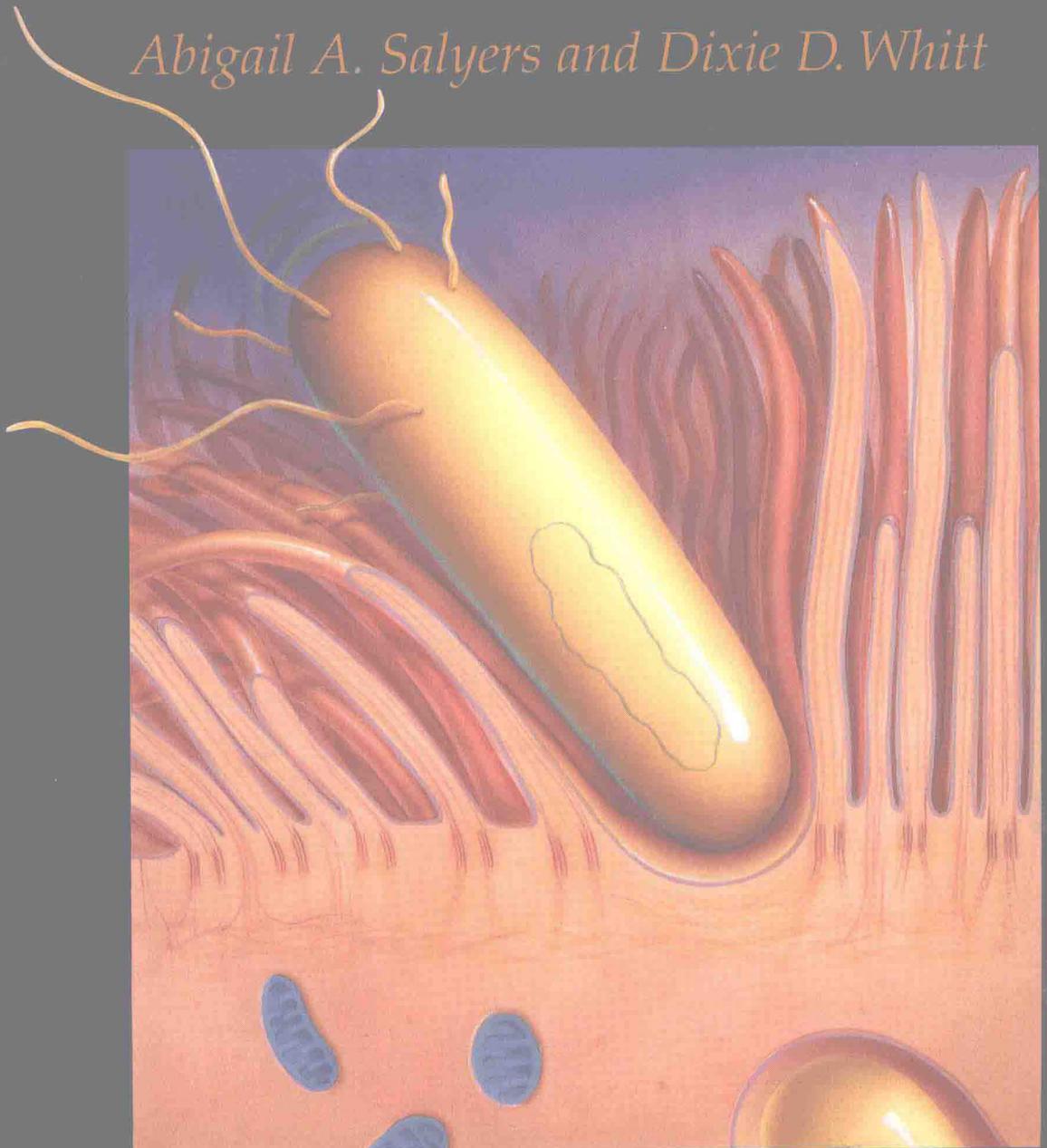


Bacterial Pathogenesis

A MOLECULAR APPROACH

Abigail A. Salyers and Dixie D. Whitt



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ASM Press
Washington, D.C.

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N.W., Washington, DC 20005

Library of Congress Cataloging-in-Publication Data

Salyers, Abigail A.

Bacterial pathogenesis : a molecular approach / Abigail A. Salyers and Dixie D.
Whitt.

p. cm.

Includes bibliographical references and index.

ISBN 1-55581-070-5

1. Bacterial diseases—Pathogenesis. 2. Virulence (Microbiology) I. Whitt, Dixie D.

II. Title.

[DNLM: 1. Bacteria—pathogenicity. 2. Bacterial Infections—etiology. 3. Bacterial
Infections—prevention & control. 4. Host-Parasite Relations. 5. Virulence.

