the assets from the MyMicrobiologyPlace website and all of the test questions from the computerized test bank. It also features class management tools, such as discussion boards and email functionality to help instructors easily teach online classes or give assignments. *www.aw-bc.com/coursecompass*



Instructor Resource DVD (IR-DVD)
0-321-72086-5 / 978-0-321-72086-3

The IR-DVD offers a wealth of media resources including all the art from the book in both JPEG and PPT formats, PowerPoint lecture outlines, computerized test bank, and answer keys all in one convenient location. The animations help bring lectures to life, while the select step-edit figures help break down complicated processes.



Instructor Manual and Test Bank
0-321-72021-0 / 978-0-321-72021-4

by W. Matthew Sattley and Christopher A. Gulvik

The Instructor Manual/Test Bank provides chapter summaries that help with class preparation as well as the answers to the end-of-chapter review and application questions. The test bank contains 3,000 questions for use in quizzes, tests, and exams.

Acknowledgments

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As hard as a publishing team may try, no textbook can ever be completely error free. Although we are confident the reader will be hard pressed to find errors in *BBOM* 13/e, any errors that do exist, either of commission or omission, are solely the responsibility of the authors. In past editions, users have been kind enough to contact us when they found an error. Users should feel free to continue to do so and to contact the authors directly about any errors, concerns, or questions they may have about the book. We will do our best to address them.

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Brief Contents

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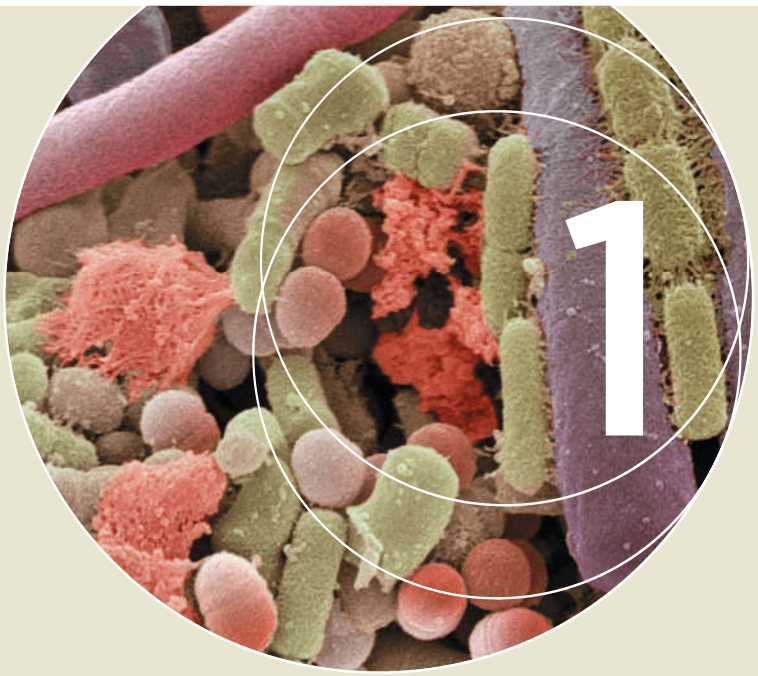


1

Microorganisms and Microbiology

Bacteria, such as these scraped from the surface of a human tongue, are independent microorganisms that live and interact with other microorganisms in microbial communities.

Microbiology is the study of microorganisms. **Microorganisms** are all single-celled microscopic organisms and include the viruses, which are microscopic but not cellular. Microbial cells differ in a fundamental way from the cells of plants and animals in that microorganisms are independent entities that carry out their life processes independently of other cells. By contrast, plant and animal cells are unable to live alone in nature and instead exist only as parts of multicellular structures, such as the organ systems of animals or the leaves of plants.

What is the science of microbiology all about? Microbiology is about microbial cells and how they work, especially the bacteria, a very large group of very small cells (**Figure 1.1**) that, collectively, have enormous basic and practical importance. Microbiology is about diversity and evolution of microbial cells, about how different kinds of microorganisms arose and why. It is also about what microorganisms do in the world at large, in soils and waters, in the human body, and in animals and plants. One way or another, microorganisms affect and support all other forms of life, and thus microbiology can be considered the most fundamental of the biological sciences.

This chapter begins our journey into the microbial world. Here we discover what microorganisms are and their impact on planet Earth. We set the stage for consideration of the structure and evolution of microorganisms that will unfold in the next chapter. We also place microbiology in historical perspective, as a process of scientific discovery. From the landmark contributions of both early microbiologists and scientists practicing today, we can see the effects that microorganisms have in medicine, agriculture, the environment, and other aspects of our daily lives.

I Introduction to Microbiology

In the first five sections of this chapter we introduce the field of microbiology, look at microorganisms as cells, examine where and how microorganisms live in nature, survey the evolutionary history of microbial life, and examine the impact that microorganisms have had and continue to have on human affairs.

1.1 The Science of Microbiology

The science of microbiology revolves around two interconnected themes: (1) understanding the living world of microscopic organisms, and (2) applying our understanding of microbial life processes for the benefit of humankind and planet Earth.

As a *basic* biological science, microbiology uses and develops tools for probing the fundamental processes of life. Scientists have obtained a rather sophisticated understanding of the chemical and physical basis of life from studies of microorganisms because microbial cells share many characteristics with cells of multicellular organisms; indeed, *all* cells have much in common. But unlike plants and animals, microbial cells can be grown to extremely high densities in small-scale laboratory cultures (Figure 1.1), making them readily amenable to rapid biochemical and genetic study. Collectively, these features make microorganisms excellent experimental systems for illuminating life processes common to multicellular organisms, including humans.

As an *applied* biological science, microbiology is at the center of many important aspects of human and veterinary medicine, agriculture, and industry. For example, although animal and plant infectious diseases are typically microbial, many microorganisms are absolutely essential to soil fertility and domestic animal welfare. Many large-scale industrial processes, such as the production of antibiotics and human proteins, rely heavily on microorganisms. Thus microorganisms affect the everyday lives of humans in both beneficial and detrimental ways.

Although microorganisms are the smallest forms of life, collectively they constitute the bulk of biomass on Earth and carry out many necessary chemical reactions for higher organisms. In the absence of microorganisms, higher life forms would never have evolved and could not now be sustained. Indeed, the very oxygen we breathe is the result of past microbial activity (as we will see in Figure 1.6). Moreover, humans, plants, and animals are intimately tied to microbial activities for the recycling of key nutrients and for degrading organic matter. It is safe to say that no

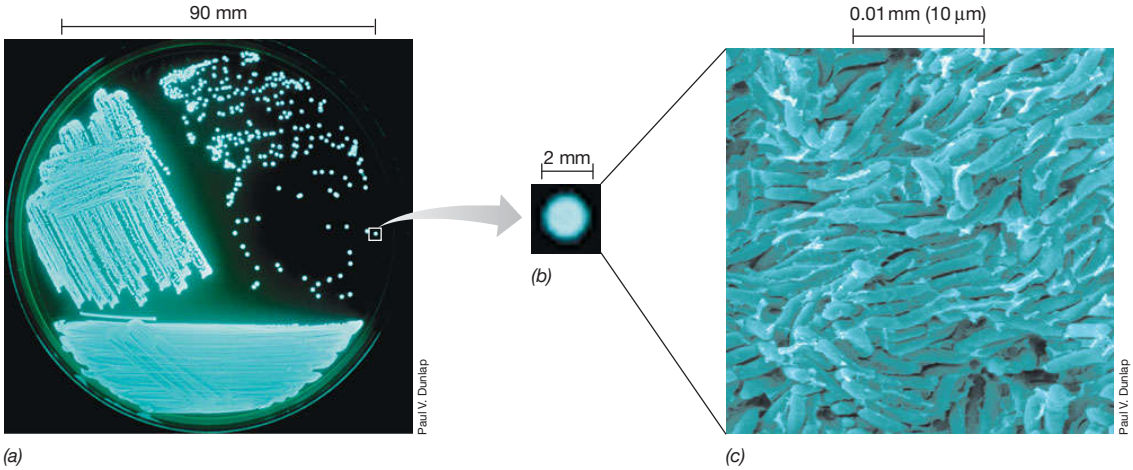


Figure 1.1 Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Scanning electron micrograph of cells of *Photobacterium*.

other life forms are as important as microorganisms for the support and maintenance of life on Earth.

Microorganisms existed on Earth for billions of years before plants and animals appeared, and we will see later that the genetic and physiological diversity of microbial life greatly exceeds that of the plants and animals. This huge diversity accounts for some of the spectacular properties of microorganisms. For example, we will see how microorganisms can live in places that would kill other organisms and how the diverse physiological capacities of microorganisms rank them as Earth's premier chemists. We will also trace the evolutionary history of microorganisms and see that three groups of cells can be distinguished by their evolutionary relationships. And finally, we will see how microorganisms have established important relationships with other organisms, some beneficial and some harmful.

We begin our study of microbiology with a consideration of the cellular structure of microorganisms.

MiniQuiz

- As they exist in nature, why can it be said that microbial cells differ fundamentally from the cells of higher organisms?
- Why are microbial cells useful tools for basic science?

1.2 Microbial Cells

A basic tenet of biology is that the **cell** is the fundamental unit of life. A single cell is an entity isolated from other such entities by a membrane; many cells also have a cell wall outside the membrane (**Figure 1.2**). The membrane defines the compartment that is the cell, maintains the correct proportions of internal constituents, and prevents leakage, while the wall lends structural strength to the cell. But the fact that a cell is a compartment does not mean that it is a *sealed* compartment. Instead, the membrane is semipermeable and thus the cell is an open, dynamic structure. Cells can communicate, move about, and exchange materials with their environments, and so they are constantly undergoing change.

Properties of Cellular Life

What essential properties characterize cells? **Figure 1.3** summarizes properties shared by all cellular microorganisms and additional properties that characterize only some of them. All cells show some form of **metabolism**. That is, they take up nutrients from the environment and transform them into new cell materials and waste products. During these transformations, energy is conserved in a form that can be drawn upon by the cell to support the synthesis of key structures. Production of the new structures culminates in the division of the cell to form two cells. The metabolic capabilities of cells can differ dramatically, but the final result of any cell's metabolic activities is to form two cells. In microbiology, we typically use the term **growth**, rather than "reproduction," to refer to the increase in cell number from cell division.

All cells undergo **evolution**, the process of descent with modification in which genetic variants are selected based on their reproductive fitness. Evolution is typically a slow process but can occur rapidly in microbial cells when selective pressure is strong. For example, we can witness today the selection for antibiotic resistance in pathogenic (disease-causing) bacteria by the indiscriminate use of antibiotics in human and veterinary medicine. Evolution is *the* overarching theme of biology, and the tenets of evolution—variation and natural selection based on fitness—govern microbial life forms just as they do multicellular life forms.

