

---

# **Microbial Ecology**

**Principles, Methods, and Applications**

---

# Microbial Ecology

Principles, Methods, and Applications

**Morris A. Levin**

*Maryland Biotechnology Institute  
University of Maryland  
Baltimore, Maryland*

**Ramon J. Seidler**

*U.S. Environmental Protection Agency  
Corvallis, Oregon*

**Marvin Rogul**

*Maryland Biotechnology Institute  
University of Maryland  
Baltimore, Maryland*

**McGraw-Hill, Inc.**

New York St. Louis San Francisco Auckland Bogotá  
Caracas Lisbon London Madrid Mexico Milan  
Montreal New Delhi Paris San Juan São Paulo  
Singapore Sydney Tokyo Toronto

Library of Congress Cataloging-in-Publication Data

Microbial ecology : principles, methods, and applications / [edited by] Morris A. Levin, Ramon J. Seidler, Marvin Rogul.  
p. cm.—(The McGraw-Hill environmental biotechnology series)

Includes index.

ISBN 0-07-037506-2

I. Microbial ecology. I. Levin, Morris A. II. Seidler, Ramon J.  
III. Rogul, Marvin. IV. Series: Environmental biotechnology.  
QR100.M516 1992  
576'.15—dc20

91-25208

Copyright © 1992 by McGraw-Hill, Inc. All rights reserved. Printed in the United States of America. Except as permitted under the United States Copyright Act of 1976, no part of this publication may be reproduced or distributed in any form or by any means, or stored in a data base or retrieval system, without the prior written permission of the publisher.

1 2 3 4 5 6 7 8 9 0 DOC/DOC 9 7 6 5 4 3 2 1

ISBN 0-07-037506-2

*The editing supervisor for this book was Stephen M. Smith and the production supervisor was Suzanne W. Babeuf. It was set in Century Schoolbook by McGraw-Hill's Professional Book Group composition unit.*

*Printed and bound by R. R. Donnelley & Sons Company.*

Information contained in this work has been obtained by McGraw-Hill, Inc., from sources believed to be reliable. However, neither McGraw-Hill nor its authors guarantees the accuracy or completeness of any information published herein and neither McGraw-Hill nor its authors shall be responsible for any errors, omissions, or damages arising out of this information. This work is published with the understanding that McGraw-Hill and its authors are supplying information but are not attempting to render engineering or other professional services. If such services are required, the assistance of an appropriate professional should be sought.

---

# Contributors

**Richard L. Anderson** USEPA Environmental Research Laboratory, Duluth, Minnesota (CHAP. 31)

**John L. Armstrong** Biotechnology/Microbial Ecology Program, USEPA Environmental Research Laboratory, Corvallis, Oregon (CHAP. 24)

**Ronald M. Atlas** University of Louisville, Louisville, Kentucky (CHAP. 2)

**Tamar Barkay** Microbial Ecology and Biotechnology Branch, USEPA Environmental Research Laboratory, Gulf Breeze, Florida (CHAP. 33)

**Gerard F. Barry** Department of Biological Sciences, Monsanto Company, St. Louis, Missouri (CHAP. 8)

**Shoshana Bascomb** Baxter Healthcare Corp., MicroScan Division, West Sacramento, California (CHAP. 6)

**Harvey Bolton, Jr.** Pacific Northwest Laboratory, Richland, Washington (CHAP. 29)

**Myron K. Brakke** Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska (CHAP. 46)

**A. Breen** Department of Microbiology, University of Tennessee, Knoxville, Tennessee; and Center for Environmental Biotechnology, Knoxville, Tennessee (CHAP. 19)

**John D. Briggs** Department of Entomology, The Ohio State University, Columbus, Ohio (CHAPS. 34, 39)

**Susan Brown** Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, Iowa (CHAP. 44)

**Jeffrey J. Byrd** Division of Natural Science and Mathematics, St. Mary's College of Maryland, St. Mary's City, Maryland (CHAP. 5)

