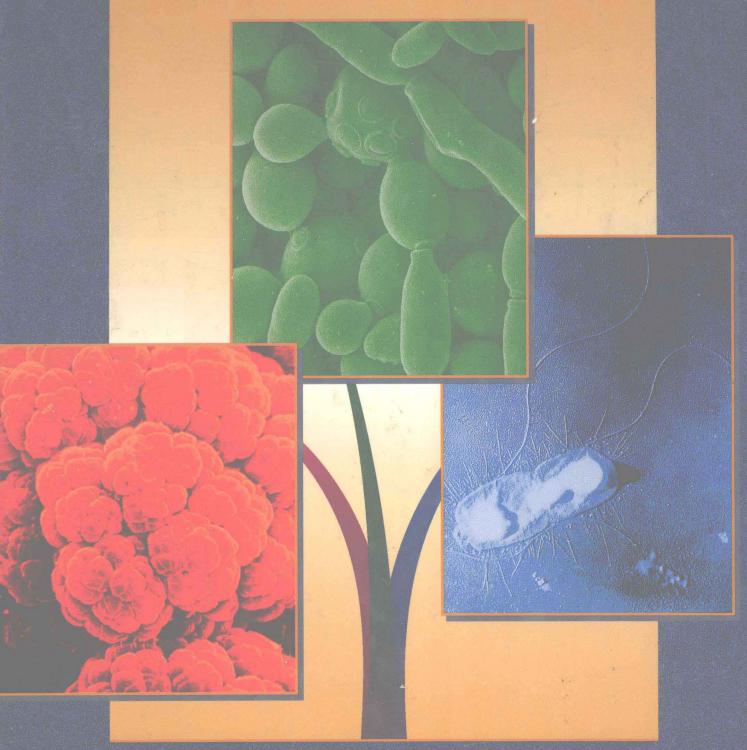
M PRINCIPLES OF ICROBIOLOGY



RONALD M. ATLAS

Second Edition

SECOND

PRINCIPLES OF MICROBIOLOGY

Ronald M. Atlas, PhD

University of Louisville Louisville, Kentucky



Dubuque, IA Bogota Boston Buenos Aires Caracas Chicago Guilford, CT London Madrid Mexico City Seoul Singapore Sydney Taipei Tokyo Toronto

Vice President and Publisher: James M. Smith

Editor: John E. Fishback

Developmental Editor: Kathleen M. Naylor Production Editor: Florence Achenbach

Art Designer: E. Rohne Rudder

Manufacturing Supervisor: David Graybill

Art: Graphic World Illustration Studio; Pagecrafters, Inc.; Tech Graphics Corp.

Cover Designer: E. Rohne Rudder

Cover Art: Bacteria (right): Escherichia coli (colorized) © Visuals Unlimited/F. Hossler

Archaean (*left*): *Methanosarcina* colony (colorized)
©Visuals Unlimited/Ralph Robinson

Eukaryote (center): Saccharomyces cerevisiae (colorized)

©Peter Arnold, Inc./David Scharf

SECOND EDITION

Copyright © 1997 by Wm. C. Brown Publishers

Previous edition copyrighted 1995 by Mosby-Year Book, Inc.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission of the publisher.

Permission to photocopy or reproduce solely for internal or personal use is permitted for libraries or other users registered with the Copyright Clearance Center, provided that the base fee of \$4.00 per chapter plus \$.10 per page is paid directly to the Copyright Clearance Center, 27 Congress Street, Salem, MA 01970. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collected works, or for resale.

Printed in the United States of America Composition by Graphic World, Inc. Lithography/color film by Accu-Color Printing/binding by Von Hoffmann Press, Inc.

Wm. C. Brown Publishers 2460 Kerper Blvd. Dubuque, Iowa 52001

Library of Congress Cataloging-in-Publication Data

Atlas, Ronald M., 1946-

Principles of microbiology / Ronald M. Atlas.

p. cm.

Includes bibliographical references and index.

ISBN 0-8151-0889-3

1. Microbiology. I. Title.

QR41.2.A843 1996

576-dc20

96-35923

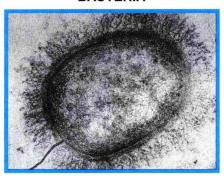
CIP

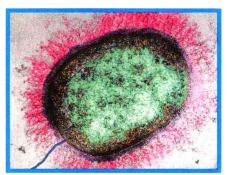
PRINCIPLES OF MICROBIOLOGY

SECOND EDITION

COLORIZING MICROGRAPHS

BACTERIA

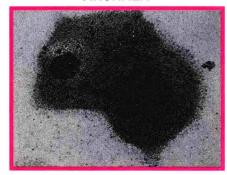




Pseudomonas aeruginosaOriginal micrograph (top)
Colorized micrograph (bottom)
Blue, flagellum (protein)

Red, glycocalyx (carbohydrate)
Gold, ribosomes (RNA)
Purple, cell wall (peptidoglycan)
Tan, cytoplasmic membrane
(phospholipid)
Green, bacterial chromosome
(DNA)

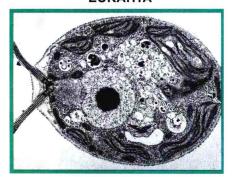
ARCHAEA

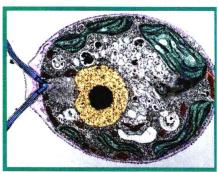




Sulfolobus brierleyi
Original micrograph (top)
Colorized micrograph (bottom)
Tan, cytoplasmic membrane
(glycolipid)
Blue, cell wall (S-layer)

EUKARYA





Chlamydomonas reinhardtii
Original micrograph (top)
Colorized micrograph (bottom)
Blue, flagellum (protein)
Gold, ribosomes (RNA)
Green, chloroplast (chlorophyll)
Tan, mitochondria (phospholipid)