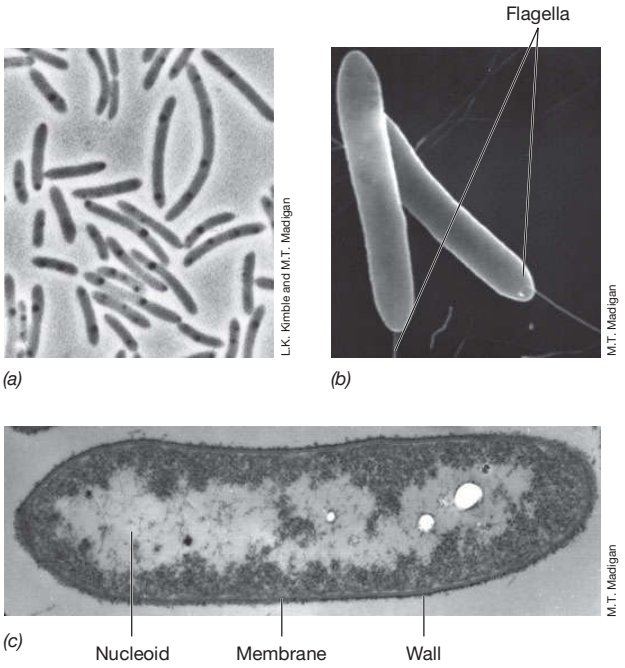


Figure 1.2 **Bacterial cells and some cell structures.** *(a)* Rod-shaped cells of the bacterium *Heliobacterium modesticaldum* as seen in the light microscope; a single cell is about 1 μm in diameter. *(b)* Scanning electron micrograph of the same cells as in part a showing flagella, structures that rotate like a propeller and allow cells to swim. *(c)* Electron micrograph of a sectioned cell of *H. modesticaldum*. The light area is aggregated DNA, the nucleoid of the cell.

Although all cells metabolize, grow, and evolve, the possession of other common properties varies from one species of cell to another. Many cells are capable of **motility**, typically by self-propulsion (Figure 1.2*b*). Motility allows cells to move away from danger or unfavorable conditions and to exploit new resources or opportunities. Some cells undergo **differentiation**, which may, for example, produce modified cells specialized for growth, dispersal, or survival. Some cells respond to chemical signals in their environment including those produced by other cells of either the same or different species. Responses to these signals may trigger new cellular activities. We can thus say that cells exhibit **communication**. As more is learned about this aspect of microbial life, it is quite possible that cell–cell communication will turn out to be a universal property of microbial cells.

Cells as Biochemical Catalysts and as Genetic Entities

The routine activities of cells can be viewed in two ways. On one hand, cells can be viewed as biochemical catalysts, carrying out the chemical reactions that constitute metabolism (**Figure 1.4**). On the other hand, cells can be viewed as genetic coding devices,

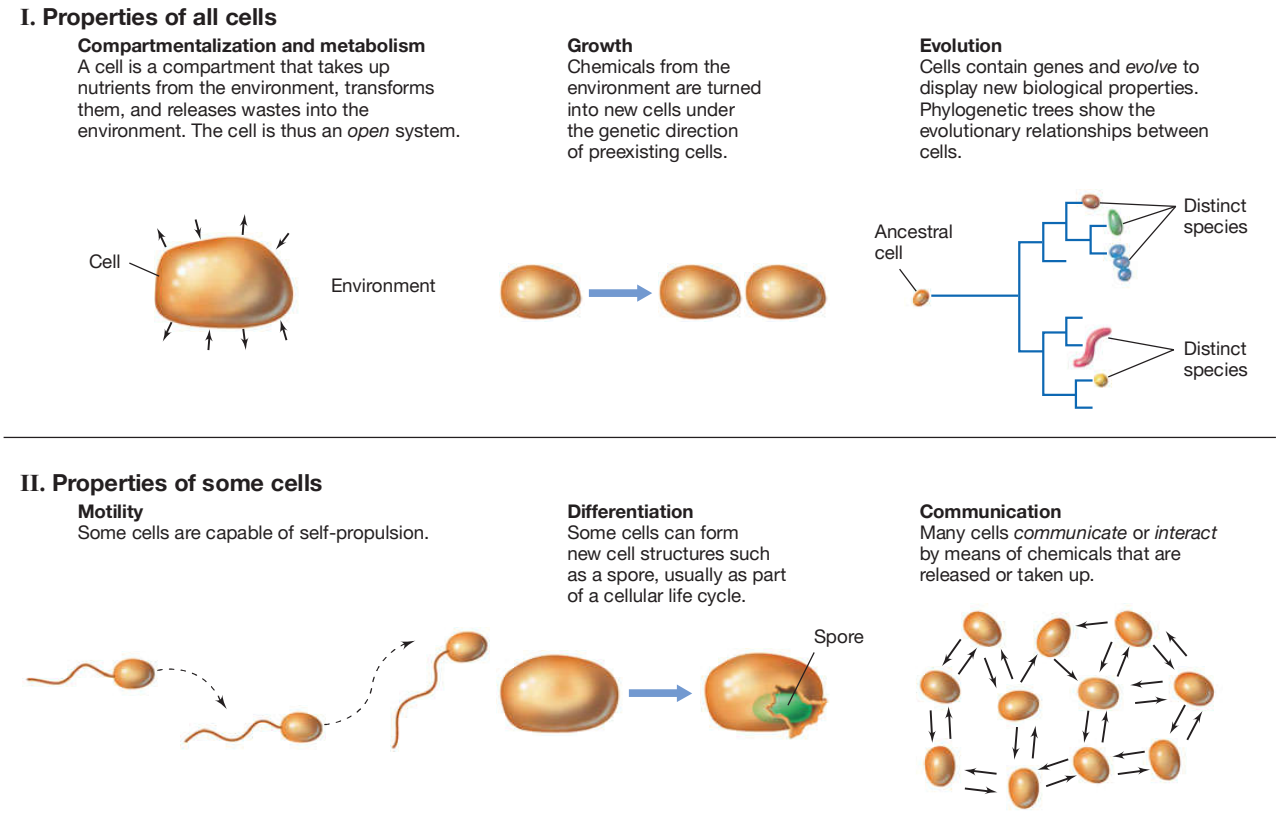


Figure 1.3 The properties of cellular life.

replicating DNA and then processing it to form the RNAs and proteins needed for maintenance and growth under the prevailing conditions. DNA processing includes two main events, the production of RNAs (transcription) and the production of proteins (translation) (Figure 1.4).

Cells coordinate their catalytic and genetic functions to support cell growth. In the events that lead up to cell division, all constituents in the cell double. This requires that a cell's catalytic machinery, its **enzymes**, supply energy and precursors for the biosynthesis of all cell components, and that its entire complement of genes (its **genome**) replicates (Figure 1.4). The catalytic and genetic functions of the cell must therefore be highly coordinated. Also, as we will see later, these functions can be regulated to ensure that new cell materials are made in the proper order and concentrations and that the cell remains optimally tuned to its surroundings.

MiniQuiz

- What does the term "growth" mean in microbiology?
- List the six major properties of cells. Which of these are universal properties of all cells?
- Compare the catalytic and genetic functions of a microbial cell. Why is neither of value to a cell without the other?

1.3 Microorganisms and Their Environments

In nature, microbial cells live in populations in association with populations of cells of other species. A population is a group of cells derived from a single parental cell by successive cell divisions. The immediate environment in which a microbial population lives is called its **habitat**. Populations of cells interact with other populations in **microbial communities** (Figure 1.5). The diversity and abundance of microorganisms in microbial communities is controlled by the resources (foods) and conditions (temperature, pH, oxygen content, and so on) that prevail in their habitat.

Microbial populations interact with each other in beneficial, neutral, or harmful ways. For example, the metabolic waste products of one group of organisms can be nutrients or even poisons to other groups of organisms. Habitats differ markedly in their characteristics, and a habitat that is favorable for the growth of one organism may actually be harmful for another. Collectively, we call all the living organisms, together with the physical and chemical components of their environment, an **ecosystem**. Major microbial ecosystems are aquatic (oceans, ponds, lakes, streams, ice, hot springs), terrestrial (surface soils, deep subsurface), and other organisms, such as plants and animals.

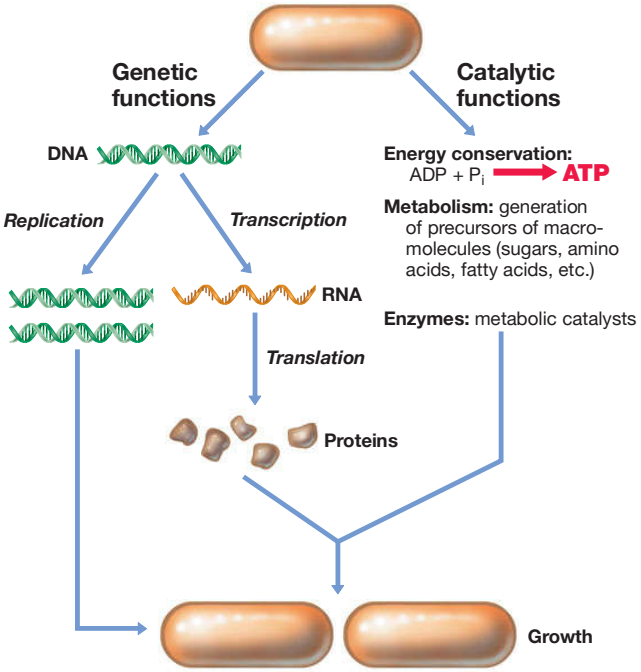
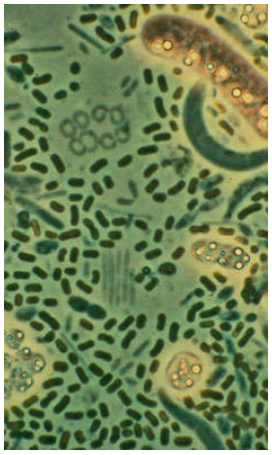


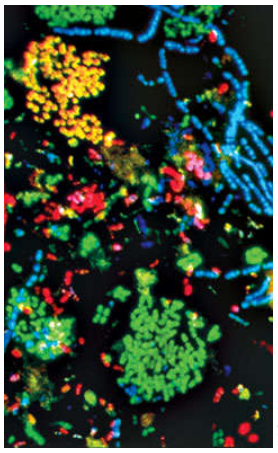
Figure 1.4 The catalytic and genetic functions of the cell. For a cell to reproduce itself there must be energy and precursors for the synthesis of new macromolecules, the genetic instructions must be replicated such that upon division each cell receives a copy, and genes must be expressed (transcribed and translated) to produce proteins and other macromolecules. Replication, transcription, and translation are the key molecular processes in cells.

An ecosystem is greatly influenced and in some cases even controlled by microbial activities. Microorganisms carrying out metabolic processes remove nutrients from the ecosystem and use them to build new cells. At the same time, they excrete waste products back into the environment. Thus, microbial ecosystems expand and contract, depending on the resources and conditions available. Over time, the metabolic activities of microorganisms gradually change their ecosystems, both chemically and physically. For example, molecular oxygen (O_2) is a vital nutrient for some microorganisms but a poison to others. If aerobic (oxygen-consuming) microorganisms remove O_2 from a habitat, rendering it anoxic (O_2 free), the changed conditions may favor the growth of anaerobic microorganisms that were formerly present in the habitat but unable to grow. In other words, as resources and conditions change in a microbial habitat, cell populations rise and fall, changing the habitat once again.

In later chapters, after we have learned about microbial structure and function, genetics, evolution, and diversity, we will return to a consideration of the ways in which microorganisms affect animals, plants, and the whole global ecosystem. This is the study of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today.



(a)



(b)

(c)

Figure 1.5 Microbial communities. *(a)* A bacterial community that developed in the depths of a small lake (Wintergreen Lake, Michigan), showing cells of various green and purple (large cells with sulfur granules) phototrophic bacteria. *(b)* A bacterial community in a sewage sludge sample. The sample was stained with a series of dyes, each of which stained a specific bacterial group. From *Journal of Bacteriology* 178: 3496–3500, Fig. 2b. © 1996 American Society for Microbiology. *(c)* Purple sulfur bacteria like that shown in part a (see also Figure 1.7*a*) that formed a dense bloom in a small Spanish lake.

MiniQuiz

- How does a microbial community differ from a microbial population?
- What is a habitat? How can microorganisms change the characteristics of their habitats?

