MICROBIAL BIOTECHNOLOGY

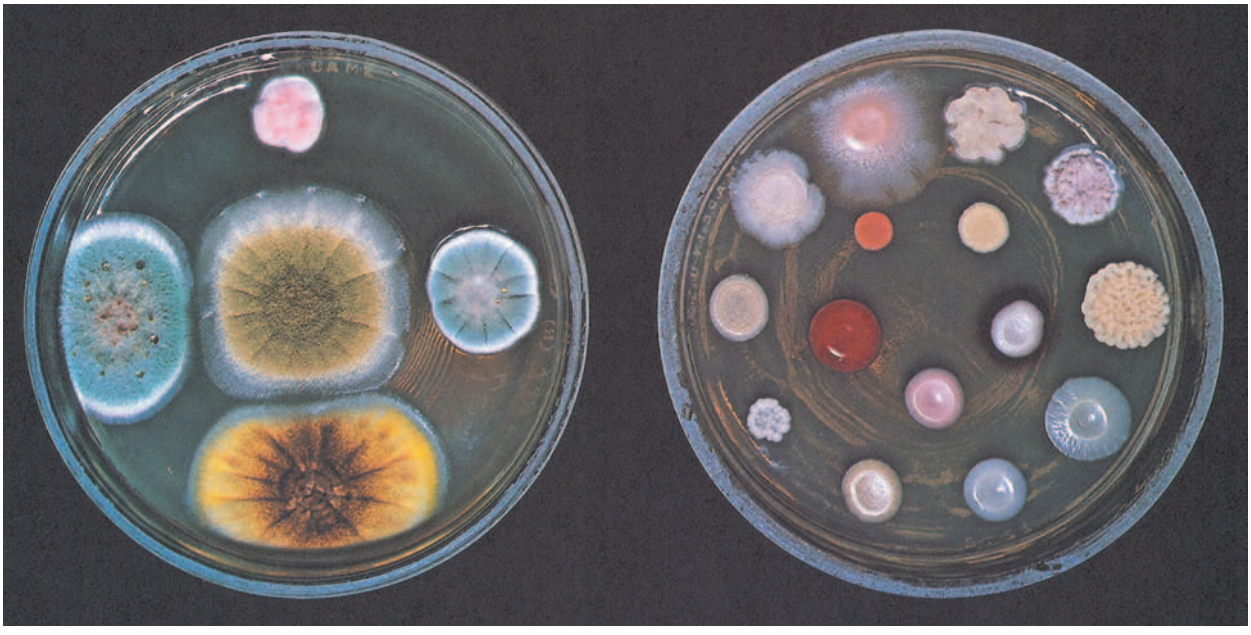
Knowledge in microbiology is growing exponentially through the determination of genomic sequences of hundreds of microorganisms and the invention of new technologies, such as genomics, transcriptomics, and proteomics, to deal with this avalanche of information.

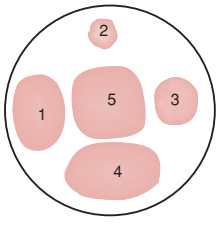
These genomic data are now exploited in thousands of applications, ranging from medicine, agriculture, organic chemistry, public health, and biomass conversion, to biomining. *Microbial Biotechnology* focuses on uses of major societal importance, enabling an in-depth analysis of these critically important applications. Some, such as wastewater treatment, have changed only modestly over time; others, such as directed molecular evolution, or "green" chemistry, are as current as today's headlines.

This fully revised second edition provides an exciting interdisciplinary journey through the rapidly changing landscape of discovery in microbial biotechnology. An ideal text for courses in applied microbiology and biotechnology, this book will also serve as an invaluable overview of recent advances in this field for professional life scientists and for the diverse community of other professionals with interests in biotechnology.

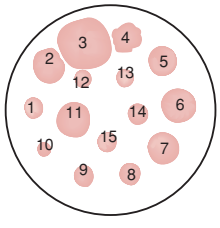
Alexander N. Glazer is a biochemist and molecular biologist and has been on the faculty of the University of California since 1964. He is a Professor of the Graduate School in the Department of Molecular and Cell Biology at the University of California, Berkeley. Dr. Glazer is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences, the American Academy of Microbiology, the American Association for the Advancement of Science, and the California Academy of Sciences. He was twice the recipient of a Guggenheim Fellowship. He was the recipient of the Botanical Society of America Darbaker Prize, 1980 and the National Academy of Sciences Scientific Reviewing Prize, 1991, a lecturer of the Foundation for Microbiology, 1996–98; and a National Guest Lecturer, New Zealand Institute of Chemistry, 1999. Dr. Glazer has authored over 250 research papers and reviews. He is a co-inventor on more than 40 U.S. patents. Since 1996, he has served as a member of the Editorial Affairs Committee of Annual Reviews, Inc.