KEY TO COLOR CODE

Chemical composition



Protein, lipoprotein



Peptidoglycan



Carbohydrate, glycoprotein, lipopolysaccharide



DNA



RNA



Lipid, phospholipid



ATP

Structure



Viral capsid, bacterial pili, flagella



Bacterial cell wall



Glycocalyx, capsule



Bacterial chromosome, plasmas, chloroplasts



Ribosomes, cytoplasm



Membranes, mitochondria, endoplasmic reticulum



Nucleus

Microorganism (taxonomic)



Bacteria



Archaea



Eukarya

Immunological



Immunoglobulins (antibodies)



Antigens



Preface

icrobiology is an extraordinarily diverse and exciting field of science that is on the cutting edge of scientific enquiry. New discoveries in microbiology are made daily many of which appear in the daily news reports as well as the scientific literature. There is a seemingly overwhelming and ever expanding state of knowledge about microorganisms—their diversity, activities, genetics, practical consequences, and biotechnological applications. This is evident in the wealth of new information contained in the second edition of Principles of Microbiology. In just the past year the complete genomes of representative bacteria, archaea, and eukaryotic microorganisms have been sequenced. Much has been learned about the molecular biology of microorganisms that provides the basis for understanding the relationships between genes and metabolic and ecologic functions. New tools have been developed for exploring previous unexplored realms of microbial diversity. We are on the verge of an unprecedented explosion in our molecular level understanding of the microbial world.

Bringing together today's discoveries with over 100 years of scientific enquiry, the second edition of Principles of Microbiology brings the scientific discipline of microbiology to its current state of the art. It focuses on the microorganisms—the unifying theme of this field of science. It captures the excitement of this contemporary and dynamic science, bringing forth the latest information available about microorganisms—their activities and relevance. It shows how microorganisms have evolved numerous strategies for carrying out essential life functions and how the activities of microorganisms contribute to the overall health and welfare of humans and the environment. It explains why some of the diverse and ubiquitous microorganisms are beneficial to humankind—describing the essential role of microorganisms for the maintenance of life on Earth-and why others are harmful-causing diseases of plants and animals. It provides insight into how events at the molecular level translate into activities of practical importance.

The second edition of *Principles of Microbiology* goes beyond the search for unifying principles to examine microbial diversity, especially at the mol-

ecular level. It provides greatly expanded coverage of microbial phylogeny and classification based on ribosomal RNA analyses. It replaces the traditional prokaryote-eukaryote paradigm with a comprehensive examination of the bacterial, archaeal, and eukaryal domains at all levels—including cell structure, metabolism, genetics, ecology, and taxonomy.

CONTENT AND ORGANIZATION OF THE BOOK

Principles of Microbiology is designed to provide a wealth of information about microorganisms in an organized manner that facilitates learning and understanding. Each chapter has the following general structure:

- Outline An outline on the opening page of each chapter sets out the scope of information that will be covered in the chapter.
- Text of chapter The text is designed to reveal the principles and diversity related to the topic of the chapter in sufficient molecular detail to develop an understanding of the topic. The style of the text is clear and readable. Terms are defined when they are first introduced. Key terms are shown in boldface or italics. The text is readable and filled with basic and practical information aimed at providing information and raising the enquiring interests of students.
- Boxes Within each chapter several boxes set off topics of special current or historical interest and methodologies used in the study of microbiology. Four types of boxes are used throughout the book: New Developments, Methodologies, Historical Perspective, and A Closer Look.
- Illustrations and micrographs Numerous micrographs and detailed illustrations throughout the chapter supplement the written text and help show the diversity and molecular biology of microorganisms. Some micrographs appear in black and white but many of the micrographs have been colorized as a pedagogical aid to connect them to the illustrations. The colors highlight specific structures and their underlying chemical compositions.

tion. In both illustrations and colorized micrographs, membranes and lipids are tan, bacterial cell walls and peptidoglycan purple, chromosomes and DNA green, ribosomes and RNA gold, carbohydrates and lipopolysaccharides red, and proteins and protein-based structures such as viral capsids blue.

- **Situational problems** Situational problems placed throughout the chapter are intended to challenge a student's creativity and to challenge him or her to think and to develop an in-depth understanding of microbiology.
- Study questions The set of review questions is intended to allow students to test their comprehension of the material they have just examined.
- Suggested supplementary readings The list of suggested readings is meant to supplement the text for more advanced courses and to sustain the interest of the student who finds a particular topic relevant to his or her purpose for having enrolled in an introductory microbiology course. Each suggested reading is annotated to describe its content.
- Sources of information on the World Wide Web The World Wide Web provides a new and exciting source of information that is upto-date and ever expanding. Information about microorganisms is added to the Web daily. The list of sources on the World Wide Web is intended to provide guidance for entering the Web and beginning an amazing exploration that reveals much information about microorganisms. Each entry is annotated to describe the content of that Web site. Surfing the Web will reveal more Web sites; users of the Web will discover that addresses often change and exploring the Web takes patience.
- Essays Each chapter ends with an essay by a prominent microbiologist describing the development and highlights of his or her scientific career. Each essay provides unique insights into the diverse fields of microbiological study. Many of the authors of these essays have served as Presidents of the American Society for Microbiology and have great perspective on what it means to be a microbiologist and the relevance of microbiology to science and human well being. Many have made breakthrough discoveries that have brought us to our current state of knowledge. These essays provide a critical perspective on the scope of microbiology and are a source of inspiration for students contemplating becoming a microbiologist.

The book has 19 chapters organized into seven major parts, two appendices, and an extensive glossary.