1.4 Evolution and the Extent of Microbial Life

Microorganisms were the first entities on Earth with the properties of living systems (Figure 1.3), and we will see that a particular group of microorganisms called the *cyanobacteria* were pivotal

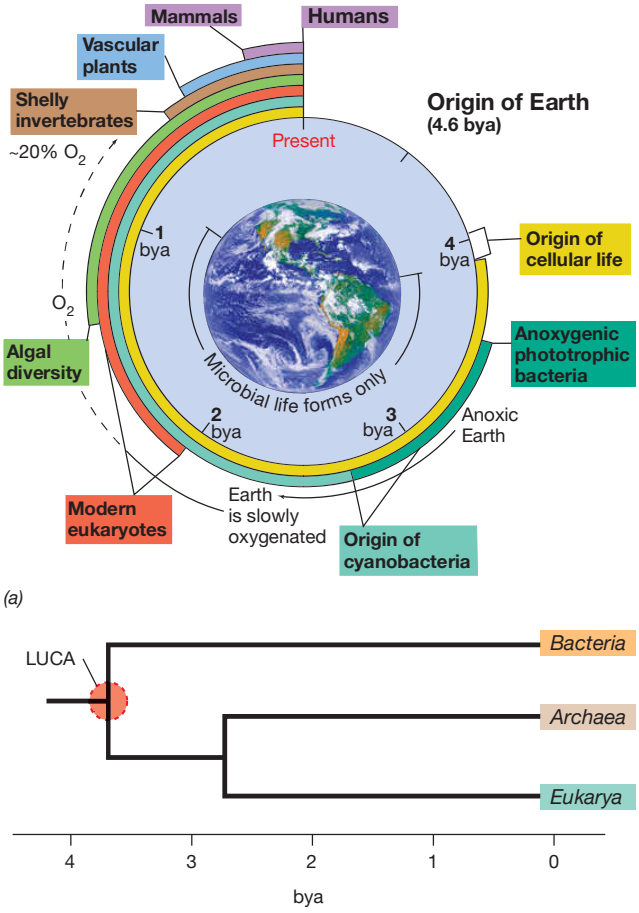


Figure 1.6 **A summary of life on Earth through time and origin of the cellular domains.** *(a)* Cellular life was present on Earth about 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 bya, but current levels of O_2 in the atmosphere were not achieved until 500–800 million years ago. Eukaryotes are nucleated cells and include both microbial and multicellular organisms. (Shelly invertebrates have shells or shell-like parts.) *(b)* The three domains of cellular organisms are *Bacteria*, *Archaea*, and *Eukarya*. The latter two lineages diverged long before nucleated cells with organelles (labeled as "modern eukaryotes" in part a) appear in the fossil record. LUCA, last universal common ancestor. Note that 80% of Earth's history was exclusively microbial.

in biological evolution because oxygen (O_2)—a waste product of their metabolism—prepared planet Earth for more complex life forms.

The First Cells and the Onset of Biological Evolution

How did cells originate? Were cells as we know them today the first self-replicating structures on Earth? Because all cells are constructed in similar ways, it is thought that all cells have descended from a common ancestral cell, the *last universal common ancestor* (LUCA). After the first cells arose from nonliving materials, a process that occurred over hundreds of millions of years, their subsequent growth formed cell populations, and these then began to interact with other populations in microbial communities. Evolution selected for improvements and diversification of these early cells to eventually yield the highly complex and diverse cells we see today. We will consider this complexity and diversity in Chapters 2 and 17–21. We consider the topic of how life originated from nonliving materials in Chapter 16.

Life on Earth through the Ages

Earth is 4.6 billion years old. Scientists have evidence that cells first appeared on Earth between 3.8 and 3.9 billion years ago, and these organisms were exclusively microbial. In fact, microorganisms were the only life on Earth for most of its history (**Figure 1.6**). Gradually, and over enormous periods of time, more complex organisms appeared. What were some of the highlights along the way?

During the first 2 billion years or so of Earth's existence, its atmosphere was anoxic; O_2 was absent, and nitrogen (N_2), carbon dioxide (CO_2), and a few other gases were present. Only microorganisms capable of anaerobic metabolisms could survive under these conditions, but these included many different types of cells, including those that produce methane, called *methanogens.* The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within a billion years of the formation of Earth. The first phototrophs were relatively simple ones, such as purple bacteria and other anoxygenic (non-oxygen-evolving) phototrophs (**Figure 1.7*a***; see also Figure 1.5), which are still widespread in anoxic habitats today. Cyanobacteria (oxygenic, or oxygen-evolving, phototrophs) (Figure 1.7*b*) evolved from anoxygenic phototrophs nearly a billion years later and began the slow process of oxygenating the atmosphere. Triggered by increases in O_2 in the atmosphere, multicellular life forms eventually evolved and continued to increase in complexity, culminating in the plants and animals we know today (Figure 1.6). We will

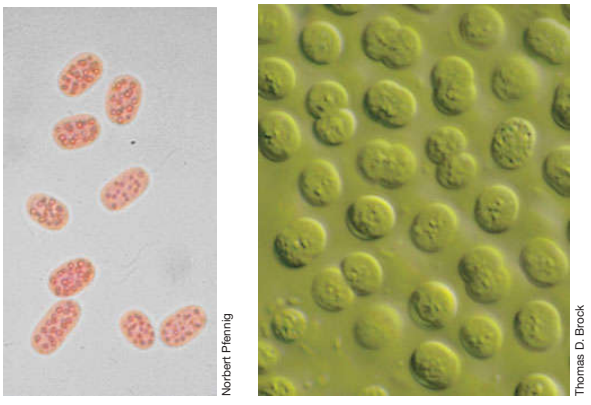


Figure 1.7 **Phototrophic microorganisms.** *(a)* Purple sulfur bacteria (anoxygenic phototrophs). *(b)* Cyanobacteria (oxygenic phototrophs). Purple bacteria appeared on Earth long before oxygenic phototrophs evolved (see Figure 1.6*a*).

explore the evolutionary history of life later, but note here that the events that unfolded beyond LUCA led to the evolution of three major lineages of microbial cells, the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.6*b*); microbial *Eukarya* were the ancestors of the plants and animals.

How do we know that evolutionary events unfolded as summarized in Figure 1.6? The answer is that we may never know that all details in our description are correct. However, scientists can reconstruct evolutionary transitions by using *biomarkers*, specific molecules that are unique to particular groups in present-day microorganisms. The presence or absence of a given biomarker in ancient rocks of a known age therefore reveals whether that particular group was present at that time.

One way or the other and over enormous periods of time (Figure 1.6), natural selection filled every suitable habitat on Earth with one or more populations of microorganisms. This brings us to the question of the current distribution of microbial life on Earth. What do we know about this important topic?

The Extent of Microbial Life

Microbial life is all around us. Examination of natural materials such as soil or water invariably reveals microbial cells. But unusual habitats such as boiling hot springs and glacial ice are also teeming with microorganisms. Although widespread on Earth, such tiny cells may seem inconsequential. But if we could count them all, what number would we reach?

Estimates of total microbial cell numbers on Earth are on the order of 2.5×10^{30} cells. The total amount of carbon present in this very large number of very small cells equals that of all plants on Earth (and plant carbon far exceeds animal carbon). But in addition, the collective contents of nitrogen and phosphorus in microbial cells is more than 10 times that in all plant biomass.

Thus, microbial cells, small as they are, constitute the major fraction of biomass on Earth and are key reservoirs of essential nutrients for life. Most microbial cells are found in just a few very large habitats. For example, most microbial cells do not reside on Earth's *surface* but instead lie underground in the oceanic and terrestrial *subsurface* (**Table 1.1**). Depths up to about 10 km under Earth's surface are clearly suitable for microbial life. We will see later that subsurface microbial habitats support diverse populations of microbial cells that make their livings in unusual ways and grow extremely slowly. By comparison to the subsurface, surface soils and waters contain a relatively small percentage of the total microbial cell numbers, and animals (including humans), which can be heavily colonized with microorganisms (see Figure 1.10), collectively contain only a tiny fraction of the total microbial cells on Earth (Table 1.1).

Because most of what we know about microbial life has come from the study of surface-dwelling organisms, there is obviously much left for future generations of microbiologists to discover and understand about the life forms that dominate Earth's biology. And when we consider the fact that surface-dwelling organisms already show enormous diversity, the hunt for new microorganisms in Earth's unexplored habitats should yield some exciting surprises.

Table 1.1 *Distribution of microorganisms in and on Earth*[a]

Habitat	*Percent of total*
Marine subsurface	66
Terrestrial subsurface	26
Surface soil	4.8
Oceans	2.2
All other habitats[b]	1.0

[a]Data compiled by William Whitman, University of Georgia, USA; refer to total numbers (estimated to be about 2.5×10^{30} cells) of *Bacteria* and *Archaea*. This enormous number of cells contain, collectively, about 5×10^{17} grams of carbon.
[b]Includes, in order of decreasing numbers: freshwater and salt lakes, domesticated animals, sea ice, termites, humans, and domesticated birds.

MiniQuiz

- What is LUCA and what major lineages of cells evolved from LUCA? Why were cyanobacteria so important in the evolution of life on Earth?
- How old is Earth, and when did cellular life forms first appear? How can we use science to reconstruct the sequence of organisms that appeared on Earth?
- Where are most microbial cells located on Earth?

1.5 The Impact of Microorganisms on Humans

Through the years microbiologists have had great success in discovering how microorganisms work, and application of this knowledge has greatly increased the beneficial effects of microorganisms and curtailed many of their harmful effects. Microbiology has thus greatly advanced human health and welfare. Besides understanding microorganisms as agents of disease, microbiology has made great advances in understanding the role of microorganisms in food and agriculture, and in exploiting microbial activities for producing valuable human products, generating energy, and cleaning up the environment.

Microorganisms as Agents of Disease

The statistics summarized in **Figure 1.8** show microbiologists' success in preventing infectious diseases since the beginning of the twentieth century. These data compare today's leading causes of death in the United States with those of 100 years ago. At the beginning of the twentieth century, the major causes of death in humans were infectious diseases caused by microorganisms called **pathogens**. Children and the aged in particular succumbed in large numbers to microbial diseases. Today, however, infectious diseases are much less deadly, at least in developed countries. Control of infectious disease has come from an increased understanding of disease processes, improved sanitary and public health practices, and the use of antimicrobial agents, such as antibiotics. As we will see from the next sections, the development of microbiology as a science can trace important aspects of its roots to studies of infectious disease.

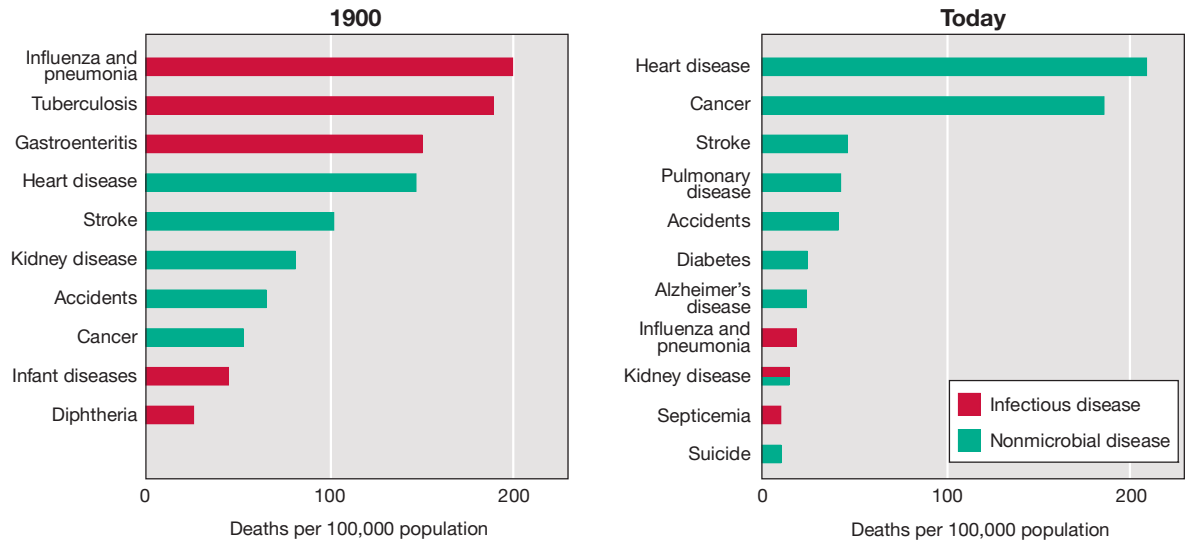


Figure 1.8 **Death rates for the leading causes of death in the United States: 1900 and today.**
Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be the result of microbial infections or systemic sources (diabetes, certain cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention and are typical of recent years.