**C. Lee Campbell** Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina (CHAP. 35)

**Rita R. Colwell** Maryland Biotechnology Institute, University of Maryland, College Park, Maryland (CHAPS. 1, 4, 5, 6)

**C. R. Cripe** USEPA Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida (CHAP. 23)

**Stephen M. Cuskey** USEPA Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida (Deceased) (CHAPS. 17, 50)

**Donald H. Dean** Department of Biochemistry, The Ohio State University, Columbus, Ohio (CHAP. 44)

**Thomas C. Dockendorff** Department of Microbiology, University of Tennessee, Knoxville, Tennessee (CHAP. 19)

**David J. Drahos** BP Technologies, Inc., Stone Mountain, Georgia (CHAP. 8)

**Anne Fairbrother** USEPA Environmental Research Laboratory, Corvallis, Oregon (CHAP. 45)

**Stephen K. Farrand** *Departments of Plant Pathology and Microbiology, University of Illinois at Urbana/Champaign, Urbana, Illinois (CHAP. 16)*

**Susan W. Fisher** *Department of Entomology, The Ohio State University, Columbus, Ohio (CHAP. 39)*

**James K. Fredrickson** *Pacific Northwest Laboratory, Richland, Washington (CHAPS. 28, 29)*

**Michael A. Gealt** *Department of Bioscience and Biotechnology, Drexel University, Philadelphia, Pennsylvania (CHAP. 15)*

**D. Haefele** *Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, Iowa (CHAP. 49)*

**Charles Hagedorn** *Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (CHAPS. 26, 28)*

**Carol A. Hendrick** *Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, Iowa (CHAP. 44)*

**Stephen C. Hern** *USEPA Environmental Monitoring Systems Laboratory, Las Vegas, Nevada (CHAPS. 22, 27)*

**William E. Holben** *Center for Microbial Ecology and Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan (CHAPS. 4, 20)*

**Mary A. Hood** *Department of Biology, University of West Florida, Pensacola, Florida (CHAPS. 20, 25)*

**Clarence I. Kado** *University of California, Davis, California (CHAP. 18)*

**James L. Kerwin** *Botany Department, University of Washington, Seattle, Washington (CHAP. 37)*

**Donald A. Klein** *Colorado State University, Fort Collins, Colorado (CHAP. 30)*

**Ivor T. Knight** *Department of Biology, James Madison University, Harrisonburg, Virginia (CHAP. 4)*

**Jonathan Lamptey** *Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, Iowa (CHAP. 44)*

**Richard E. Lenski** *Center for Microbial Ecology, Michigan State University, East Lansing, Michigan (CHAP. 9)*

**Morris A. Levin** *Maryland Biotechnology Institute, University of Maryland, Baltimore, Maryland (CHAP. 1)*

**Cynthia Liebert** *Technology Research, Inc., USEPA Environmental Research Laboratory, Gulf Breeze, Florida (CHAP. 33)*

**Bruce Lighthart** *USEPA Environmental Research Laboratory, Corvallis, Oregon (CHAP. 22)*

**J. Lindemann** *Lindemann Consulting, El Cerrito, California (CHAP. 49)*

**S. E. Lindow** *Department of Plant Pathology, University of California, Berkeley, California (CHAP. 49)*

**Sarah A. McIntire** *Biology Department, Texas Woman's University, Denton, Texas (CHAP. 10)*

**Russel H. Melnts** *Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon (CHAP. 48)*

**Robert V. Miller** *Department of Microbiology, Oklahoma State University, Stillwater, Oklahoma (CHAPS. 7, 11)*

**Timothy J. Miller** *Department of Molecular Genetics, SmithKline Beckman Animal Health Products, King of Prussia, Pennsylvania (CHAP. 43)*

**Richard Y. Morita** *Department of Microbiology, College of Science and College of Oceanography, Oregon State University, Corvallis, Oregon (CHAP. 21)*

**O. A. Ogunseltan** *Department of Microbiology, University of Tennessee, Knoxville, Tennessee; and Center for Environmental Biotechnology, Knoxville, Tennessee (CHAP. 19)*

**Ronald H. Olsen** *Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan (CHAP. 17)*

**Susan B. O'Morchoe** *Department of Biochemistry and Biophysics and the Program in Molecular Biology, Stritch School of Medicine, Loyola University of Chicago, Maywood, Illinois (CHAP. 13)*

**J. G. Packard** *Graduate Program in Ecology, University of Tennessee, Knoxville, Tennessee; and Center for Environmental Biotechnology, Knoxville, Tennessee (CHAP. 19)*