QZ 65 S186b 1994]

QR201.B34S24 1994

616'.014—dc20

DNLM/DLC

for Library of Congress

94-1468
CIP

10 9 8 7 6 5

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Printed in the United States of America

Cover and interior design: Susan Brown Schmidler

Cover illustration: Terese Winslow

Bacterial Pathogenesis
A MOLECULAR APPROACH

We dedicate this book to Dwayne C. Savage, the originator of the course that gave rise to this book and one of the first people to advocate the idea that bacterial disease is a complex, multifactoral interaction between the host and the bacteria.

Preface

The application of molecular techniques to the study of bacterial pathogenesis has made possible discoveries that are changing the way scientists view the bacterium-host interactions, but most of these new insights have not yet made their way into basic texts on medical microbiology. In fact, the revolution in bacterial pathogenesis has been largely inaccessible even to research scientists trained in other areas, because although thousands of research papers have appeared in print, these and the occasional review articles are written primarily for specialists in the field. The only books available on the subject have been collections of papers presented at scientific meetings or specialized texts covering a single area in depth. The purpose of this text is to bring together, in readable form, information about recent advances in our understanding of how bacteria cause disease. It is the first text designed to provide an introduction to bacterial pathogenesis for advanced undergraduates, graduate students, and medical students interested in infectious diseases.

Research on the molecular basis of bacterial pathogenesis of necessity transcends established boundaries between cell biology, bacteriology, and immunology. Approaches and concepts from all of these areas have been used to answer the basic question of how bacteria establish an infection and to solve the practical problem of how to design safe and effective vaccines. Thus, in writing this text, we have included information from a variety of areas normally covered as separate disciplines in separate texts. The first eight chapters are intended to provide the necessary background for understanding the material covered in chapters on individual diseases. Throughout the text, we have tried to give a sense of the excitement and challenges offered by this new field. There are few things more discouraging to a student than the impression given by many texts that everything important has already been done in the area. Accordingly, we have gone out of our way to indicate the still unanswered questions and the problems remaining to be solved.

No attempt has been made to cover every known bacterial pathogen (the approach popularly known as the "bug parade") or even to cover every disease caused by the bacterial species that have been included. Instead, we have chosen

to provide an in-depth treatment of those diseases that have been most intensively studied at the molecular level and that have provided new insights into the bacterium-host interaction. In general, we have tried to avoid reproducing what is already well covered in available texts on medical microbiology and have focused instead on topics not covered in these texts. This criterion has caused us to omit some medically important diseases and bacterial pathogens. Also omitted are detailed discussions of standard diagnostic tests, except in cases where molecular approaches are being taken or special problems have arisen.

Most chapters contain boxed items. Generally these include material that does not fit conveniently in the body of the text itself: practical examples of the material being covered, historical notes of interest, humorous aspects of infectious diseases, and controversial issues. Some cover unexpected new research findings that establish new connections or suggest new aspects of bacterial virulence. At the end of each chapter are a brief summary of the chapter contents, a set of selected readings, and a set of questions. The summary is intended as a review of the main points made in the chapter. The selected readings are designed to give students an entry point into current literature. They are not intended as a complete list of current literature in the area or as a list of "classic" papers. In most cases, only papers published after 1989 are cited. The questions at the end of the chapter have two purposes. Some are designed to help the student review material covered in the chapter and to assess mastery of the subject. Others are open-ended questions, designed to encourage students to think critically about issues covered in the chapter. Answers to the question are given at the end of the book (see Appendix 4).