Although many infectious diseases can now be controlled, microorganisms can still be a major threat, particularly in developing countries. In the latter, microbial diseases are still the major causes of death, and millions still die yearly from other microbial diseases such as malaria, tuberculosis, cholera, African sleeping sickness, measles, pneumonia and other respiratory diseases, and diarrheal syndromes. In addition to these, humans worldwide are under threat from diseases that could emerge suddenly, such as bird or swine flu, or Ebola hemorrhagic fever, which are primarily animal diseases that under certain circumstances can be transmitted to humans and spread quickly through a population. And if this were not enough, consider the threat to humans worldwide from those who would deploy microbial bioterrorism agents! Clearly, microorganisms are still serious health threats to humans in all parts of the world.

Although we should obviously appreciate the powerful threat posed by pathogenic microorganisms, in reality, most microorganisms are not harmful to humans. In fact, most microorganisms cause no harm but instead are beneficial—and in many cases even essential—to human welfare and the functioning of the planet. We turn our attention to these microorganisms now.

Microorganisms, Digestive Processes, and Agriculture

Agriculture benefits from the cycling of nutrients by microorganisms. For example, a number of major crop plants are legumes. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the root nodules, these bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) that the plants use as a nitrogen source for growth (**Figure 1.9**). Thanks to the activities of these nitrogen-fixing bacteria, the legumes have no need for costly and polluting nitrogen fertilizers. Other bacteria cycle sulfur compounds, oxidizing toxic sulfur species such as hydrogen sulfide (H_2S) into sulfate (SO_4^{2-}), which is an essential plant nutrient (Figure 1.9*c*).

Also of major agricultural importance are the microorganisms that inhabit ruminant animals, such as cattle and sheep. These important domesticated animals have a characteristic digestive vessel called the *rumen* in which large populations of microorganisms digest and ferment cellulose, the major component of plant cell walls, at neutral pH (Figure 1.9*d*). Without these symbiotic microorganisms, cattle and sheep could not thrive on cellulose-rich (but otherwise nutrient-poor) food, such as grass and hay. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.

The ruminant digestive system contrasts sharply with that of humans and most other animals. In humans, food enters a highly acidic stomach where major digestive processes are chemical rather than microbial. In the human digestive tract, large microbial populations occur only in the colon (large intestine), a structure that comes after the stomach and small intestine and which lacks significant numbers of cellulose-degrading bacteria. However, other parts of the human body can be loaded with bacteria. In addition to the large intestine, the skin and oral cavity (**Figure 1.10**) contain a significant normal microbial flora, most of which benefits the host or at least does no harm.

In addition to benefiting plants and animals, microorganisms can also, of course, have negative effects on them. Microbial diseases of plants and animals used for human food cause major

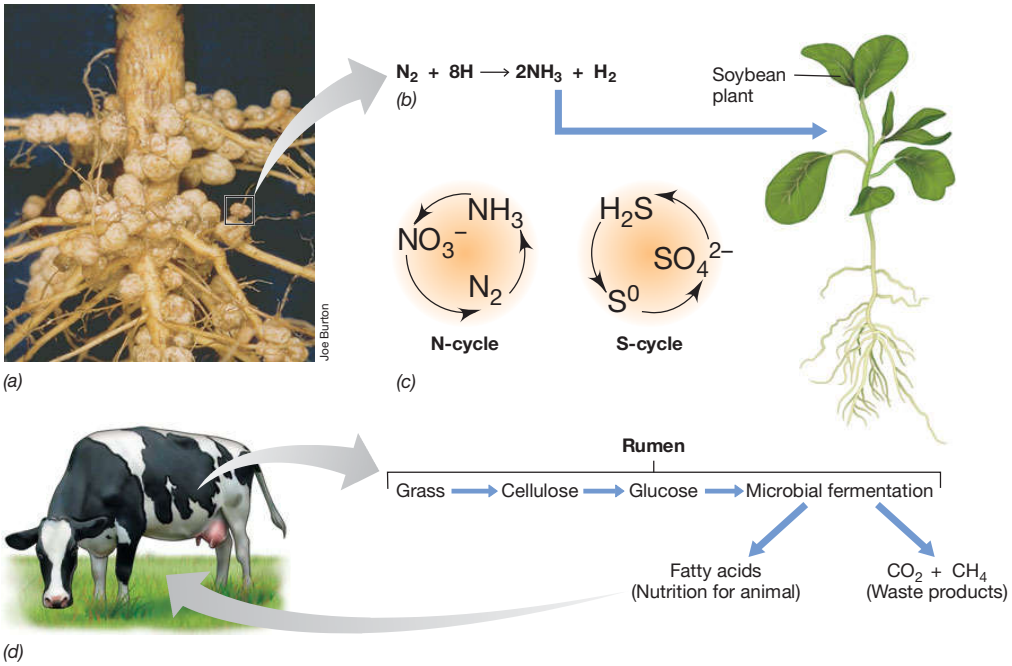


Figure 1.9 **Microorganisms in modern agriculture.** *(a, b)* Root nodules on this soybean plant contain bacteria that fix molecular nitrogen (N_2) for use by the plant. *(c)* The nitrogen and sulfur cycles, key nutrient cycles in nature. *(d)* Ruminant animals. Microorganisms in the rumen of the cow convert cellulose from grass into fatty acids that can be used by the animal.

economic losses in the agricultural industry every year. In some cases a food product can cause serious human disease, such as when pathogenic *Escherichia coli* or *Salmonella* is transmitted from infected meat, or when microbial pathogens are ingested with contaminated fresh fruits and vegetables. Thus microorganisms significantly impact the agriculture industry both positively and negatively.

Microorganisms and Food, Energy, and the Environment

Microorganisms play important roles in the food industry, including in the areas of spoilage, safety, and production. After plants and animals are produced for human consumption, the products must be delivered to consumers in a wholesome form. Food spoilage alone results in huge economic losses each year. Indeed, the canning, frozen food, and dried-food industries were founded as means to preserve foods that would otherwise easily undergo microbial spoilage. Food safety requires constant monitoring of food products to ensure they are free of pathogenic microorganisms and to track disease outbreaks to identify the source(s).

However, not all microorganisms in foods have harmful effects on food products or those who eat them. For example, many dairy products depend on the activities of microorganisms, including the fermentations that yield cheeses, yogurt, and buttermilk. Sauerkraut, pickles, and some sausages are also products of microbial fermentations. Moreover, baked goods and alcoholic beverages rely on the fermentative activities of yeast, which generate carbon dioxide (CO_2) to raise the dough and alcohol as a key ingredient, respectively. Many of these fermentations are discussed in Chapter 14.

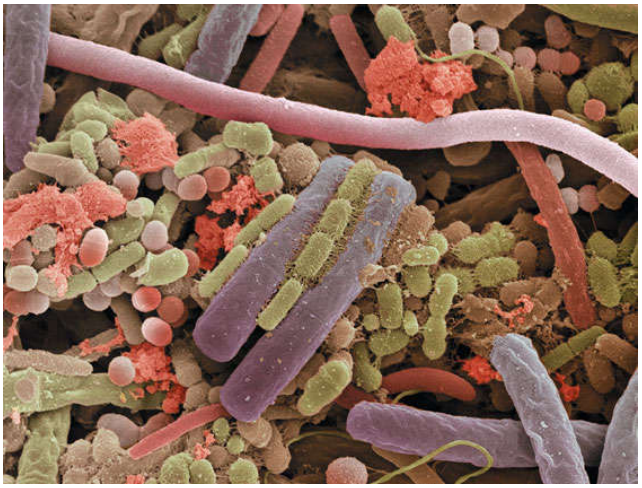


Figure 1.10 **Human oral bacterial community.** The oral cavity of warm-blooded animals contains high numbers of various bacteria, as shown in this electron micrograph (false color) of cells scraped from a human tongue.

(a) (b)

Figure 1.11 Biofuels. *(a)* Natural gas (methane) is collected in a funnel from swamp sediments where it was produced by methanogens and then ignited as a demonstration experiment. *(b)* An ethanol plant in the United States. Sugars obtained from corn or other crops are fermented to ethanol for use as a motor fuel extender.

Some microorganisms produce *biofuels*. Natural gas (methane) is a product of the anaerobic degradation of organic matter by methanogenic microorganisms (**Figure 1.11**). Ethyl alcohol (ethanol), which is produced by the microbial fermentation of glucose from feedstocks such as sugarcane or cornstarch, is a major motor fuel in some countries (Figure 1.11*b*). Waste materials such as domestic refuse, animal wastes, and cellulose can also be converted to biofuels by microbial activities and are more efficient feedstocks for ethanol production than is corn. Soybeans are also used as biofuel feedstocks, as soybean oils can be converted into biodiesel to fuel diesel engines. As global oil production is waning, it is likely that various biofuels will take on a greater and greater part of the global energy picture.

Microorganisms are used to clean up human pollution, a process called *microbial bioremediation*, and to produce commercially valuable products by *industrial microbiology* and *biotechnology*. For example, microorganisms can be used to consume spilled oil, solvents, pesticides, and other environmentally toxic pollutants. Bioremediation accelerates cleanup in either of two ways: (1) by introducing specific microorganisms to a polluted environment, or (2) by adding nutrients that stimulate preexisting microorganisms to degrade the pollutants. In both cases the goal is to accelerate metabolism of the pollutant.

In industrial microbiology, microorganisms are grown on a large scale to make products of relatively low commercial value, such as antibiotics, enzymes, and various chemicals. By contrast, the related field of biotechnology employs *genetically engineered* microorganisms to synthesize products of high commercial value, such as human proteins. **Genomics** is the science of the identification and analysis of genomes and has greatly enhanced biotechnology. Using genomic methods, biotechnologists can access the genome of virtually any organism and search in it for genes encoding proteins of commercial interest.

At this point the influence of microorganisms on humans should be apparent. Microorganisms are essential for life and their activities can cause significant benefit or harm to humans. As the eminent French scientist Louis Pasteur, one of the founders of microbiology, expressed it: "The role of the infinitely small in nature is infinitely large." We continue our introduction to the microbial world in the next section with an historical overview of the contributions of Pasteur and a few other key scientists.

MiniQuiz

- List two ways in which microorganisms are important in the food and agricultural industries.
- Which biofuel is widely used in many countries as a motor fuel?
- What is biotechnology and how might it improve the lives of humans?