**Norberto Palleroni** *New York University Medical School, New York, New York (CHAP. 1)*

**P. H. Pritchard** *USEPA Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida (CHAP. 23)*

**David C. Sands** *Department of Plant Pathology, Montana State University, Bozeman, Montana (CHAPS. 34, 35)*

**Dennis J. Saye** *Department of Biochemistry and Biophysics and the Program in Molecular Biology, Stritch School of Medicine, Loyola University of Chicago, Maywood, Illinois (CHAP. 13)*

**G. S. Saylor** *Department of Microbiology and Graduate Program in Ecology, University of Tennessee, Knoxville, Tennessee; and Center for Environmental Biotechnology, Knoxville, Tennessee (CHAP. 19)*

**Ramon J. Seidler** *USEPA Environmental Research Laboratory, Corvallis, Oregon (CHAPS. 14, 22, 27)*

**John A. Shaddock** *Office of the Dean, Texas Veterinary Medical Center, College of Veterinary Medicine, Texas A&M University, College Station, Texas (CHAP. 38)*

**Lyle Shannon** *Department of Biology, University of Minnesota, Duluth, Minnesota (CHAP. 31)*

**Jessup M. Shively** *Department of Biological Sciences, Clemson University, Clemson, South Carolina (CHAP. 42)*

**Joel P. Siegel** *Center for Economic Entomology, Medical Entomology Program, Illinois National History Survey, Champaign, Illinois (CHAP. 38)*

**J. Skujiņš** *Department of Biology, Utah State University, Logan, Utah (CHAP. 47)*

**Anne Spacie** *Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana (CHAP. 36)*

**Linda D. Stetzenbach** *Environmental Research Center, University of Nevada, Las Vegas, Nevada (CHAPS. 22, 27)*

**Gregory J. Stewart** *Department of Biology, University of South Florida, Tampa, Florida (CHAP. 12)*

**Guenther Stotzky** *Department of Biology, New York University, New York, New York (CHAPS. 29, 40)*

**Frieda B. Taub** *School of Fisheries, College of Ocean and Fishery Sciences, University of Washington, Seattle, Washington (CHAP. 32)*

**James M. Tiedje** *Center for Microbial Ecology and Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan (CHAPS. 4, 20)*

**Nancy J. Tomes** *Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, Iowa (CHAP. 44)*

**James L. Van Etten** *Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska (CHAP. 48)*

**Peter A. Vandenberg** *MicroLife Technics, Sarasota, Florida (CHAP. 41)*

**Anne K. Vidaver** *Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska (CHAP. 40)*

**Michael V. Walter** *Research and Development, Texaco Inc., Beacon, New York (CHAP. 14)*

**Sara F. Wright** *USDA-ARS Soil Microbial Systems Laboratory, Beltsville, Maryland (CHAP. 3)*

**Gerben J. Zylstra** *Center for Agricultural Molecular Biology, Rutgers University, New Brunswick, New Jersey (CHAP. 17)*

---

# Preface

Elevated expenditures for biotechnology research dealing with environmentally oriented products (e.g., pesticides and waste-treatment products) have resulted in large numbers of petitions for permits and licenses at federal and state regulatory agencies to conduct field tests involving the release of genetically engineered microorganisms. As data bases and other more traditional sources of information are reviewed, it is becoming increasingly apparent to researchers and regulators that ecological measurements and information are the most essential elements in assessing the risks of such releases.

Frustration and difficulties in finding this material have served to sensitize investigators and government officials to the need for a repository of factual information and current methodology in microbial ecology. This book represents a response by microbiologists and allied scientists to bring this knowledge together in a guide to researchers and regulators alike.

The text compiles, describes, and references procedures and concepts being used by environmental scientists in microbial ecology. The need for specific, reliable, and effective methods is essential to the development of protocols for evaluating releases of microbial pest control agents and other environmental applications of either naturally occurring or genetically altered microorganisms.