PART I SCIENTIFIC STUDY OF MICROORGANISMS

Part 1 reviews the scientific study of microorganisms. It introduces the microorganisms and the methods and methodologies used for their study. It presents a brief overview of the microbial world, exploring the realm of studies on microorganisms. It sets the stage for the three domains of bacteria, archaea, and eukarya. Overall, it gives a perspective on microbiology with its many vistas.

Chapter I Development of Microbiology as a Scientific Discipline

Chapter 1 provides an overview of the microorganisms that are the focus of this textbook. It traces the development of microbiology as a scientific discipline, showing how scientists think and how they use the scientific method for studies on microorganisms. It gives a historical perspective to microbiology, highlighting the contributions of noteworthy microbiologists such as Louis Pasteur and Robert Koch.

Chapter 2 Methods for Studying Microorganisms

Chapter 2 reviews the methodologies used by microbiologists. The science of microbiology depends on the ability to make observations using these methods. The chapter discusses the various forms of microscopy that are used to view microorganisms, the culture methods employed for studying microorganisms, and the development of molecular methodologies that have contributed to the understanding of microorganisms.

PART 2 MICROBIAL PHYSIOLOGY— CELLULAR BIOLOGY

Part 2, on microbial physiology and cell biology, examines the structure and function of cells of microorganisms. It explores many of the fundamental properties of living systems, showing how microorganisms have developed diverse solutions for meeting essential requirements for life. It provides a great deal of biochemical and molecular level detail on the diverse structures and metabolic functions of bacterial, archaeal, and eukaryal cells.

Chapter 3 Organization and Structure of Microorganisms

Chapter 3 covers the organization of bacterial, archaeal, and eukaryotic cells. The emphasis is placed on bacterial and archaeal cells, which are often covered only cursorily in general biology

classes. The chapter compares structures that have evolved in different organisms to serve similar functions, emphasizing the differences between bacterial, archaeal, and eukaryotic cells, many of which have important practical implications. It highlights the design of cellular structure and reveals how cells meet the essential requirements for life.

Chapter 4 Cellular Metabolism: Generation of Cellular Energy

Chapter 4 treats the bioenergetics of cellular metabolism, indicating how the principles of chemistry apply to biological systems. It focuses on the flow of energy through cellular metabolism and diverse strategies that occur among microorganisms for generating ATP.

Chapter 5 Cellular Metabolism: Biosynthesis of Macromolecules

Chapter 5 covers the metabolic reactions involved in forming cell biomass by autotrophic and heterotrophic metabolism. It treats the transformations of materials that are necessary for the formation of new cells and shows how cells can use simple starting substrates to make complex cell structures.

PART 3 MICROBIAL GENETICS—MOLECULAR BIOLOGY

Part 3, covering microbial genetics and molecular biology, examines topics of great contemporary interest. It focuses on the structure and functioning of DNA and RNA. It leads from the basic structure of DNA and expression of genetic information to the practical field of recombinant DNA technology that forms the basis of the biotechnological revolution. The chapters in this part provide great molecular detail that underpin our current understanding of life.

Chapter 6 DNA Replication and Gene Expression

Chapter 6 examines the role of DNA in heredity and control of cellular functions. It demonstrates how the discovery of the structure of DNA started the revolution in our understanding of the functioning of cells and led to the field of biotechnology. It examines the molecular basis of heredity and how DNA controls protein synthesis, relating genetics to the functioning of the cell. As in other chapters it compares the molecular biology of bacteria, archaea, and eukaryotes.

Chapter 7 Genetic Mutation, Recombination, and Mapping

Chapter 7 discusses the genetic changes that alter hereditary information. It shows the molecular events involved in recombination. It establishes the principles underlying the development of recombinant DNA technology, giving the basis for genetic engineering and its practical importance.

PART 4 MICROBIAL REPLICATION AND GROWTH

Part 4 examines microbial growth and replication. It shows that microorganisms have enormous potentials for population growth. It also examines the factors that control the rates of microbial reproduction.

Chapter 8 Viral Replication

Chapter 8 is about viruses. It covers the replication of viruses, distinguishing viruses from living organisms, and showing why viruses depend on host cells for their replication. It describes the stages of viral replication and the strategies employed for the replication of different viruses.

Chapter 9 Bacterial Growth and Reproduction

Chapter 9 treats bacterial growth and reproduction. It examines the consequences of bacterial reproduction by binary fission, showing that exponential increases of bacterial cell numbers occur due to reproduction by binary fission. The chapter also discusses the influences of various environmental factors, such as temperature, on bacterial growth rates.

Chapter 10 Control of Microbial Growth

Chapter 10 deals with the basis for control of microbial growth and the abilities of physical and chemical factors to kill or prevent the growth of microorganisms. It relates the modes of action of various antimicrobial agents to fundamental properties of microbial physiology and the abilities to control unwanted microbial growth.

PART 5 MICROORGANISMS AND HUMAN DISEASES

Part 5, about microorganisms and human disease, covers topics of importance related to human health. It emphasizes the relationship between the defenses of the human body and the virulence factors of pathogenic microorganisms. It describes how diseases are spread and how the transmission of pathogens can be controlled. It describes the molecular level events that underpin infection, disease, pathogenesis, and the body's defenses against pathogenic microorganisms.

Chapter II Immunology

Chapter 11 examines immunology and the defenses of the body against infections and diseases.

It discusses the innate and specific defense systems that protect the human body from infection, highlighting the complex nature of the body's lines of defense against disease. It reveals the intricacies of the integrated network of interactions of the immune system underlying molecular basis for the body's resistance to invasion by foreign substances. It also describes the consequences of failures of the immune system, including consequences of failures such as allergies and AIDS.