II Pathways of Discovery in Microbiology

The future of any science is rooted in its past accomplishments. Although microbiology claims very early roots, the science did not really develop in a systematic way until the nineteenth century. Since that time, microbiology has expanded in a way

Table 1.2 *Giants of the early days of microbiology and their major contributions*

Investigator	*Nationality*	*Dates*[a]	*Contributions*
Robert Hooke	English	1664	Discovery of microorganisms (fungi)
Antoni van Leeuwenhoek	Dutch	1684	Discovery of bacteria
Edward Jenner	English	1798	Vaccination (smallpox)
Louis Pasteur	French	Mid- to late 1800s	Mechanism of fermentation, defeat of spontaneous generation, rabies and other vaccines, principles of immunization
Joseph Lister	English	1867	Methods for preventing infections during surgeries
Ferdinand Cohn	German	1876	Discovery of endospores
Robert Koch	German	Late 1800s	Koch's postulates, pure culture microbiology, discovery of agents of tuberculosis and cholera
Sergei Winogradsky	Russian	Late 1800s to mid-1900s	Chemolithotrophy and chemoautotrophy, nitrogen fixation, sulfur bacteria
Martinus Beijerinck	Dutch	Late 1800s to 1920	Enrichment culture technique, discovery of many metabolic groups of bacteria, concept of a virus

[a]The year in which the key paper describing the contribution was published, or the date range in which the investigator was most scientifically active.

unprecedented by any of the other biological sciences and has spawned several new but related fields. We retrace these pathways of discovery now and discuss a few of the major contributors (**Table 1.2**).

1.6 The Historical Roots of Microbiology: Hooke, van Leeuwenhoek, and Cohn

Although the existence of creatures too small to be seen with the naked eye had long been suspected, their discovery was linked to the invention of the microscope. Robert Hooke (1635–1703), an English mathematician and natural historian, was also an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated, among many other things, the fruiting structures of molds (**Figure 1.12**). This was the first known description of microorganisms. The first person to see bacteria was the Dutch draper and amateur microscope builder Antoni van Leeuwenhoek (1632–1723). In 1684, van Leeuwenhoek, who was well aware of the work of Hooke, used extremely simple microscopes of his own construction (**Figure 1.13**) to examine the microbial content of natural substances.

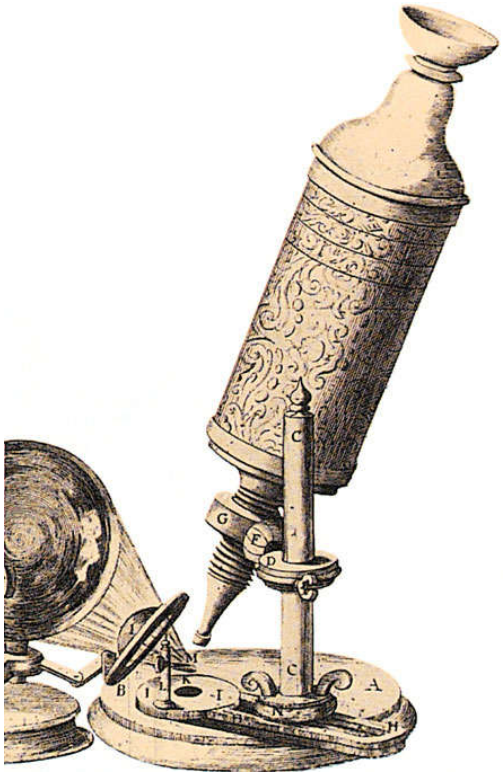
Van Leeuwenhoek's microscopes were crude by today's standards, but by careful manipulation and focusing he was able to see bacteria, microorganisms considerably smaller than molds (molds are fungi). He discovered bacteria in 1676 while studying pepper–water infusions. He reported his observations in a series of letters to the prestigious Royal Society of London, which published them in 1684 in English translation. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.13*b*, and a photo taken through such a microscope is shown in Figure 1.13*c*.

As years went by, van Leeuwenhoek's observations were confirmed by many others. However, primarily because of the lack of experimental tools, little progress in understanding the nature and importance of the tiny creatures was made for almost 150 years. Only in the nineteenth century did improved microscopes and some simple tools for growing microoorganisms in the laboratory become available, and using these, the extent and nature of microbial life became more apparent.

In the mid- to late nineteenth century major advances in the science of microbiology were made because of the attention given to two major questions that pervaded biology and medicine at the time: (1) Does spontaneous generation occur? and (2) What is the nature of infectious disease? Answers to these seminal questions emerged from the work of two giants in the fledgling field of microbiology: the French chemist Louis Pasteur and the German physician Robert Koch. But before we explore their work, let us briefly consider the groundbreaking efforts of a German botanist, Ferdinand Cohn, a contemporary of Pasteur and Koch, and the founder of the field we now call *bacteriology*.

Ferdinand Cohn (1828–1898) was born in Breslau (now in Poland). He was trained as a botanist and became an excellent microscopist. His interests in microscopy led him to the study of unicellular algae and later to bacteria, including the large sulfur bacterium *Beggiatoa* (**Figure 1.14**). Cohn was particularly interested in heat resistance in bacteria, which led to his discovery that some bacteria form endospores. We now know that bacterial endospores are formed by differentiation from the mother (vegetative) cell (Figure 1.3) and that endospores are extremely heat-resistant. Cohn described the life cycle of the endospore-forming bacterium *Bacillus* (vegetative cell → endospore → vegetative cell) and showed that vegetative cells but not endospores were killed by boiling.

Cohn is credited with many other accomplishments. He laid the groundwork for a system of bacterial classification, including an early attempt to define a bacterial species, an issue still unresolved today, and founded a major scientific journal of plant and microbial biology. He strongly advocated use of the techniques and research of Robert Koch, the first medical microbiologist. Cohn devised simple but effective methods for preventing the contamination of culture media, such as the use



(a)

(b)

Figure 1.12 **Robert Hooke and early microscopy.** *(a)* A drawing of the microscope used by Robert Hooke in 1664. The lens was fitted at the end of an adjustable bellows (G) and light focused on the specimen by a separate lens (1). *(b)* This drawing of a mold that was growing on the surface of leather, together with other drawings and accompanying text published by Robert Hooke in *Micrographia* in 1665, were the first descriptions of microorganisms. The round structures contain spores of the mold. Compare Hooke's microscope with that of van Leeuwenhoek's shown in Figure 1.13.

of cotton for closing flasks and tubes. These methods were later used by Koch and allowed him to make rapid progress in the isolation and characterization of several disease-causing bacteria (Section 1.8).

MiniQuiz

- What prevented the science of microbiology from developing before the era of Hooke and van Leeuwenhoek?
- What major discovery emerged from Cohn's study of heat resistance in microorganisms?

1.7 Pasteur and the Defeat of Spontaneous Generation

The late nineteenth century saw the science of microbiology blossom. The theory of spontaneous generation was crushed by the brilliant work of the Frenchman Louis Pasteur (1822–1895).

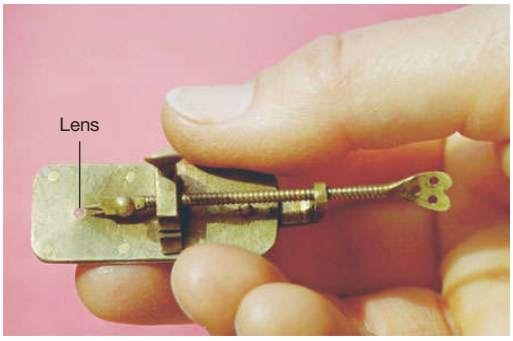
Optical Isomers and Fermentations

Pasteur was a chemist by training and was one of the first to recognize the significance of *optical isomers*. A molecule is optically active if a pure solution or crystal diffracts light in only one direction. Pasteur studied crystals of tartaric acid that he separated by hand into those that bent a beam of polarized light to the left and those that bent the beam to the right (**Figure 1.15**). Pasteur found that the mold *Aspergillus* metabolized D-tartrate, which bent light to the right, but did not metabolize its optical isomer, L-tartrate. The fact that a living organism could discriminate between optical isomers was of profound significance to Pasteur, and he began to see living organisms as inherently asymmetric entities.

Pasteur's thinking on the asymmetry of life carried over into his work on fermentations and, eventually, spontaneous generation. At the invitation of a local industrialist who was having problems making alcohol from the fermentation of beets, Pasteur studied the mechanism of the alcoholic fermentation, at that time thought to be a strictly chemical process. The yeast cells in the fermenting broth were thought to be a complex chemical substance formed by the fermentation. Although ethyl alcohol does not form optical isomers, one of the side products of beet fermentation is amyl alcohol, which does, and Pasteur tested the fermenting juice and found the amyl alcohol to be of only one optical isomer. From his work on tartrate metabolism this suggested to Pasteur that the beet fermentation was a biological process. Microscopic observations and other simple but rigorous experiments convinced Pasteur that the alcoholic fermentation was catalyzed by living organisms, the yeast cells. Indeed, in Pasteur's own words: ". . . fermentation is associated with the life and structural integrity of the cells and not with their death and decay." From this foundation, Pasteur began a series of classic experiments on spontaneous generation, experiments that are forever linked to his name and to the science of microbiology.

Spontaneous Generation

The concept of **spontaneous generation** had existed since biblical times and its basic tenet can be easily grasped. For example, if



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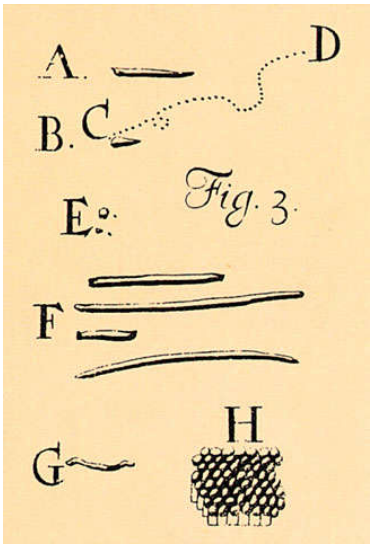

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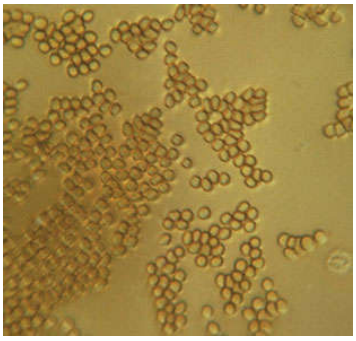


Brian J. Ford

(c)

Figure 1.13 **The van Leeuwenhoek microscope.** *(a)* A replica of Antoni van Leeuwenhoek's microscope. *(b)* Van Leeuwenhoek's drawings of bacteria, published in 1684. Even from these simple drawings we can recognize several shapes of common bacteria: A, C, F, and G, rods; E, cocci; H, packets of cocci. *(c)* Photomicrograph of a human blood smear taken through a van Leeuwenhoek microscope. Red blood cells are clearly apparent.

food is allowed to stand for some time, it putrefies. When examined microscopically, the putrefied food is seen to be teeming with bacteria and perhaps even maggots and worms. From where do these organisms not apparent in the fresh food originate? Some people said they developed from seeds or germs that entered the food from air. Others said they arose spontaneously from nonliving materials, that is, by *spontaneous generation*. Who was right? Keen insight was necessary to solve this controversy, and this was exactly the kind of problem that appealed to Louis Pasteur.

Pasteur became a powerful opponent of spontaneous generation. Following his discoveries about fermentation, Pasteur predicted that microorganisms observed in putrefying materials are also present in air and that putrefaction resulted from the activities of microorganisms that entered from the air or that had been present on the surfaces of the containers holding the decaying materials. Pasteur further reasoned that if food were treated in such a way as to destroy all living organisms contaminating it, that is, if it were rendered **sterile** and then protected from further contamination, it should not putrefy.