One of the most daunting aspects of providing a text of this type was the knowledge that no two people, however conscientious and well intentioned, could adequately and accurately convey all of the new information in the variety of areas covered without some outside help. Accordingly, each chapter has been reviewed by at least one expert in the area being covered. Many of these reviewers were kind enough to be complimentary, and all gave encouragement and constructive criticism. We extend special thanks to the following reviewers for taking the time to do such a thorough job: Alan G. Barbour, M.D., University of Texas Health Science Center; Stephen B. Calderwood, M.D., Massachusetts General Hospital; Virginia L. Clark, Ph.D., University of Rochester Medical Center; P. Patrick Cleary, Ph.D., University of Minnesota; R. John Collier, Ph.D., Harvard Medical School; Michael P. Doyle, Ph.D., University of Georgia; B. Brett Finlay, Ph.D., University of British Columbia; Vincent A. Fischetti, Ph.D., Rockefeller University; Peter H. Gilligan, Ph.D., University of North Carolina Hospital; Paul A. Gulig, Ph.D., University of Florida; Anne Morris Hooke, Ph.D., Miami University; Barbara H. Iglewski, Ph.D., University of Rochester Medical School; Ralph R. Isberg, Ph.D., Tufts University; James R. Johnson, M.D., University of Minnesota; David M. Lyerly, Ph.D., Virginia Polytechnic Institute and State University; Anthony T. Maurelli, Ph.D., Uniformed Services University of the Health Sciences; Virginia L. Miller, Ph.D., University of California at Los Angeles; Stephen B. Morse, Ph.D., Centers for Disease Control and Prevention; Steve L. Moseley, Ph.D., University of Washington; Irving Nachamkin, Ph.D., University of Pennsylvania; Alison D. O'Brien, Ph.D., Uniformed Services University of the Health Sciences; David H. Persing, M.D., Ph.D., Mayo Clinic; Daniel A. Portnoy, Ph.D., University of Pennsylvania; Thomas M. Shinnick, Ph.D., Centers for Disease Control and Prevention; Ronald K. Taylor, Ph.D., Dartmouth Medical Center; Elaine Tuomanen, M.D., Rockefeller University; Janis J. Weis, Ph.D., University of Utah; Tracy D. Wilkins, Ph.D., Virginia Polytechnic Institute and State University. We are also grateful to Stanley Falkow of Stanford University for his consistent encouragement and helpful comments during the long and sometimes painful process of writing and revising.

We thank the University of Illinois students who took Microbiology 326 and endured the earlier, much rougher, versions of this text. Their enthusiasm and comments helped teach us how to write such a text for a student audience. It is largely due to their encouragement that we decided to forsake our normally conservative, deadpan style of scientific writing and try for a livelier, occasionally humorous, style. We also thank Patrick Fitzgerald, Director of ASM Press, for his enthusiasm, for his many helpful suggestions, and for managing to convince so many leading figures in the field to read and critique parts of this book. Dr. Anne Morris Hooke deserves special thanks for her many helpful suggestions at different stages in the writing process. A.S. thanks the people in her laboratory for patiently suffering through the various stages of the writing process and thanks Professor Arnie Levine of Princeton University and the people in his laboratory for their help and advice during the year she spent on sabbatical in that laboratory. D.W. thanks Greg for continued support and patience during what at times seemed like a project without end.

Foreword

Thirty years ago, as a new Ph.D. assistant professor, my initial teaching assignment was to teach a medical microbiology course. At my first medical school curriculum committee meeting, both basic and clinical scientists called the course "Bugs and Drugs." As a medical bacteriologist, I was offended by this designation. I was young enough at the time to say so explicitly to the members of the committee. The biochemist sneered that a great deal was known about *Escherichia coli*, but most of the information about bacterial pathogens was "primitive." The chief of surgery spoke to me kindly but reinforced the idea that very little progress in understanding bacterial pathogens had been made since antibiotics had made their appearance. A number of people in medicine, in basic science, and, certainly, in granting agencies responded the same way. Several very influential basic scientists, one a Nobel laureate, suggested that studying the genetics of typhoid and dysentery bacilli was a waste of my time. "Who cares anymore?" asked the Nobelist rhetorically.

In the late 1960s and continuing into the early 1980s, attitudes began to change. It was not just that there were R-plasmids interfering with the "drugs;" there was also a realization that some of the most urgent health care crises were caused by infectious "bugs," such as legionnaire's disease, toxic shock syndrome, Lyme disease, and HIV. Compare the table of contents of a medical microbiology text of 15 years ago and a contemporary text. Some of the organisms prevail in their importance, and some were not even dreamed of. Ulcers are caused by the bacterium *Helicobacter pylori*. The crises continue. There is a resurgence of serious infectious diseases such as mycobacterial disease and group A streptococcal disease. There has been a spawning of symposia dealing with the specter of "emerging infectious diseases." Most people in the world continue to die of infectious diseases. In the United States it is likely that just as many people die today of infectious diseases as they did before the advent of antibiotics; they simply die of different infectious agents than before. This unhappy event will continue until we understand more about microbial disease.

In the 1970s a revolution was brought about by recombinant DNA and other advances in molecular biology, cell biology, and immunology. Those of us who

had struggled to study the biology of pathogens in the 1960s found that the resolving power of the holy trinity of bacterial genetics—transduction, transformation, and conjugation—was simply not good enough to dissect the genes important in pathogenicity from those more numerous bacterial genes concerned with what Lew Thomas called “their own business [of] browsing and recycling the rest of life.” Molecular cloning, the use of transposon mutagenesis, DNA sequencing, and all of the other wonderful methods so nicely described in the early chapters of this book changed this. There was a virtual explosion of knowledge about the biology of pathogenic microorganisms. The subtleties and nuances of the host-microbe interaction are scientifically speaking breathtaking. A field of study, now generally called microbial pathogenesis, has emerged and excited microbiologists, physicians, and scientists from a legion of different disciplines.