Chapter 12 Epidemiology and Public Health: Disease Transmission, Diagnosis, and Prevention

Chapter 12 gives an epidemiological perspective to selected human diseases caused by microorganisms. It examines the underlying principles of disease transmission and how understanding the basis of infectious disease can be used to block disease transmission. It includes a discussion of how vaccines are used to control and to eliminate specific diseases. It includes in depth coverage of emerging infections and problems arising from the evolution of antibiotic resistant microorganisms.

Chapter 13 Medical Microbiology: Pathogenesis and Pathology of Infectious Diseases

Chapter 13 covers the basis of pathogenesis of infectious diseases. It examines the molecular level properties of pathogenic microorganisms that contribute to their abilities to cause disease and the physiological changes that occur as a result of microbial infections. It also examines the basis for diagnosing various diseases.

PART 6 APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Part 6 examines applied and environmental microbiology, emphasizing some of the practical aspects of microbiology. It shows the essential functions played by microorganisms in ecology and the practical uses of microorganisms in biotechnology.

Chapter 14 Microbial Ecology and Environmental Microbiology

Chapter 14 examines the interactions among microorganisms and the roles of microorganisms in global biogeochemical cycling. It also discusses the importance of microorganisms for the maintenance of environmental quality, including the essential uses of microorganisms for degrading wastes and pollutants.

Chapter 15 Industrial Microbiology and Biotechnology

Chapter 15 is about biotechnology, including the economic uses of microorganisms for producing

foods, antibiotics, and numerous other products; recombinant DNA technology; and traditional practices employed in industrial microbiology.

PART 7 MICROBIAL DIVERSITY

Part 7 is a survey of microorganisms that describes their great diversity.

Chapter 16 Microbial Systematics: Evolution, Phylogeny and Classification

Chapter 16 examines the evolution of microorganisms and how rRNA analyses provide the means of developing phylogenetic classification systems. It discusses the molecular methodologies that are used to reveal evolutionary relatedness. It describes the new taxonomic organization driven by those molecular analyses and discusses the most recent phylogenetic classification of microorganisms. It describes the major evolutionary lineages within the bacterial, archaeal, and eukaryal domains of life.

Chapter 17 Bacterial Diversity

Chapter 17 provides a survey of the bacteria, revealing their great diversity in form and function. It is a very extensive chapter owing to the great diversity of the bacteria. The chapter describes the phenotypic characteristics of diverse bacteria that are observed in nature.

Chapter 18 Archaeal Diversity

Chapter 18 provides a survey of the archaea. It describes the taxonomy and ecology of the archaea and the unique physiologies of these microorganisms.

Chapter 19 Biodiversity of Eukaryotic Microorganisms: Fungi, Algae, and Protozoa

Chapter 19 gives a brief overview of eukaryotic microorganisms. It describes the diversity of the fungi, algae, and protozoa.

APPENDICES

The appendices provide a framework for review and study.

Appendix I Groups of Microorganisms Described in Bergey's Manual

Appendix II Chemistry for the Microbiologist

Glossary

An extensive glossary of microbiological terms serves as a guide to the terminology used by microbiologists.

ACKNOWLEDGMENTS

Many individuals have contributed to the writing and development of Principles of Microbiology. Some informally shared ideas about teaching microbiology, which augmented my own two decades of teaching introductory microbiology and bacteriology courses. Larry Parks, a colleague and microbial physiologist, worked exhaustively with me, helping to focus the presentation of material. Michel Atlas, my wife and health sciences reference librarian, also read each new draft for clarity of presentation and inclusion of the latest information. Kathleen Naylor, John Fishback, Florence Achenbach, and Liz Rudder oversaw the development and production of the book, including the illustrations and design.

The following individuals provided essays:

Alice S. Huang, New York University Norm Pace, University of California Moselio Schaechter, Tufts University Hans Schlegel, University of Göttingen John Ingraham, University of California—Davis David Schlessinger, Washington University Sam Kaplan, University of Texas Ken Berns, Cornell University Medical College Holger W. Jannasch, Woods Hole Oceanographic Institution John Sherris, University of Washington Carol Nacy, EntreMed, Inc. Gail Houston Cassell, University of Alabama— Birmingham Stan Falkow, Stanford University Rita R. Colwell, University of Maryland Jean Brenchley, *Penn State* Erko Stackenbrandt, Technical University— Braunschweig R.G.E. Murray, University of Western Ontario Carl Woese, University of Illinois Joan Bennett, Tulane University

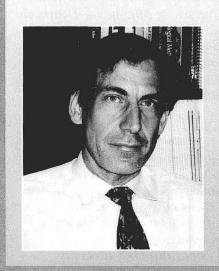
The following individuals provided reviews of the manuscript:

Delia Anderson, University of Southern Mississippi Kenneth L. Anderson, California State University, Los Angeles
Robert E. Andrews, Jr., Iowa State University
Robert K. Antibus, Clarkson University
Richard Bernstein, San Francisco State University
Prakash H. Bhuta, Eastern Washington University
James L. Botsford, New Mexico State University
Mary Burke, Oregon State University
Marcia Cordts, Cornell University
Michael Dalbey, University of California—Santa
Cruz