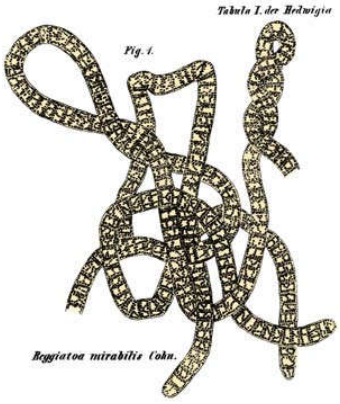
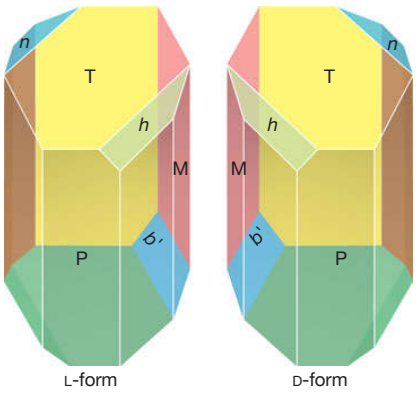


Figure 1.14 **Drawing by Ferdinand Cohn of large filamentous sulfur-oxidizing bacteria *Beggiatoa mirabilis*.** The small granules inside the cells consist of elemental sulfur, produced from the oxidation of hydrogen sulfide (H_2S). Cohn was the first to identify the granules as sulfur in 1866. A cell of *B. mirabilis* is about 15 μm in diameter. Compare with Figure 1.22*b*. *Beggiatoa* moves on solid surfaces by a gliding mechanism and in so doing, cells often twist about one another.



(a) (b)

Figure 1.15 **Louis Pasteur's drawings of tartaric acid crystals from his famous paper on optical activity.** *(a)* Left-handed crystal (bends light to the left). *(b)* Right-handed crystal (bends light to the right). Note that the two crystals are mirror images of one another, a hallmark of optical isomers.

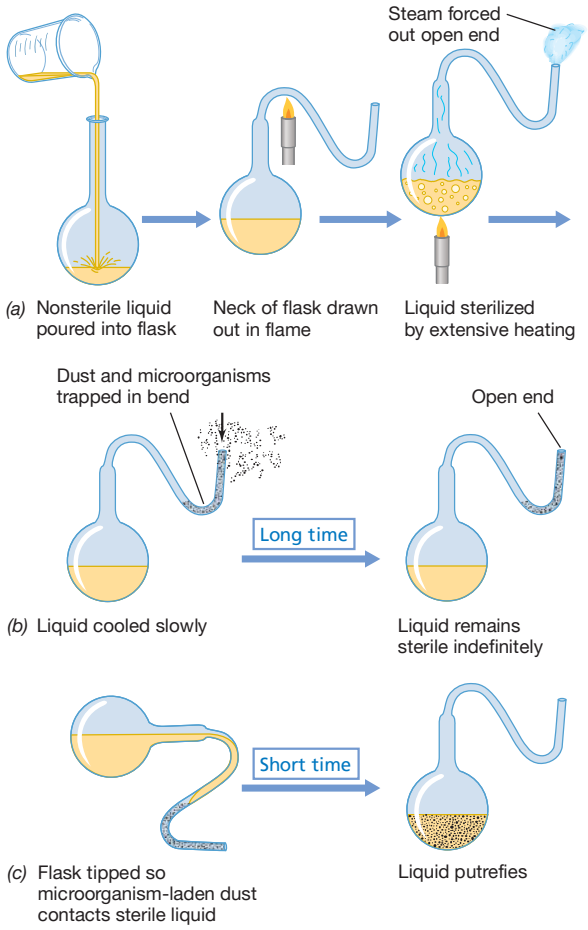


Figure 1.16 **The defeat of spontaneous generation: Pasteur's swan-necked flask experiment.** In *(c)* the liquid putrefies because microorganisms enter with the dust.

Pasteur used heat to eliminate contaminants. Killing all the bacteria or other microorganisms in or on objects is a process we now call *sterilization*. Proponents of spontaneous generation criticized such experiments by declaring that "fresh air" was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing a swan-necked flask, now called a *Pasteur flask* (**Figure 1.16**). In such a flask nutrient solutions could be heated to boiling and sterilized. However, after the flask was cooled, air was allowed to reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and causing putrefaction.

The teeming microorganisms observed after particulate matter was allowed to enter at the end of this simple experiment (Figure 1.16*c*) effectively settled the controversy, and microbiology was able to bury the idea of spontaneous generation for good and move ahead on firm footing. Incidentally, Pasteur's work also led to the development of effective sterilization procedures that were eventually refined and carried over into both basic and applied microbiological research. Food science also owes a debt to Pasteur, as his principles are applied today in the preservation of milk and many other foods by heat treatment (pasteurization). www.microbiologyplace.com **Online Tutorial 1.1: Pasteur's Experiment**

Other Accomplishments of Louis Pasteur

Pasteur went on to many other triumphs in microbiology and medicine. Some highlights include his development of vaccines for the diseases anthrax, fowl cholera, and rabies during a very scientifically productive period from 1880 to 1890. Pasteur's work on rabies was his most famous success, culminating in July 1885 with the first administration of a rabies vaccine to a human, a young French boy named Joseph Meister who had been bitten by a rabid dog. In those days, a bite from a rabid animal was invariably fatal. News spread quickly of the success of Meister's vaccination, and of one administered shortly thereafter to a young shepherd boy, Jean Baptiste Jupille (**Figure 1.17**). Within a

(a)

M.T. Madigan

(b)

Figure 1.17 **Louis Pasteur and some symbols of his contributions to microbiology.** *(a)* A French 5-franc note honoring Pasteur. The shepherd boy Jean Baptiste Jupille is shown killing a rabid dog that had attacked children. Pasteur's rabies vaccine saved Jupille's life. In France, the franc preceded the euro as a currency. *(b)* The Pasteur Institute, Paris, France. Today this structure, built for Pasteur by the French government, houses a museum that displays some of the original swan-necked flasks used in his experiments.

year several thousand people bitten by rabid animals had traveled to Paris to be treated with Pasteur's rabies vaccine.

Pasteur's fame from his rabies research was legendary and led the French government to establish the Pasteur Institute in Paris in 1888 (Figure 1.17*b*). Originally established as a clinical center for the treatment of rabies and other contagious diseases, the Pasteur Institute today is a major biomedical research center focused on antiserum and vaccine research and production. The medical and veterinary breakthroughs of Pasteur were not only highly significant in their own right but helped solidify the concept of the germ theory of disease, whose principles were being developed at about the same time by a second giant of this era, Robert Koch.

MiniQuiz

- Define the term sterile. How did Pasteur's experiments using swan-necked flasks defeat the theory of spontaneous generation?
- Besides ending the controversy over spontaneous generation, what other accomplishments do we credit to Pasteur?

1.8 Koch, Infectious Disease, and Pure Culture Microbiology

Proof that some microorganisms cause disease provided the greatest impetus for the development of microbiology as an independent biological science. Even as early as the sixteenth century it was thought that something that induced disease could be transmitted from a diseased person to a healthy person. After the discovery of microorganisms, it was widely believed that they were responsible, but definitive proof was lacking. Improvements in sanitation by Ignaz Semmelweis and Joseph Lister provided indirect evidence for the importance of microorganisms in causing human diseases, but it was not until the work of a German physician, Robert Koch (1843–1910) (**Figure 1.18**), that the concept of infectious disease was given experimental support.

The Germ Theory of Disease and Koch's Postulates

In his early work Koch studied anthrax, a disease of cattle and occasionally of humans. Anthrax is caused by an endospore-forming bacterium called *Bacillus anthracis.* By careful microscopy and by using special stains, Koch established that the bacteria were always present in the blood of an animal that was succumbing to the disease. However, Koch reasoned that the mere association of the bacterium with the disease was not proof of cause and effect. He sensed an opportunity to study cause and effect experimentally using anthrax. The results of this study formed the standard by which infectious diseases have been studied ever since.

Koch used mice as experimental animals. Using appropriate controls, Koch demonstrated that when a small amount of blood from a diseased mouse was injected into a healthy mouse, the latter quickly developed anthrax. He took blood from this second animal, injected it into another, and again observed the characteristic disease symptoms. However, Koch carried this experiment a critically important step further. He discovered that the anthrax bacteria could be grown in nutrient fluids *outside the host* and that even after many transfers in laboratory culture, the bacteria still caused the disease when inoculated into a healthy animal.

Figure 1.18 Robert Koch. The German physician and microbiologist is credited with founding medical microbiology and formulating his famous postulates.

On the basis of these experiments and others on the causative agent of tuberculosis, Koch formulated a set of rigorous criteria, now known as **Koch's postulates**, for definitively linking a specific microorganism to a specific disease. Koch's postulates state the following:

1. The disease-causing organism must always be present in animals suffering from the disease but not in healthy animals.
2. The organism must be cultivated in a pure culture away from the animal body.
3. The isolated organism must cause the disease when inoculated into healthy susceptible animals.
4. The organism must be isolated from the newly infected animals and cultured again in the laboratory, after which it should be seen to be the same as the original organism.

Koch's postulates, summarized in **Figure 1.19**, were a monumental step forward in the study of infectious diseases. The postulates not only offered a means for linking the cause and effect of an infectious disease, but also stressed the importance of *laboratory culture* of the putative infectious agent. With these postulates as a guide, Koch, his students, and those that followed them discovered the causative agents of most of the important

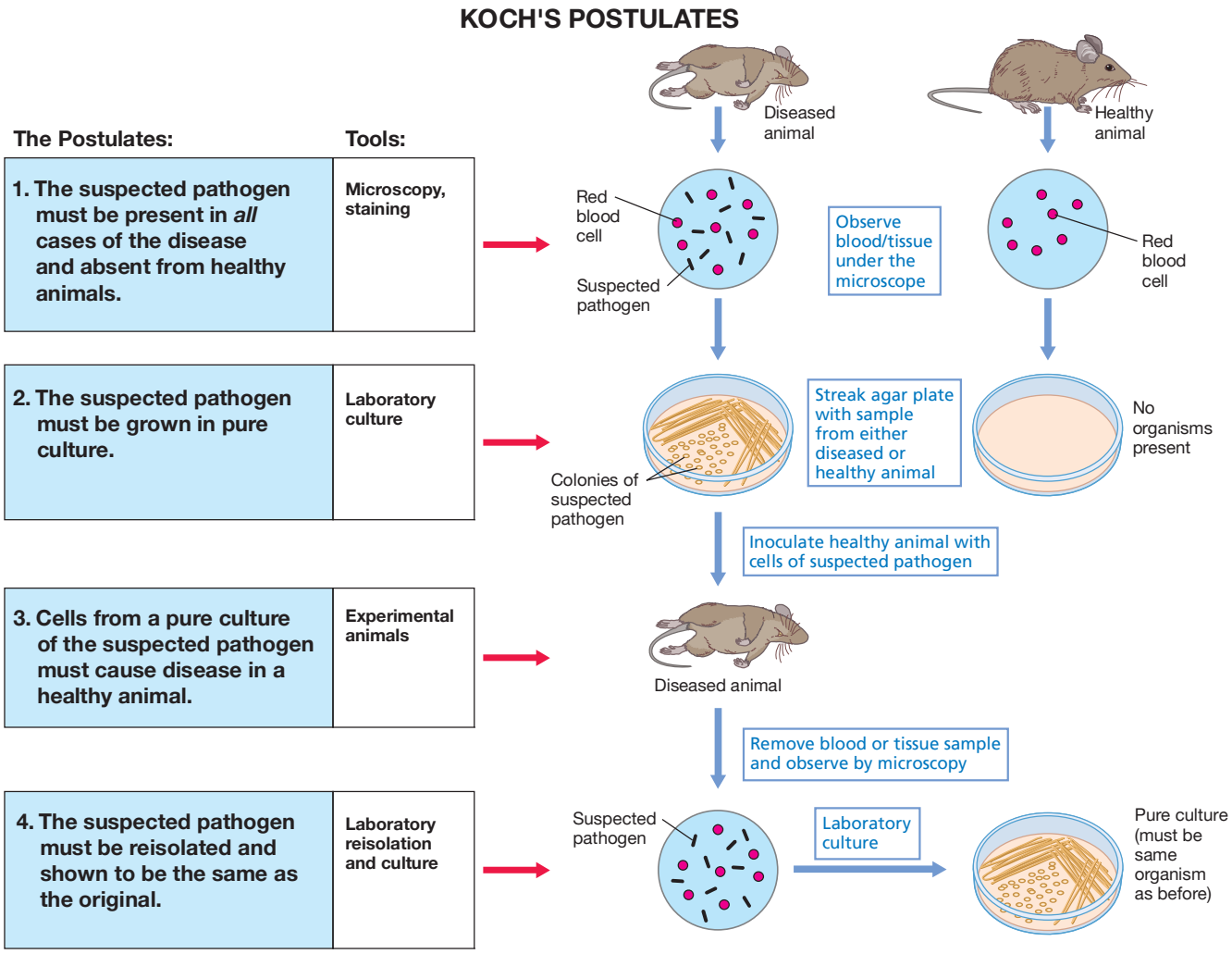


Figure 1.19 **Koch's postulates for proving cause and effect in infectious diseases.** Note that following isolation of a pure culture of the suspected pathogen, the cultured organism must both initiate the disease and be recovered from the diseased animal. Establishing the correct conditions for growing the pathogen is essential; otherwise it will be missed.