An advisory group consisting of representatives from the biotechnology scientific community, federal agencies involved in regulating biotechnology products, and public interest groups helped formulate the boundaries of this book, establish its organization, and select the experts who would be responsible for overseeing each of its six parts. The editors wish to acknowledge the many valuable contributions of the advisory group, which consisted of Dr. Mary Ann Danello (Food and Drug Administration), Dr. Robert Frederick and Dr. Elizabeth Milewski (EPA), Dr. Mary Gant (Executive Office of the President; OSTP), Dr. Doug McCormick (*Bio/Technology*), Dr. Margaret Mellon (Environmental Law Institute), and Dr. Richard Parry, Jr. (USDA). The editors, in addition, wish to gratefully acknowledge the financial support of the EPA's Office of Research and Development. However, this book does not represent the official position or opinion



of the U.S. Environmental Protection Agency or any agency with which a contributing author may be affiliated.

The selection of part coordinators was especially difficult since there are many persons who have made major contributions to the field of microbial and molecular ecology. The efforts of these coordinators in selecting chapter authors and reviewing the chapters were instrumental in the successful completion of this project.

Finally, the editors thank Dr. Edwin L. Schmidt, Dr. M. J. Sadonsky, and Dr. B. K. Kinkle, who reviewed the entire manuscript and provided many constructive comments to individual authors and part coordinators. Their efforts significantly improved the quality of individual chapters and the overall content of the end product.

*Morris A. Levin  
Ramon J. Seidler  
Marvin Rogul*

---

# Contents

Contributors	xxiii
Preface	xxvii

Introduction	1
Background	2
Data Quality	4
Importance of Quality Assurance and Control	4
Implementation of Quality Assurance Procedures	5
Recording Activities	5
Quality Control	6
Organization of This Book	6
References	8

## Part 1 Detection, Identification, Classification, and Enumeration

Chapter 1. Overview: Historical Perspective, Present Status, and Future Directions	11
Introduction	11
Available Methods	12
Ideal Methods	13
Comparison of Methods	14
Cultural: <i>Escherichia coli</i> As an Example	14
Heterotrophic Bacteria	16
Application of Methods	19
Actinomycetes As a Prototype	20
Conclusions	24
References	25

Chapter 2. Detection and Enumeration of Microorganisms Based upon Phenotype	29
Detection and Enumeration of Viable Microorganisms	30
Sampling and Processing	31
Viable Plating Procedures	32
MPN Method	35

Genetically Engineered Markers	37
Pigmentation and Bioluminescence	37
Antibiotic Resistance and Heavy Metal Tolerance	38
Substrate Utilization	39
References	41
 Chapter 3. Immunological Techniques for Detection, Identification, and Enumeration of Microorganisms in the Environment	 45
Introduction	45
Immunoglobulins	46
Antigenic Stimulation of Antibodies	46
Immunoglobulin Molecules	46
Affinity of Antibodies to Antigens	47
Microbial Antigens	47
Microbes in the Environment	47
Electrophoresis	48
Monoclonal versus Polyclonal Antibodies	49
Animal Responses	50
Monoclonal Antibody Production	52
ELISA	53
Fusion Procedure	54
Intrasplenic Immunization	55
Commercial or University Biotechnology Centers As Producers of Antibodies	55
Antibody Standardization and Purification	55
Adsorbed Polyclonal Antiserum	55
Purification of Antibodies	56
Testing Whether Monoclonal Antibodies Recognize the Same Antigenic Site	56
Selected Assays	57
Immunofluorescence	57
ELISA	58
Precipitation and Agglutination Tests	58
Dot-Immunoblot Tests	59
Immunomagnetic Particles to Extract Bacteria from Mixed Cultures	59
References	60
 Chapter 4. Nucleic Acid Hybridization Techniques for Detection, Identification, and Enumeration of Microorganisms in the Environment	 65
Introduction	65
General Principles	66
Strategies for Probe Construction	70
Total Genomic DNA	70
Cloned Restriction Fragments	71
Synthetic Oligodeoxynucleotide Probes	72
Labeling Techniques and Hybridization Formats	73
The Mixed-Phase Format	74
Radioactive Labels	76