Michael W. Dennis, Eastern Montana College Alan A. DiSpirito, Iowa State University Merrill Emmett, University of Colorado—Denver Douglas Eveleigh, Rutgers University James Fishback, Kansas University Medical Center Linda E. Fisher, University of Michigan—Dearborn John W. Fitzgerald, University of Georgia Harold F. Foerster, Sam Houston State University William Gibbons, South Dakota State University Van H. Grosse, Columbus College Patricia Hartzell, University of California— Los Angeles George D. Hegeman, Indiana University-Bloomington John G. Holt, Michigan State University Scott W. Hooper, University of Mississippi Valeria Howard, Bismarck State College Douglas I. Johnson, University of Vermont David Kafkewitz, Rutgers University Scott T. Kellogg, University of Idaho Kenneth Keudell, Western Illinois University Brian K. Kinkle, University of Cincinnati Arthur L. Koch, Indiana University—Bloomington Michael Konkel, Washington State University Robin Kurtz, University of Wisconsin-Madison John J. Lee, City College of New York Alan C. Leonard, Florida Institute of Technology Susanne Lindgren, Oregon Health Sciences University Banadakoppa T. Lingappa, College of the Holy Cross Robert J.C. McLean, Southwest Texas State Robert G.E. Murray, University of Western Ontario Richard L. Myers, Southwest Missouri State University David R. Nelson, University of Rhode Island Nina T. Parker, Minot State University Kenneth Pidock, Wilkes University David Rose, Trenton State College Deborah D. Ross, Indiana University—Purdue Joseph Ross, Xavier University Sara Silverstone, California State University— Bakersfield Garriet W. Smith, University of South Carolina— Henry G. Spratt, Jr., Southeast Missouri State University John Sternick, Mansfield University Robert S. Stewart, Jr., Stephen F. Austin State Uni-Robert G. Taylor, Eastern New Mexico University Eugene D. Weinberg, Indiana University-Bloomington Gary R. Wilson, McMurray University Patricia L. Visser, Albion College Bruce A. Voyles, Grinnell College

Steven Woeste, Scholl College

DIVERSITY OF A MICROBIOLOGIST



Ronald M. Atlas University of Louisville

Ronald M. Atlas was born in New York City in 1946. He received a B.S. degree from the State University of New York at Stony Brook in 1968, an M.S. from Rutgers University in 1970 and a Ph.D. from Rutgers University in 1972. After one year as a National Research Council Research Associate at the let Propulsion Laboratory he joined the faculty of the University of Louisville in 1973. He is a member of the American Academy of Microbiology and was the recipient of the American Society for Microbiology award in Applied and Environmental Sciences. He currently is professor of biology at the University of Louisville.

Looking back I can see the path with all its twists and turns that has led me to my current position in microbiology. It has been a career path full of serendipity and surprises. My fascination with science and microorganisms began early. By the seventh grade I was carrying out experimental investigations at home on the effects of electromagnetic radiation on plants and of plant hormones on microorganisms and entering projects in science fairs. After my junior-year in high school I spent a summer at Cornell University in a National Science Foundation program that allowed me to take two courses in microbiology—one general survey course and the other an experimental methods course that allowed me to carry out investigative studies. The lectures on microbial ecology by Martin Alexander must have had a major impact as I remember them to this day. Mine, like all careers of microbiologists, is punctuated with mentors and memories.

Despite this early interest in science and microbiology I had no intention of becoming a microbiologist. My thoughts were on medicine and saving humanity from disease. My images were of diseases like tuberculosis and polio, as those diseases were still prevalent in the neighborhood in New York City where I grew up. A career as a physician, not as a scientist, seemed the likely career path as I went off to college. In fact, although I majored in biology, the only course in microbiology that I took as an undergraduate was a seminar course taught by Edward Battley where my one contribution was a paper on alcoholic fermentation followed by an evening of sampling a great variety of wines. Battley served as my undergraduate advisor and would later suggest that I explore graduate studies

at Rutgers.

While my career path was aimed at medicine, my real interest at Stony Brook was learning about the world. It was the 1960s, filled with protests about everything, and I was part of that quest for a better world. I spent weekends in Greenwich Village with the poet Alan Ginsburg. I was at Woodstock. I wandered through Europe and experienced the diversity of humanity. I met my wife at a corner of the 1967 Montreal Expo. I stood at Nietzsche's Oxford and Divinity street. And then I decided to go to graduate school and become a microbiologist.

At Rutgers I was assigned to the Department of Biochemistry and Microbiology in the Agriculture school, which later became Cook College. But all graduate students took the same introductory course—a year long course taught by 60 faculty members in microbiology. The course covered the breadth of microbiology. Each lecture was specialized and in depth. Diverse unconnected topics followed one another. We were left to our own and our discussions with individual faculty to form a coherent picture of microbiology. I was fortunate to be guided by David Pramer, who placed me in the laboratory of Richard Bartha to do my research and who both then and now has guided my career. Mentoring is an essential part of career development.

Working in Bartha's laboratory was a strange mix of formality and friendship. Bartha was born in Hungary and educated at the University of Göttingen in Germany, working in the laboratory of Hans Schlegel. He brought European formality to the laboratory at Rutgers. As students we feared him and always respectfully addressed him as Dr. Bartha or Professor Bartha. But students also frequently gathered at his house for dinners, and my wife and I even spent a week camping with him and his family: it was not until I had successfully defended my Ph.D. dissertation and he had presented me the option of whether to continue the formality of the relationship, that I first called him Richard.

During my graduate years I learned a great deal about what was involved in being a microbiologist. There were the courses but more importantly there was the laboratory. Bartha had a wonderful way of teaching students methods and then sitting back while the results were generated. Initially I tried to work on two projects-one a physiological project on the requirement for nickel by hydrogen-utilizing bacteria and the other an ecological project on the microbial utilization of petroleum hydrocarbons. I had little success with either and Bartha was clearly concerned by my lack of progress. David Pramer later described how Bartha asked the faculty to remove me from the program because I had been there a year and had yet to publish a paper. Fortunately I was given more time to develop as a scientist.