infectious diseases of humans and domestic animals. These discoveries led to the development of successful treatments for the prevention and cure of many of these diseases, thereby greatly improving the scientific basis of clinical medicine and human health and welfare (Figure 1.8).

Koch and Pure Cultures

To satisfy the second of Koch's postulates, the suspected pathogen must be isolated and grown away from other microorganisms in laboratory culture; in microbiology we say that such a culture is *pure*. The importance of this was not lost on Robert Koch in formulating his famous postulates, and to accomplish this goal, he and his associates developed several simple but ingenious methods of obtaining and growing bacteria in **pure culture**.

Koch started by using solid nutrients such as a potato slice to culture bacteria, but quickly developed more reliable methods, many of which are still in use today. Koch observed that when a solid surface was incubated in air, bacterial colonies developed, each having a characteristic shape and color. He inferred that each colony had arisen from a single bacterial cell that had fallen on the surface, found suitable nutrients, and multiplied. Each colony was a population of identical cells, or in other words, a pure culture, and Koch quickly realized that solid media provided an easy way to obtain pure cultures. However, because not all organisms grow on potato slices, Koch devised more exacting and reproducible nutrient solutions solidified with gelatin and, later, with agar—laboratory techniques that remain with us to this day (see the Microbial Sidebar, "Solid Media, Pure Cultures, and the Birth of Microbial Systematics").

Solid Media, Pure Cultures, and the Birth of Microbial Systematics

Robert Koch was the first to grow bacteria on solid culture media. Koch's early use of potato slices as solid media was fraught with problems. Besides the problem that not all bacteria can grow on potatoes, the slices were frequently overgrown with molds. Koch thus needed a more reliable and reproducible means of growing bacteria on solid media, and he found the answer for solidifying his nutrient solutions in agar.

Koch initially employed gelatin as a solidifying agent for the various nutrient fluids he used to culture bacteria, and he kept horizontal slabs of solid gelatin free of contamination under a bell jar or in a glass box (see Figure 1.20*c*). Nutrient-supplemented gelatin was a good culture medium for the isolation and study of various bacteria, but it had several drawbacks, the most important of which was that it did not remain solid at 37°C, the optimum temperature for growth of most human pathogens. Thus, a different solidifying agent was needed.

Agar is a polysaccharide derived from red algae. It was widely used in the nineteenth century as a gelling agent. Walter Hesse, an associate of Koch, first used agar as a solidifying agent for bacteriological culture media (Figure 1). The actual suggestion that agar be used instead of gelatin was made by Hesse's wife, Fannie. She had used agar to solidify fruit jellies. When it was tried as a solidifying agent for microbial media, its superior gelling qualities were immediately evident. Hesse wrote to Koch about this discovery, and Koch quickly adapted agar to his own studies, including his classic studies on the isolation of the bacterium *Mycobacterium tuberculosis,* the cause of the disease tuberculosis (see text and Figure 1.20).

Agar has many other properties that make it desirable as a gelling agent for microbial culture media. In particular, agar remains solid at 37°C and, after melting during the sterilization process, remains liquid to about 45°C, at which time it can be poured into sterile vessels. In addition, unlike gelatin, agar is not degraded by most bacteria and typically yields a transparent medium, making it easier to differentiate bacterial colonies from inanimate particulate matter. For these reasons, agar found its place early in the annals of microbiology and is still used today for obtaining and maintaining pure cultures.

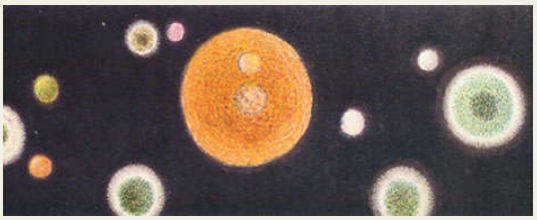
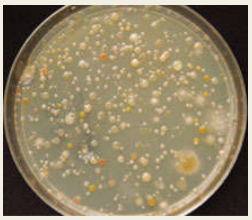


Figure 1 A hand-colored photograph taken by Walter Hesse of colonies formed on agar. The colonies include those of molds and bacteria obtained during Hesse's studies of the microbial content of air in Berlin, Germany, in 1882. From Hesse, W. 1884. "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen," in Struck, H. (ed.), *Mittheilungen aus dem Kaiserlichen Gesundheitsamte.* August Hirschwald.



Paul V. Dunlap

Figure 2 Photo of a Petri dish containing colonies of marine bacteria. Each colony contains millions of bacterial cells descended from a single cell.

In 1887 Richard Petri, a German bacteriologist, published a brief paper describing a modification of Koch's flat plate technique (Figure 1.20*c*). Petri's enhancement, which turned out to be amazingly useful, was the development of the transparent double-sided dishes that bear his name (Figure 2). The advantages of Petri dishes were immediately apparent. They could easily be stacked and sterilized separately from the medium, and, following the addition of molten culture medium to the smaller of the two dishes, the larger dish could be used as a cover to prevent contamination. Colonies that formed on the surface of the agar in the Petri dish retained access to air without direct exposure to air and could easily be manipulated for further study. The original idea of Petri has not been improved on to this day, and the Petri dish, constructed of either glass or plastic, is a mainstay of the microbiology laboratory.

Koch quickly grasped the significance of pure cultures and was keenly aware of the implications his pure culture methods had for classifying microorganisms. He observed that colonies that differed in color, morphology, size, and the like (see Figure 2) bred true and could be distinguished from one another. Cells from different colonies typically differed in size and shape and often in their temperature or nutrient requirements as well. Koch realized that these differences among microorganisms met all the requirements that biological taxonomists had established for the classification of larger organisms, such as plant and animal species. In Koch's own words (translated from the German): "All bacteria which maintain the characteristics which differentiate one from another when they are cultured on the same medium and under the same conditions, should be designated as species, varieties, forms, or other suitable designation." Such insightful thinking was important for the rapid acceptance of microbiology as a new biological science, rooted as biology was in classification at the time of Koch. It has since had a profound impact on the diagnosis of infectious diseases and the field of microbial diversity.

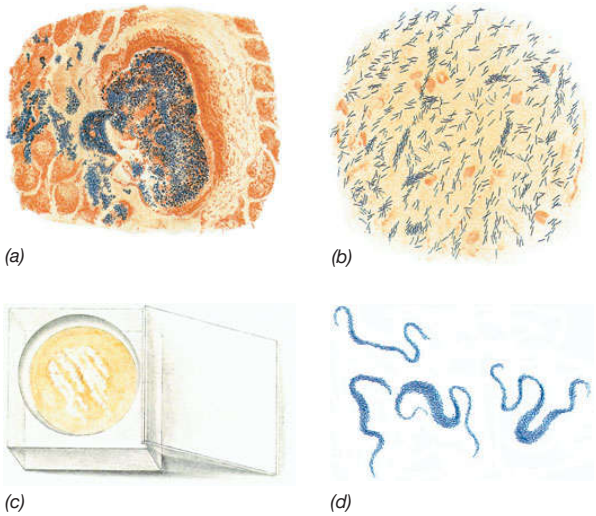


Figure 1.20 Robert Koch's drawings of *Mycobacterium tuberculosis.* *(a)* Section through infected lung tissue showing cells of *M. tuberculosis* (blue). *(b) M. tuberculosis* cells in a sputum sample from a tubercular patient. *(c)* Growth of *M. tuberculosis* on a glass plate of coagulated blood serum stored inside a glass box to prevent contamination. *(d) M. tuberculosis* cells taken from the plate in part c and observed microscopically; cells appear as long cordlike forms. Original drawings from Koch, R. 1884. "Die Aetiologie der Tuberkulose." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte* 2:1–88.

Tuberculosis: The Ultimate Test of Koch's Postulates

Koch's crowning accomplishment in medical bacteriology was his discovery of the causative agent of tuberculosis. At the time Koch began this work (1881), one-seventh of all reported human deaths were caused by tuberculosis (Figure 1.8). There was a strong suspicion that tuberculosis was a contagious disease, but the suspected agent had never been seen, either in diseased tissues or in culture. Koch was determined to demonstrate the cause of tuberculosis, and to this end he brought together all of the methods he had so carefully developed in his previous studies with anthrax: microscopy, staining, pure culture isolation, and an animal model system.

As is now well known, the bacterium that causes tuberculosis, *Mycobacterium tuberculosis,* is very difficult to stain because of the large amounts of a waxy lipid present in its cell wall. But Koch devised a staining procedure for *M. tuberculosis* cells in tissue samples; using this method, he observed blue, rod-shaped cells of *M. tuberculosis* in tubercular tissues but not in healthy tissues (**Figure 1.20**). However, from his previous work on anthrax, Koch realized that he must *culture* this organism in order to prove that it was the cause of tuberculosis.

Obtaining cultures of *M. tuberculosis* was not easy, but eventually Koch was successful in growing colonies of this organism on a medium containing coagulated blood serum. Later he used agar, which had just been introduced as a solidifying agent (see the Microbial Sidebar). Under the best of conditions, *M. tuberculosis* grows slowly in culture, but Koch's persistence and patience eventually led to pure cultures of this organism from human and animal sources.

From this point it was relatively easy for Koch to use his postulates (Figure 1.19) to obtain definitive proof that the organism he had isolated was the cause of the disease tuberculosis. Guinea pigs can be readily infected with *M. tuberculosis* and eventually succumb to systemic tuberculosis. Koch showed that diseased guinea pigs contained masses of *M. tuberculosis* cells in their lungs and that pure cultures obtained from such animals transmitted the disease to uninfected animals. Thus, Koch successfully satisfied all four of his postulates, and the cause of tuberculosis was understood. Koch announced his discovery of the cause of tuberculosis in 1882 and published a paper on the subject in 1884 in which his postulates are most clearly stated. For his contributions on tuberculosis, Robert Koch was awarded the 1905 Nobel Prize for Physiology or Medicine. Koch had many other triumphs in medicine, including discovering the organism responsible for the disease cholera and developing methods to diagnose exposure to *M. tuberculosis* (the tuberculin test).