Nonradioactive Labels and Alternative Formats	80
Polymerase Chain Reaction	82
Recovery of Nucleic Acids from Environmental Samples	83
Soil and Sediment	84
Water	85
References	86
 Chapter 5. Microscopy Applications for Analysis of Environmental Samples	 93
Introduction	93
Morphology-Based Identification	93
Epifluorescent Microscopy	95
Total Count	95
Viable Cell Detection and Enumeration	99
Image Analysis	103
Fluorescent Antibody Microscopy	103
Electron Microscopy	105
Summary	106
References	107
 Chapter 6. Application of Numerical Taxonomy in Microbial Ecology	 113
Introduction	113
Data Acquisition and Preparation	114
Manual Input of Data	115
Automated Acquisition of Data	116
Output Format	116
Data Preparation	116
Clustering Models	117
Choice of OTVs and Attributes	117
Measure of Resemblance between Pairs of OTVs	118
Methods of Cluster Formation	119
Portraying Results of Clustering Techniques	120
Statistical Packages Available	125
Effect of Choice of Clustering Techniques on Classifications	125
Validation of Classifications	125
Identification Models	129
Identification Models for Binary Data	129
Identification Models for Continuous Data	131
Criteria for Acceptability of Identification	131
Additional Testing	132
Software Available for Identification	132
Assessment of Performance of Identification Systems	133
Commercial Identification Systems	133
Conclusions	134
References	135

## Part 2 Genetic Transfer and Stability

<b>Chapter 7. Overview: Methods for the Evaluation of Genetic Transport and Stability in the Environment</b>	<b>141</b>
Introduction	141
Background Development, Significance, History: Mechanisms of Gene Transfer in Bacteria	142
General Characteristics of Methods to Be Covered	144
Phase 1: Idealized Laboratory Studies for the Determination of Maximal Potentials	144
Phase 2: Laboratory Simulations—Microcosm Studies	144
Phase 3: Environmental Studies	145
Environmental Implications and Considerations	145
Cell Concentration and Probability of Association	146
Temperature	146
Particulate Matter	147
Barriers to the Entry of DNA into the Recipient Cell	147
Restriction—Modification	148
Incompatibility	149
Superinfection Immunity	149
Fertility Inhibition	150
DNA Replication Compatibility	150
Recombination	150
Consideration of Potentials for Gene Expression	151
Other Factors That May Affect Gene Stability	152
Mutation	152
Transposition	153
Conclusions	154
Overview of Part 2	154
References	155
 <b>Chapter 8. Assessment of Genetic Stability</b>	 <b>161</b>
Introduction	161
Principles	162
Current Practices and Trends	163
Monitoring Gene Presence	163
Introduction of Monitoring Element	166
Methods	167
Chromosomal Insertion Procedures	167
Verification of Recipient Strain Identity-Integrity	172
Gene Insertion Verification	175
Insert Purity	175
Insert Stability	176
Summary and Conclusions	177
References	179

<b>Chapter 9. Relative Fitness: Its Estimation and Its Significance for Environmental Applications of Microorganisms</b>	<b>183</b>
Introduction	183
Definitions and Principles	185
Specific Methods	187
Assumptions	192
Summary and Conclusions	196
References	198
 <b>Chapter 10. Analysis of Conjugation in Bacteria</b>	 <b>199</b>
Introduction and History	199
Introduction	199
History	199
General Considerations	208
Gram-Negative Organisms	209
Gram-Positive Organisms	212
Mobilization	213
Biological-Environmental Parameters	213
Specific Procedures	216
Liquid Matings	216
Surface Matings	217
Plate Matings	217
Qualitative Methods	218
Transiently Heterozygous Donor Strains	218
Summary and Conclusions	219
References	220
 <b>Chapter 11. Methods for Evaluating Transduction: An Overview with Environmental Considerations</b>	 <b>225</b>
Introduction: A Historical View of Transduction	225
Principles	227
Lysogeny	228
Generalized Transduction	231
Cotransduction	232
Specialized Transduction	237
Diversity of Transduction As a Mechanism of Gene Transfer in Bacteria	238
Methods	238
Enumeration of Phage	239
Isolation of Bacteriophage from Nature	240
Induction of Lysogens	240
A General Method for Transductional Analysis	241
Variables to Be Considered in Designing a Specific Transduction Assay	243
Conclusions	244
References	244