Bartha went off on a sabbatical at Woods Hole with Holger Jannasch and I continued to

muddle around the laboratory. I still didn't understand what it took to successfully carry out a scientific investigation could withstand critical peer review. I decided to focus my efforts on the oil degradation project and specifically the investigation of the factors limiting petroleum biodegradation in the oceans. This was just over a year after the Torrey Canyon oil spill and there was great public interest in the environment. My naivete proved useful. I didn't know that there were questions that weren't to be asked because of scientific dogma. By taking the wrong path I made new discoveries and that inspired me to work harder. I began to work day and night, tied to the laboratory bench by a quest for discovery. Our Saint Bernard dog, Bernie, would lay outside the laboratory door, patiently waiting, perhaps even to save me if an experiment went awry. Many of the experiments were with flammable solvents and more than one of Bartha's students had blown up the laboratory, on one occasion forcing Bartha to escape through the window and lower himself two stories using a rope. Fortunately there were no injuries and I was never responsible. My wife and many of the other graduate student spouses gathered each night in the department's conference room. Between experimental procedures I would join them and we would eat, talk, and drink. Our social life developed around the University and we still have many good friends from those days.

In retrospect it's not hard to understand why our work on oil biodegradation was so important. The fact that low nutrient concentrations in the oceans limit the rates of hydrocarbon biodegradation should have

been obvious. That overcoming those limitations could speed up the removal of petroleum pollutants also should have been clear. But it wasn't, and my first scientific presentation before the American Chemical Society showing that petroleum biodegradation in the oceans is nutrient limited was so controversial that the meeting had to be adjourned and the next scheduled paper cancelled. And who would have predicted that from our work bioremediation would emerge as a major biotechnological solution for cleaning up the environment of many pollutants; or that twenty years later I would work with Exxon to apply what I had discovered as a graduate student to the bioremediation of the Exxon Valdez Alaskan oil spill? While those outcomes were unpredicted in 1972, it was clear by the time I finished graduate studies that I was on the road to becoming a productive scientist. Bartha's patience was rewarded with ten publications from my graduate studies. Moreover we had established a collaborative relationship that continues to this day.

finishing graduate studies I took a postdoctoral position at the Jet Propulsion Laboratory of California Institute of Technology in an Antarctic research program that was part of the NASA Mars Viking lander project. The idea was to use the Antarctic dry valleys as a test site for detection systems that would be sent to Mars. Unfortunately several aircraft that were supposed to carry me to the Antarctic either crashed or developed mechanical problems: so I was left working in a freezer room in the laboratory in Pasadena. At lunch we engaged in great philosophical discussions asking what is life? and how could we design a univer-

sal experimental system that would detect all life-any time and any place? One day I proposed that I take the system, which measured the conversion of carbon dioxide to organic matter (one of the few universal reactions of living systems), to the Arctic. The idea was accepted and I was off to the Naval Arctic Research Laboratory at Point Barrow. Alaska. There besides testing the life detection system that eventually was sent to Mars, I renewed my studies on oil biodegradation for the Office of Naval Research.

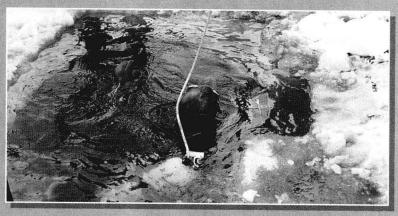
I continued working in Alaska after moving to the University of Louisville. I spent summers working with graduate students exploring the microbial populations of tundra and coastal waters. We expanded our studies to work through the winter. We began diving under the ice-even when surface air temperatures were -50° C to study the diversity of microbial communities and the abilities of indigenous microbial populations to degrade pollutants. Many of these studies employed numerical taxonomy to characterize diverse microbial populations, requiring extensive laboratory and computer analyses. I found myself managing a laboratory of eighteen people with all the inherent personnel and fiscal problems.

While the research was going well and was well funded, I felt left out of the molecular biology revolution. My research program was still focused on biochemistry and ecology. Therefore I encouraged some graduate students to begin studying the environmental fate of recombinant bacteria. The aim was to detect and contain genetically engineered microorganisms that might deliberately be released into the environ-

ment. We explored methods for containing genetically engineered microorganisms by using suicide vectors, working together with Asim Bej, Mike Perlin, and Sorin Molin. We struggled to increase the sensitivity for detecting microorganisms in soil and water. We couldn't do better than about 10,000 bacteria per gram of soil. Then one of my graduate students, Robert Steffan, received a vial of enzyme and some suggestions on how it might help us. It turned out to be tag polymerase and we were soon running polymerase chain reactions (PCR). I have never asked where the enzyme came from. I was just thrilled that we could pioneer the environmental applications of PCR. Within a year I could proclaim that we could detect a single genetically engineered microorganism in one hundred grams of soil or one liter of water. Along with several other students and colleagues we would use PCR to detect pathogens and indicator organisms in waters, including the bacterium Legionella and the protozoan Giardia. We even figured out how to use PCR for differentiating live from dead microorganisms. We used PCR to identify areas of significant health risks. Not only had I moved into the realm of molecular biology but I had also managed to join environmental and health related research.

With the research successes came local, national, and international recognition. There were requests for presentations at scientific meetings and I and my students began frequently traveling around the world. I was ill-prepared for the travel demands, which have grown to almost one trip per week. There were also requests to serve on various committees. Juggling time became a major challenge. At one point I was serving on twenty committees at the University of Louisville alone: member of the faculty senate, head of the arts and sciences personnel committee. head of the biology department's graduate committee, and chair of the University's academic excellence committee. The time commitment was enormous and drew me away from teaching and research. Later, I became Associate Dean of Arts and Sciences College and was even more removed from the aspects of academic life that I enjoy. After three years in that administrative post I began my return to the laboratory and classroom.