Koch's Postulates Today

For human diseases in which an animal model is available, it is relatively easy to use Koch's postulates. In modern clinical medicine, however, this is not always so easy. For instance, the causative agents of several human diseases do not cause disease in any known experimental animals. These include many of the diseases associated with bacteria that live only *within* cells, such as the rickettsias and chlamydias, and diseases caused by some viruses and protozoan parasites. So for most of these diseases cause and effect cannot be unequivocally proven. However, the clinical and epidemiological (disease tracking) evidence for virtually every infectious disease of humans lends all but certain proof of the specific cause of the disease. Thus, although Koch's postulates remain the "gold standard" in medical microbiology, it has been impossible to satisfy all of his postulates for every human infectious disease.

MiniQuiz

- How do Koch's postulates ensure that cause and effect of a given disease are clearly differentiated?
- What advantages do solid media offer for the isolation of microorganisms?
- What is a pure culture?

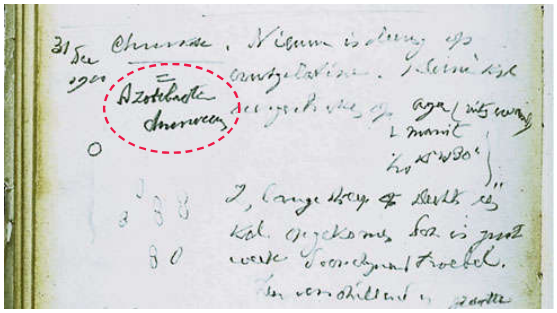
1.9 The Rise of Microbial Diversity

As microbiology moved into the twentieth century, its initial focus on basic principles, methods, and medical aspects broadened to include studies of the microbial diversity of soil and water and the metabolic processes that organisms in these habitats carried out. Two giants of this era included the Dutchman Martinus Beijerinck and the Russian Sergei Winogradsky.

Martinus Beijerinck and the Enrichment Culture Technique

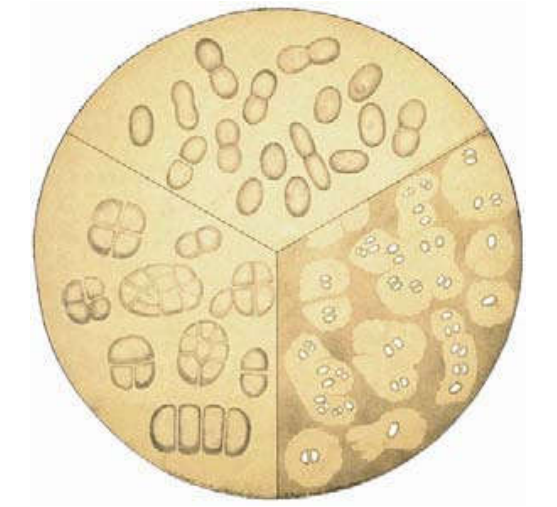
Martinus Beijerinck (1851–1931), a professor at the Delft Polytechnic School in Holland, was originally trained in botany, so he began his career in microbiology studying plants. Beijerinck's greatest contribution to the field of microbiology was his clear formulation of the **enrichment culture technique**. In enrichment cultures microorganisms are isolated from natural samples using highly selective techniques of adjusting nutrient and incubation conditions to favor a particular metabolic group of organisms. Beijerinck's skill with the enrichment method was readily apparent when, following Winogradsky's discovery of the process of nitrogen fixation, he isolated the aerobic nitrogen-fixing bacterium *Azotobacter* from soil (**Figure 1.21**).

Using the enrichment culture technique, Beijerinck isolated the first pure cultures of many soil and aquatic microorganisms, including sulfate-reducing and sulfur-oxidizing bacteria, nitrogen-fixing root nodule bacteria (Figure 1.9), *Lactobacillus* species, green algae, various anaerobic bacteria, and many others. In his studies of tobacco mosaic disease, Beijerinck used selective filtering techniques to show that the infectious agent (a virus) was smaller than a bacterium and that it somehow became incorporated into cells of the living host plant. In this insightful work, Beijerinck not only described the first virus, but also the basic principles of virology, which we present in Chapters 9 and 21.



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

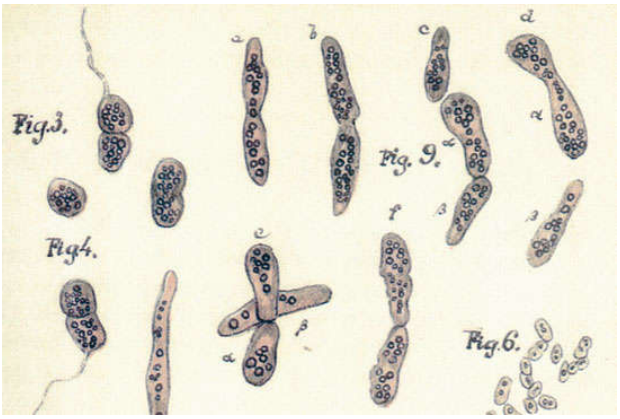
(a)



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

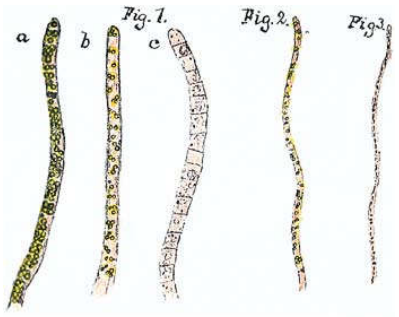
(b)

Figure 1.21 Martinus Beijerinck and *Azotobacter*. *(a)* A page from the laboratory notebook of M. Beijerinck dated 31 December 1900 describing the aerobic nitrogen-fixing bacterium *Azotobacter chroococcum* (name circled in red). Compare Beijerinck's drawings of pairs of *A. chroococcum* cells with the photomicrograph of cells of *Azotobacter* in Figure 17.18a. *(b)* A painting by M. Beijerinck's sister, Henriëtte Beijerinck, showing cells of *Azotobacter chroococcum*. Beijerinck used such paintings to illustrate his lectures.



From *Microbiologie du Sol*, used with permission

(a)



From Winogradsky, S. 1949. *Microbiologie du Sol*. Masson, Paris.

(b)

Figure 1.22 Sulfur bacteria. The original drawings were made by Sergei Winogradsky in the late 1880s and then copied and hand-colored by his wife Hèléne. *(a)* Purple sulfur phototrophic bacteria. Figures 3 and 4 show cells of *Chromatium okenii* (compare with photomicrographs of *C. okenii* in Figures 1.5a and 1.7a). *(b) Beggiatoa*, a sulfur chemolithotroph (compare with Figure 1.14).

Sergei Winogradsky, Chemolithotrophy, and Nitrogen Fixation

Sergei Winogradsky (1856–1953) had interests similar to Beijerinck's—the diversity of bacteria in soils and waters—and was highly successful in isolating several key bacteria from natural samples. Winogradsky was particularly interested in bacteria that cycle nitrogen and sulfur compounds, such as the nitrifying bacteria and the sulfur bacteria (**Figure 1.22**). He showed that these bacteria catalyze specific chemical transformations in nature and

proposed the important concept of **chemolithotrophy**, the oxidation of *inorganic* compounds to yield energy. Winogradsky further showed that these organisms, which he called *chemolithotrophs*, obtained their carbon from CO_2. Winogradsky thus revealed that, like phototrophic organisms, chemolithotrophic bacteria were *autotrophs*.

Winogradsky performed the first isolation of a nitrogen-fixing bacterium, the anaerobe *Clostridium pasteurianum,* and as just mentioned, Beijerinck used this discovery to guide his isolation of aerobic nitrogen-fixing bacteria years later (Figure 1.21). Winogradsky lived to be almost 100, publishing many scientific papers and a major monograph, *Microbiologie du Sol* (*Soil Microbiology*). This work, a milestone in microbiology, contains drawings of many of the organisms Winogradsky studied during his lengthy career (Figure 1.22).

MiniQuiz

- What is meant by the term "enrichment culture"?
- What is meant by the term "chemolithotrophy"? In what way are chemolithotrophs like plants?

1.10 The Modern Era of Microbiology

In the twentieth century, the field of microbiology developed rapidly in two different yet complementary directions—*applied* and *basic*. During this period a host of new laboratory tools became available, and the science of microbiology began to mature and spawn new subdisciplines. Few of these subdisciplines were purely applied or purely basic. Instead, most had both discovery (basic) and problem-solving (applied) components. **Table 1.3** summarizes these major subdisciplines of microbiology that arose in the twentieth century.

Several microbiologists are remembered for their key contributions during this period. In the early twentieth century many remained focused on medical aspects of microbiology, and even today, many dedicated microbiologists grapple with the impacts of microorganisms on human, animal, and plant disease. But following World War II, an exciting new emphasis began to take hold with studies of the genetic properties of microorganisms. From roots in microbial genetics has emerged "modern biology," driven by molecular biology, genetic engineering, and genomics. This molecular approach has revolutionized scientific thinking in the life sciences and has driven experimental approaches to the most compelling problems in biology. Some key Nobel laureates and their contributions to the molecular era of microbiology are listed in **Table 1.4**.

Many of the advances in microbiology today are fueled by the genomics revolution; that is, we are clearly in the era of "molecular microbiology." Rapid progress in DNA sequencing technology and improved computational power have yielded huge amounts of genomic information that have supported major advances in medicine, agriculture, biotechnology, and microbial ecology. For example, to obtain the sequence of the entire genome of a bacterium takes only a few hours (although sequence analysis is a much more time-consuming process). The fast-paced field of genomics has itself spawned highly focused new subdisciplines, such as *transcriptomics, proteomics,* and *metabolomics,* which explore, respectively, the patterns of RNA, protein, and metabolic expression in cells. The concepts of genomics, transcriptomics, proteomics, and metabolomics are all developed in Chapter 12.

All signs point to a continued maturation of molecular microbiology as we enter a period where technology is almost ahead of our ability to formulate exciting scientific questions. In fact, microbial research today is very close to defining the *minimalist genome*—the minimum complement of genes necessary for a living cell. When such a genetic blueprint is available, microbiologists should be able to define, at least in biochemical terms, the prerequisites for life. When that day arrives, can the laboratory creation of an actual living cell from nonliving components, that is, spontaneous generation under controlled laboratory conditions, be far off? Almost certainly not. Stay tuned, as much exciting science is on the way!

Table 1.3 *The major subdisciplines of microbiology*

Subdiscipline	*Focus*
I. Basic emphases[a]	
Microbial physiology	Nutrition, metabolism
Microbial genetics	Genes, heredity, and genetic variation
Microbial biochemistry	Enzymes and chemical reactions in cells
Microbial systematics	Classification and nomenclature
Virology	Viruses and subviral particles
Molecular biology	Nucleic acids and protein
Microbial ecology	Microbial diversity and activity in natural habitats; biogeochemistry
II. Applied emphases[a]	
Medical microbiology	Infectious disease
Immunology	Immune systems
Agricultural/soil microbiology	Microbial diversity and processes in soil
Industrial microbiology	Large-scale production of antibiotics, alcohol, and other chemicals
Biotechnology	Production of human proteins by genetically engineered microorganisms
Aquatic microbiology	Microbial processes in waters and wastewaters, drinking water safety

[a]None of these subdisciplines are devoted entirely to basic science or applied science. However, the subdisciplines listed in I tend to be more focused on discovery and those in II more focused on solving specific problems or synthesizing commercial products from microbial sources.

MiniQuiz

- For each of the following topics, name the subdiscipline of microbiology that focuses on it: metabolism, enzymology, nucleic acid and protein synthesis, microorganisms and their natural environments, microbial classification, inheritance of characteristics.