<b>Chapter 12. Natural Transformation and Its Potential for Gene Transfer in the Environment</b>	<b>253</b>
Historical Perspectives and Introduction	254
Mechanism of Natural Transformation	258
Development of Competence	259
DNA Binding	261
DNA Uptake	262
Recombination	263
Current Practices	266
Trends in Natural Transformation	270
Specific Methods for Measuring Environmental Transformation—	
Strain-Specific Assays	272
Selection of Chromosomal Antibiotic Resistance Mutations in Bacteria	273
Juni Lysis Procedure	274
Preparation of Purified DNA	274
Determining DNA Concentration	274
Plate Transformation Method (Qualitative Assay)	275
Filter Transformation Assay (Quantitative Assay)	276
Generic Transformation Screen Using Multimeric Plasmid DNA	276
References	278
 <b>Chapter 13. Evaluating the Potential for Genetic Exchange in Natural Freshwater Environments</b>	 <b>283</b>
Introduction	283
Principles	285
Establishing Model Systems	285
Test Systems in Situ	289
Enumeration of Recombinant Organisms	291
Effect of Environmental Parameters	296
Methods	297
Conjugation in Fresh Water	297
Transduction in Fresh Water	300
Transformation in a Freshwater Environment	306
Conclusion	306
References	306
 <b>Chapter 14. Measurement of Conjugal Gene Transfer in Terrestrial Ecosystems</b>	 <b>311</b>
Introduction and Historical Perspective	311
Current Practices	312
Specific Methods to Evaluate Conjugal DNA Transfer	314
Laboratory Techniques	314
Soil Slurries	316
Terrestrial Microcosms	317
Summary and Conclusion	324
References	325

<b>Chapter 15. Gene Transfer in Wastewater</b>	<b>327</b>
Introduction	327
Gene Dissemination in Wastewater	328
Waste Treatment	329
Plasmid Transmission in Wastewater	330
Mobilization in a Laboratory Waste-Treatment Facility (LWTF)	332
Conclusion	340
References	340
<b>Chapter 16. Conjugal Gene Transfer on Plants</b>	<b>345</b>
Introduction and History	345
General Considerations and Issues	347
Donors and Recipients	347
Markers for Selection and Counterselection	349
Mating Substrates	351
Environmental Conditions	353
Questions of Purpose	354
Experimental Designs	355
Preparation of Inocula	355
Inoculations	355
Incubations	356
Recovery of Bacteria from Inoculated Plants	357
Selections	357
Screens	358
Controls	358
Enumeration and Expression of Mating Frequencies	359
Summary and Conclusions	360
References	360
<b>Chapter 17. Construction of Plasmids for Use in Survival and Gene Transfer Research</b>	<b>363</b>
Introduction and General Considerations	363
Benchmark Plasmids	364
Plasmids with a Unique DNA Sequence for Identification of Released Organisms and Indigenous DNA Recipients	366
Other Vector Systems Useful for Environmental Testing	368
Summary and Conclusions	368
References	368
<b>Chapter 18. Lux and Other Reporter Genes</b>	<b>371</b>
Introduction	371
Vector Construction	372
Basic Requirements	372
Broad-Host-Range Vectors	373
Vector Mobilization	374



Assessing Microbial Expression by Use of Reporter Genes	375
Antibiotic Resistance Genes	375
Other Reporter Genes	378
Optimization of Gene Expression	382
Use of Superpromoters	382
Use of the $\Omega$ Translation Enhancer	383
Methods and Applications	383
Antibiotic Resistance	383
Summary and Conclusions	388
References	389
 Chapter 19. Practical Considerations of Nucleic Acid Hybridization and Reassociation Techniques in Environmental Analysis	 393
Introduction	393
Molecular Basis of Hybridization Reactions	394
DNA Hybridization Procedures	397
Colony Hybridization	397
Dot-Slot Hybridization	399
Southern Hybridization	399
Common Hybridization Protocol Steps	400
Binding of Target DNA	400
Prehybridization	400
Hybridization	401
Washing the Filter	401
Amount of Probe Used	402
Incubation Time	402
Single- vs. Double-Stranded Probes	402
Length of Probe	403
Detection and Quantitation	404
Other Considerations	404
Filter Choice	404
Reprobing	404
MPN-DNA Hybridization	405
Probe and Target Isolation and Purification	406
Direct Recovery of DNA from Environmental Samples	407
Quantitation	412
Isolation of Probe DNA	412
Probe Labeling	414
Applications for Solution Hybridization and DNA Reassociation	
Kinetic Analysis	416
Conclusion	418
References	418
 Part 3 Fate and Transport	
Chapter 20. Overview of Fate and Transport of Microbes	423
Introduction	432