While I now limit my committee activities within the University, I continue to carry



Diving under the ice off Point Barrow, Alaska, to study microbial activities and microbial community diversity in marine waters and sediments.

out extensive service at the national and international level. I will never forget how my hand trembled when I voted to approve the first human gene therapy experiments as a member of the National Institutes of Health Recombinant DNA Advisory Committee (RAC). That vote followed two years of discussions about safety, ethics, and science. Those debates often were heated-as I discovered when I failed to notice the CNN cameras capturing me clashing with a woman representing handicapped groups over whether medical researchers should try to find a cure for blindness. I argued that bringing our understanding of molecular biology to the treatment of disease represented a historic step that would better human health. Besides my service on the RAC and various other government boards, I chair the American Society for Microbiology Public and Scientific Affairs Board Environment Committee. That activity frequently leads me to Washington where I testify before Congress on appropriations for science and advise the Administration and Congress on topics ranging from environmental biotechnology, to safe drinking water, and protecting the world against the use of biological weapons. Hardly a day goes by without dozens of faxes and E-mail messages about national and international events on which the American Society for Microbiology offers advice. Helping shape government policy on such important topics is an unexpected role for a scientist, but one that a handful of microbiologists like myself carry out with great devotion.

While developing an active research and service program I carry out an extensive teaching program-frequently racing between the classroom and the airport. As the only microbiologist in a biology department I have responsibility for all the undergraduate and graduate courses in microbiology. At the undergraduate level I teach courses for biology majors, premeds, nurses, and others. At the graduate level I teach courses in food microbiology, industrial microbiolgy, and microbial ecology. My students demand that I relate microbiology to their real world

experiences and career aspirations. I find myself connecting fundamental information about microorganisms with practical aspects of microbiology-explaining the applied consequences of microbial physiology, ecology, and molecular biology in practical terms such as disease treatment to which students can relate. Unlike many colleagues who think teaching interferes with research I have always found that it is the interactions with students that drives me further in quest of knowledge and forces me to stay current with the full scope of microbiological information.

My interest in teaching has also led me to write textbooks. When I began teaching microbial ecology only a few textbooks had been written in that field and I tried teaching using only handouts and assigned readings. My students objected and I soon wrote a microbial ecology textbook with Richard Bartha. It took several years and thousands of our own dollars but eventually we had a firstrate book that is now headed for its fourth edition. Soon I wrote my own textbooks for introductory microbiology courses-trying to provide practical connections to fundamental microbiological knowledge. With each new book there are new challenges. It gets more difficult to decide what information to include, especially with the explosion of molecular biology, and how to communicate that information in relevant terms.

With each new book, like this one, I have to learn more and more. I turn into a student, realizing the need for further education. My quest for discovery and integrating knowledge seemingly never ends. Such is the life and diversity of being a microbiologist.



Collecting water samples in Prince William Sound, Alaska, to study microbial degradation of oil.

Contents in brief

SCIENTIFIC STUDY OF MICROORGANISMS PART I 1 Development of Microbiology as a Scientific Discipline, 2 Methods for Studying Microorganisms, 44 MICROBIAL PHYSIOLOGY—CELLULAR BIOLOGY PART 2 3 Organization and Structure of Microorganisms, 84 Cellular Metabolism: Generation of Cellular Energy, 146 Cellular Metabolism: Biosynthesis of Macromolecules, 198 MICROBIAL GENETICS-MOLECULAR BIOLOGY PART 3 DNA Replication and Gene Expression, 234 Genetic Mutation, Recombination, and Mapping, 296 MICROBIAL REPLICATION AND GROWTH PART 4 Viral Replication, 366 Bacterial Growth and Reproduction, 414 Control of Microbial Growth, 454 PART 5 **MICROORGANISMS AND HUMAN DISEASES** 11 Immunology, 504 Epidemiology and Public Health: Disease Transmission, Diagnosis, and Prevention, 578 13 Medical Microbiology: Pathogenesis and Pathology of Infectious Diseases, 670 PART 6 APPLIED AND ENVIRONMENTAL MICROBIOLOGY Microbial Ecology and Environmental Microbiology, 738 Industrial Microbiology and Biotechnology, 820 MICROBIAL DIVERSITY PART 7 16 Microbial Systematics: Evolution, Phylogeny, and Classification, 888 Bacterial Diversity, 958 18 Archaeal Diversity, 1056 19 Biodiversity of Eukaryotic Microorganisms: Fungi, Algae, and Protozoa, 1098 APPEND X I, 1152 APPEND X 11, 1159

GLOSSARY, 1186

CREDITS, 1246

INDEX, 1250



Contents

PART ONE



SCIENTIFIC STUDY OF MICROORGANISMS

I DEVELOPMENT OF MICROBIOLOGY AS A SCIENTIFIC DISCIPLINE, 2

Microorganisms: The Unifying Focus of Microbiology, 3

Structural Organization of Microorganisms: Bacterial, Archaeal, and Eukaryotic Cells, 3

Box 1-1: Evolution of Microorganisms, 6 Box 1-2: Largest Prokaryotic Cells, 8

Microorganisms with Prokaryotic Cells—Archaea and Bacteria, 8

Microorganisms with Eukaryotic Cells—Fungi, Algae, and Protozoa, 9

Microorganisms Lacking Cells—Viruses, 10

Science of Microbiology, 11

Scientific Method, 11

Early Observation of Microorganisms, 12 Establishing that Microorganisms are Living Organisms, 13

Theory of Spontaneous Generation, 13 Pasteur and the Final Refutation of

Spontaneous Generation—Birth of Microbiology as a Science, 14

Demonstrating that Microorganisms Cause

Human Disease, 16

Koch and the Scientific Demonstration that Microorganisms Cause Disease, 16

Koch's Postulates, 18

Establishing that Chemicals Can Control Microbial Infections, 20

Wilcrobial infections, 20

First Synthetic Antimicrobial Drugs, 20

Discovery of Antibiotics, 21

Examining the Immune Response and Preventing Disease by Immunization, 22

Development of Immunization, 22

Developing an Understanding of the Immune Response, 24 Establishing the Roles of Microorganisms in Nature, 25