Hiroshi Nikaido is a biochemist and microbiologist. He received his M.D. from Keio University in Japan in 1955 and became a faculty member at Harvard Medical School in 1963, before moving to University of California in 1969. He is a Professor of Biochemistry and Molecular Biology in the Department of Molecular and Cell Biology at the University of California, Berkeley. Dr. Nikaido is a Fellow of the American Academy of Arts and Sciences and the American Academy of Microbiology. He was the recipient of a Guggenheim Fellowship, NIH Senior International Fellowship, Paul Ehrlich prize (1969), Hoechst-Roussel Award of American Society for Microbiology (1984), and Freedom-to-Discover Award for Distinguished Research in Infectious Diseases from Bristol-Myers Squibb (2004). He was an Editor of *Journal of Bacteriology* from 1998 to 2002. Dr. Nikaido has authored nearly 300 research papers and reviews.





MOLDS
1 *Penicillium chrysogenum*
2 *Monascus purpurea*
3 *Penicillium notatum*
4 *Aspergillus niger*
5 *Aspergillus oryzae*



YEASTS
1 *Saccharomyces cerevisiae*
2 *Candida utilis*
3 *Aureobasidium pullulans*
4 *Trichosporon cutaneum*
5 *Saccharomycopsis capsularis*
6 *Saccharomycopsis lipolytica*
7 *Hanseniaspora guilliermondii*
8 *Hansenula capsulata*
9 *Saccharomyces carlsbergensis*
10 *Saccharomyces rouxii*
11 *Rhodotorula rubra*
12 *Phaffia rhodozyma*
13 *Cryptococcus laurentii*
14 *Metschnikowia pulcherrima*
15 *Rhodotorula pallida*

Cultures of molds and yeasts on nutrient agar in glass Petri dishes. From H. Phaff, Industrial microorganisms, Scientific American, September 1981. Copyright © 1981 by Scientific American, Inc. All rights reserved.

MICROBIAL BIOTECHNOLOGY

Fundamentals of Applied Microbiology, Second Edition

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We dedicate this book to Eva and Kishiko,

for the gift of years of support, tolerance, and patience.

Contents in Brief

Advances of particular relevance and importance will be posted periodically on the website www.cambridge.org/glazer.

Contents

Preamble

Il n'y a pas des sciences appliquées . . . mais il y'a des applications de la science. (*There are no applied sciences . . . but there are the applications of science.)*

– Louis Pasteur

Microorganisms are the most versatile and adaptable forms of life on Earth, and they have existed here for some 3.5 billion years. Indeed, for the first 2 billion years of their existence, prokaryotes alone ruled the biosphere, colonizing every accessible ecological niche, from glacial ice to the hydrothermal vents of the deep-sea bottoms. As these early prokaryotes evolved, they developed the major metabolic pathways characteristic of all living organisms today, as well as various other metabolic processes, such as nitrogen fixation, still restricted to prokaryotes alone. Over their long period of global dominance, prokaryotes also changed the earth, transforming its anaerobic atmosphere to one rich in oxygen and generating massive amounts of organic compounds. Eventually, they created an environment suited to the maintenance of more complex forms of life.

Today, the biochemistry and physiology of bacteria and other microorganisms provide a living record of several billion years' worth of genetic responses to an ever-changing world. At the same time, their physiologic and metabolic versatility and their ability to survive in small niches cause them to be much less affected by the changes in the biosphere than are larger, more complex forms of life. Thus, it is likely that representatives of most of the microbial species that existed before humans are still here to be explored.

Such an exploration is by no means a purely academic pursuit. The many thousands of microorganisms already available in pure culture and the thousands of others yet to be cultured or discovered represent a large fraction of the total gene pool of the living world, and this tremendous genetic diversity is the raw material of genetic engineering, the direct manipulation of the heritable characteristics of living organisms. Biologists are now able to greatly accelerate the acquisition of desired traits in an organism by directly modifying its genetic makeup through the manipulation of its DNA, rather than through the traditional methods of breeding and selection at the level of

the whole organism. The various techniques of manipulation summarized under the rubric of "recombinant DNA technology" can take the form of removing genes, adding genes from a different organism, modifying genetic control mechanisms, and introducing synthetic DNA, sometimes enabling a cell to perform functions that are totally new to the living world. In these ways, new stable heritable traits have by now been introduced into all forms of life. One result has been a significant enhancement of the already considerable practical value of applied microbiology. Applied microbiology covers a broad spectrum of activities, contributing to medicine, agriculture, "green" chemistry, exploitation of sources of renewable energy, wastewater treatment, and bioremediation, to name but a few. The ability to manipulate the genetic makeup of organisms has led to explosive progress in all areas of this field.