Initially a good deal of effort was spent identifying genes that might be important in pathogenicity. The general approach was to clone the gene, mutate it in a defined way, return the gene to the organism of interest to see if pathogenicity was affected, and then restore the gene function to see if pathogenicity returned. This simplified form of molecular Koch’s postulates is explained wonderfully in this book and its implications expanded over my original version. Yet as time passed, it became clear that bacteriologists could no longer pay lip service to the “host” of the oft-cited “host-parasite relationship.” It was necessary to study the cell biology of eucaryotic cells if one was to fully understand intracellular parasitism and the full consequences of what occurs when a microbe attaches to its specific receptor on the surface of a host target cell. Cell biologists have tried wisely for most of their careers to keep microbes out of their tissue cultures. Pathogens or not, bacteria growing in animal cell cultures create difficulties. Yet, the cell biologists recognized that microbes knew more about the niceties of animal cell biology than they did or had ever expected. Microorganisms exploit normal host cell mechanisms for their own end. Understand how the microbe does it, and you may recognize how the cell does it normally. Similarly, the immunological response to a pathogen was no longer restricted to studies performed by individuals trained solely in immunology. Microbiologists began to realize that many of the widely held views of the immune response to bacteria were based on the use of heat-killed microbes or bits of their purified proteins injected intraperitoneally into an inbred strain of mouse. Many facets of the immune response to pathogenic agents are currently being reexamined.

Anyone involved in the field of microbial pathogenesis knows that after every seminar, symposium talk, or lecture, at least one person approaches and asks where he or she can read more about the field of microbial pathogenicity. Although there have been several collections of review articles or an occasional useful overview, there has not been a general text on the subject available. That is no longer the case. This wonderful book by Abigail Salyers and Dixie Whitt fills the need for a textbook designed to introduce students at all levels into the field of bacterial pathogenesis. The authors have done a splendid job of capturing the rich excitement that pervades the field, they have portrayed the intradisciplinary nature of the field, and they have woven enough cell biology and cellular immunology into their text to do justice to the breadth of this experimental specialty. Thus, the initial subject material provides an overview of basic concepts of host-parasite relationships, which is written with better fidelity and insight than that seen in many textbooks designed for medical students, and for infectious diseases specialists, for that matter. The authors do not feel compelled to mention all possible, potential, or recognized pathogenic microorganisms, but those that they do choose to focus on are important instructive examples. Each chapter is a good synthesis of history, current research, and the central ideas that are affecting the research. I admire the fact that Salyers and Whitt have made this a personal text;

they virtually converse with the reader, and they share their opinions and support their favorite ideas (sometimes shamelessly). Good for them! They have tried earnestly to be factually correct. Although I never spoke directly with or wrote the authors during the course of their efforts, Patrick Fitzgerald of the American Society for Microbiology Books Division sent to me many of the anonymous reviews of individual chapters. With each new version, I could see that the authors tried to address any factual errors and to adopt constructive comments. They quite correctly ignored some of the more forceful opinions that they change their style of doing things. It is an impossible task to write a contemporary textbook and to keep up with each new piece of work or the latest fad. Yet, at least in the areas that I know well, *Bacterial Pathogenesis: A Molecular Approach* is an accurate picture of our current thinking. The writing is clear and crisp; it is a marvelous vehicle to teach with and learn from. Heretofore, all of the courses in bacterial pathogenesis of which I am aware, and certainly my own, were an unhappy amalgam of reprints, preprints, and symposium proceedings. Now there is a comprehensive, well-organized text, complete with provocative questions that permit students to obtain a fundamental foundation in the basics of medical microbiology and the biology of pathogenicity. Students at all levels will profit from reading this book. I am equally certain that even those who are active participants in the bacterial pathogenesis field will learn a great deal of new information by reading this text and, if they can be objective, think about some concepts in a different vein.

I am indebted to the authors for the extraordinary amount of work that they have invested in providing such a rich teaching medium. They have given me and other teachers and students the right substrate. Now as teachers, it is our job, with the help of our students, to provide the correct amount of catalyst to make the reaction go as efficiently as possible.

Stanley Falkow
*Stanford University, Stanford, California, and
The Rocky Mountain Laboratories,
Hamilton, Montana*

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