Developing an Understanding of Microbial Genetics and the Molecular Basis of Heredity, 26

Proving that DNA is the Hereditary Molecule, 27

Demonstrating the Structure of DNA—The Molecular Basis of Heredity, 28

Contemporary Microbiology, 30

Essay: Microbiology in the 1990s and Beyond: Challenges and Rewards, 40

2 METHODS FOR STUDYING MICROORGANISMS, 44

Microscopy, 45

Light Microscopy, 45

Properties of Light, 46

Distortions of Microscope Lenses, 47

Magnification, 48

Resolution, 48

Wavelength, 49

Numerical Aperture, 49

Oil Immersion Lens, 49

Contrast, 50

Simple Staining Procedures, 50

Differential Staining Procedures, 51

Types of Light Microscopes, 52

Brightfield Microscope, 52

Fluorescence Microscope, 53

Darkfield Microscope, 54

Phase Contrast Microscope, 54

Nomarski Differential Interference Contrast

Microscope, 54

Confocal Scanning Microscope, 56

Electron Microscopy, 58

Preparation of Specimens, 58

Staining, 58

Thin Sectioning, 58

Freeze Etching, 58

Transmission Electron Microscope, 60 Scanning Electron Microscope, 61

Culture of Microorganisms, 63

Culture Media, 63

Defined and Complex Media, 63

Selective and Differential Media, 63

Enrichment Culture, 64

Establishing a Pure Culture, 65

Sterilization, 65

Aseptic Technique, 66

Streak Plate, 67

Spread Plate, 68 Pour Plate, 68

Maintaining and Preserving Pure Cultures, 69

Characterization and Identification, 70

Identification Based on Physiological and

Metabolic Characteristics, 70

Serological Identification, 70

Gene Probe Identification, 71

Enumeration of Bacteria, 72

Viable Count Procedures, 73

Viable Plate Count, 73

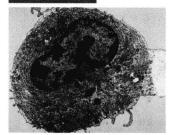
Most Probable Number (MPN) Procedures, 74

Direct Count Procedures, 74

Turbidimetric Procedures, 76

Essay: Climbing Around in the Big Tree: Molecular Microbial Ecology, 79

PART TWO



MICROBIAL PHYSIOLOGY— CELLULAR BIOLOGY

3 ORGANIZATION AND STRUCTURE OF MICROORGANISMS, 84

Cells: Basic Organizational Units of Living Systems, 85

Cytoplasmic Membrane: Movement of Material Into and Out of Cells, 89

Structure and Chemical Composition of the Cytoplasmic Membrane, 89

Box 3-1: Cell Size, 90

Biodiversity and Membrane Structure, 90 Cytoplasmic Membranes of Bacterial Cells, 92 Cytoplasmic Membranes of Archaeal Cells, 93

Box 3-2: Recovery of Cells and Analysis of Cellular Constituents, 94

Cytoplasmic Membranes of Eukaryotic Cells, 94

Transport Across the Cytoplasmic Membrane, 95 Passive Processes: Diffusion, 96

Passive Diffusion, 96

Osmosis, 97

Facilitated Diffusion, 97

Activity Energy-linked Transport Processes, 98

Active Transport, 98

Protonmotive Force, 99

Sodium-Potassium Pump, 100

Group Translocation—Phosphoenolpyruvate: Phosphotransferase System, 100

Binding Protein Transport, 102

Box 3-3: Mesosomes: Real Structures or

Artifacts?, 103

Cytosis—Eukaryotic Specific Transport, 103

External Structures That Protect the Cell, 104

Bacterial Cell Walls and Envelopes, 104

Chemical Composition of the Bacterial Cell

Wall: Peptidoglycan, 105

Effect of Lysozyme on the Bacterial Cell

Wall, 106

Box 3-4: Development of the Gram Stain

Procedure, 107

Effect of Penicillin on the Bacterial Cell

Wall, 108

Gram-positive Bacterial Cell Wall, 108

Gram-negative Bacterial Cell Wall and Cell

Envelope, 109

Outer Membrane, 109

Periplasm, 111

Box 3-5: Assembly of Gram-negative Cell Wall, 112

Cell Walls of Archaea, 114

Cell Walls Of Eukaryotic Microorganisms, 114

Algal Cell Walls, 114

Fungal Cell Walls, 115

Protozoan Cell Walls, 115

Bacterial Capsules, Slime Layers, and S

Layers, 115

Cellular Genetic Information, 116

Bacterial and Archaeal Chromosomes, 116

Plasmids, 118

Nucleus and Chromosomes of Eukaryotic

Cells, 118

Nucleus, 118

Chromosomes, 118

Ribosomes and Protein Synthesis: Information Flow in Cells, 120

Sites of Cellular Energy Transformations where ATP is Generated, 121

Sites of ATP Generation in Bacterial and

Archaeal Cells, 122

Bacterial and Archaeal Cytoplasmic

Membrane, 122

Bacterial Internal Membranes, 123

Sites of ATP Generation in Eukaryotic Cells, 123

Mitochondria, 123

Chloroplasts, 124

Coordinated Material Movement and Storage in Cells, 125

Material Movement Out of Bacterial Cells, 125 Material Storage in Bacterial Cells: Inclusion

Bodies, 126

Network of Membrane-bound Organelles in

Eukaryotic Cells, 127

Endoplasmic Reticulum, 127

Golgi Apparatus, 128

Lysosomes, 129

Microbodies, 129

Vacuoles, 129

Cytoskeleton, 129

Structures Involved with Movement of Cells, 130 Bacterial Flagella, 130