The purpose of this book is to provide a rigorous, unified treatment of all facets of microbial biotechnology, freely crossing the boundaries of formal disciplines in order to do so: microbiology supplies the raw materials; genomics, transcriptomics, and proteomics provide the blueprints; biochemistry, chemistry, and process engineering provide the tools; and many other scientific fields serve as important reservoirs of information. Moreover, unlike a textbook of biochemistry, microbiology, molecular biology, organic chemistry, or some other vast basic field, which must concentrate solely on teaching general principles and patterns in order to provide an overview, this one will continually emphasize the importance of diversity and uniqueness. In applied microbiology, one is frequently likely to seek the unusual: a producer of a novel antibiotic, a parasitic organism that specifically infects a particularly widespread and noxious pest, a hyperthermophilic bacterium that might serve as a source of enzymes active above 100°C. In sum, this book examines the fundamental principles and facts that underlie current practical applications of bacteria, fungi, and other microorganisms; describes those applications; and examines future prospects for related technologies.

The stage on which microbial biotechnology performs today is vastly different from that portrayed in the first edition of this book, published 12 years ago. The second edition has been extensively rewritten to incorporate the avalanche of new knowledge. What are some of the most influential of these recent advances?

- Hundreds of prokaryotic and fungal genomes have been fully sequenced, and partial genomic information is available for many more organisms available in pure culture.
- The understanding of the phylogenetic and evolutionary relationships among microorganisms now rests on the objective foundation provided by this large body of sequence data. These data have also revealed the mosaic and dynamic aspects of microbial genomes.
- Environmental DNA libraries offer a glimpse of the immensity and functional diversity of the microbial world and provide rapid access to genes from tens of thousands of yet-uncultured microorganisms.

- Extensive databases of annotated sequences along with sophisticated computational tools allow rapid access to the burgeoning body of information and reveal potential functions of new sequences.
- The polymerase chain reaction coupled with versatile techniques for the generation of recombinant organisms allows exploitation of sequence information to create new molecules or organisms with desired properties.
- Genomics, transcriptomics, and metabolomics use powerful new techniques to map how complex cell functions arise from coordinated regulation of multiple genes to give rise to the interdependent pathways of metabolism and to the integration of the sensory inputs that ensure proper functioning of cells in responding to environmental change.
- In the past 10 years, these developments have also changed the processes used in all of the "classical" areas of biotechnology – for instance, in the production of amino acids, antibiotics, polymers, and vaccines.
- The growing human population of the earth, equipped with the ability to effect massive environmental change by applying ever-increasing technological sophistication, is placing huge and unsustainable demands on natural resources. Microbial biotechnology is of increasing importance in contributing to the generation of crops with resistance to particular insect pests, tolerance to herbicides, and improved ability to survive drought and high levels of salt. The urgent need to minimize the discharge of organic chemical pollutants into the environment along with the need to conserve declining reserves of petrochemicals has led to the advent of "green" chemistry with attendant rapid growth in the use of biocatalysts. The future of the use of biomass as a renewable source of energy is critically dependent on progress in efficient direct microbial conversion of complex mixtures of polysaccharides to ethanol. The treatment of wastewater, a critical contribution of microorganisms to maintaining the life-support systems of the planet, is an important area for future innovation.

The application of biotechnology to medicine, agriculture, the chemical industry, and the environment is changing all aspects of everyday life, and the pace of that change is increasing. Thus, basic understanding of the many facets of microbial biotechnology is important to scientists and nonscientists alike. We hope that both will find this book a useful source of information. Although a strong technical background may be necessary to assimilate the fine points described herein, we have tried to make the fundamental concepts and issues accessible to readers whose background in the life sciences is quite modest. The attempt is vital, for only an *informed* public can distinguish desirable biotechnological options from the undesirable, those likely to succeed from those likely to result in costly failure.

Acknowledgments

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The combined efforts of all of these individuals have contributed a great deal to the accuracy and aesthetic quality of this book. The authors are responsible for any imperfections that remain.