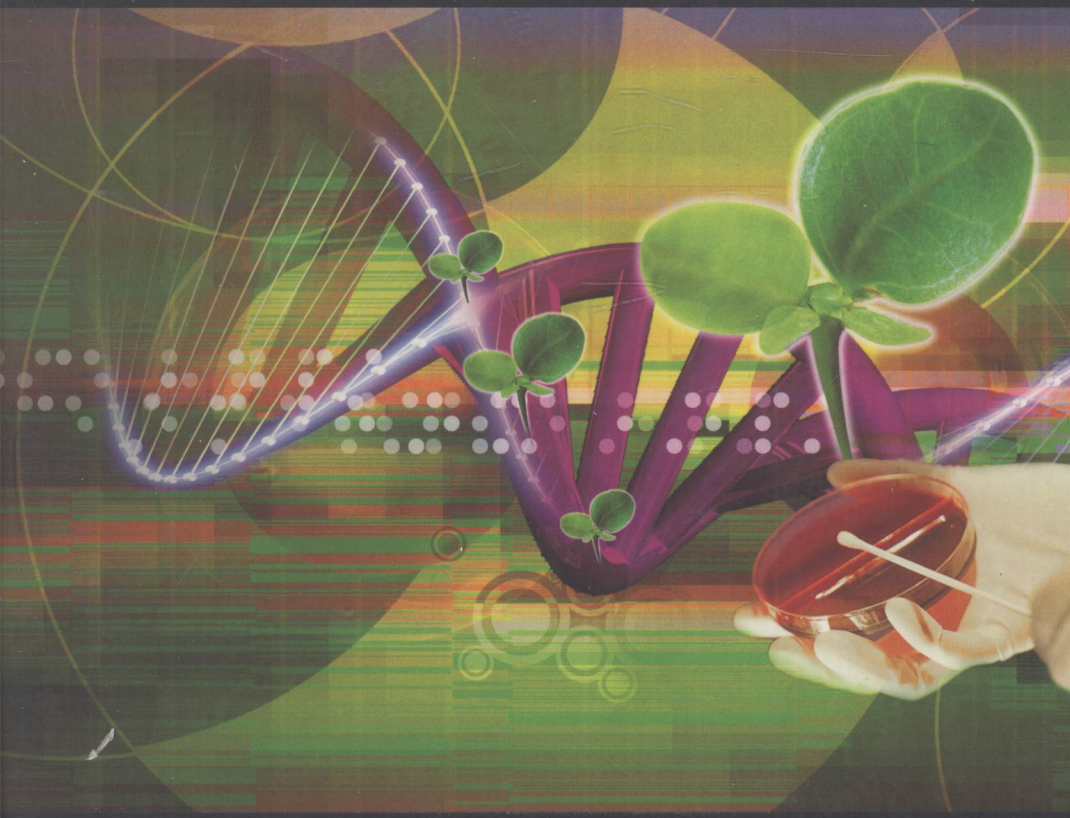
Microbial Biotechnology

Principles and Applications

Second Edition



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Preface

Life science research and industry is developing rapidly all over the world. Microbial biotechnology is increasingly being regarded as a core subject in most university and polytechnic life science courses. There are already a number of excellent general textbooks on microbiology and biotechnology in the market that deal with the basic principles of the field. In order to complement this, this book aims to focus on the various applications of microbial biotechnological principles. A teaching-based format is adopted, whereby working problems, as well as answers to frequently asked questions, will supplement the main text. This textbook also includes real life examples on how the application of microbial biotechnological principles has achieved breakthroughs in both research and industrial production.

The first edition of the textbook was divided into seven parts:

Part I deals with the *Principles of Microbial Biotechnology*, which contains *Screening for Microbial Products, Bioprocess Technology, Enzymology, Manipulation of Genes, Applications of Bioinformatics and Biocomputing to Microbiological Research,* and *Real Time Polymerase Chain Reaction.*

Part II focuses on the *Food Production Involving Microorganisms and Their Products*, which includes the subheadings *Fermented Foods, Food Involving Yeast* and *Ethanol Fermentation, Fungal Solid State Cultivation, Food Ingredients, Enzyme Modified Food Products*, and *Regulation of Foods Involving Genetically Engineered Microorganisms.*

Part III deals with *Microbes in Agrobiotechnology*, and is subdivided into *Microbes and Livestock* and *Transgenic Plants*.

Part IV probes into the area of *Microbes in Medical Biotechnology*, under the subheadings of *Diagnostic Clinical Microbiology and Microbes: Friends or Foes*?

Part V elaborates on the roles of *Microbes in Environmental Biotechnology*. These include *Municipal Wastewater Treatment, Industrial Wastewater Treatment, Municipal and Industrial Solid Waste Treatment*, and *Regulatory Issues on Application of Natural and Genetic Engineered Microbes in Environmental Biotechnology*.

Part VI explores the potential of *Microbes in Alternative Energy*.

Part VII provides a glimpse of *Patenting Inventions in Microbiology*.

In this second edition of the textbook, most chapters were revised and two additional chapters were included. They are: Chapter 6 "Real Time Polymerase Chain Reaction (RTPCR)" and Chapter 9 "Fungal Solid State Cultivation". RTPCR is becoming a widely used molecular biology technique in biotechnology research and application, and it is our belief that a special chapter should be devoted to this subject. Growing fungi on wet substrate (solid state) is common in fermentation production of food. The inclusion of the chapter on "Fungal Solid State Cultivation" compliments the other chapters in the "Food" section of this textbook.

Although this book is written for university undergraduates and polytechnic students, it contains sufficient details to be used as a reference book for postgraduate students and lecturers. It may also serve as a source book for corporate planners, managers and applied research personnel.

Lee Yuan Kun
Editor

Contents

Part I

Principles of Microbial Biotechnology

The use of microorganisms for large-scale industrial purposes has a long history, which is long before the realization of the activities of the microorganisms. For centuries, beer, wine, vinegar, soy sauce and other fermented foods were produced through spontaneous fermentation of natural occurring microorganisms or the use of carry-over microbial seeds from the previous batch of production. The quality and productivity of these early products were very often inconsistent. The development of scientific screening and isolation methods allows the selection of desirable natural occurring or mutated microorganisms for specific purposes. These methods, coupled with the advancement of the technical know-how in large-scale sterilization of culture media, in provision of adequate oxygen supply and in mixing homogeneity of the culture systems, enable the exploitation of both anaerobic (yeast and some bacteria) and aerobic microorganisms (fungi and some bacteria). Common examples are: the development of large-scale processes for the production of citric acid, amino acids and antibiotics; in improved biotransformation of steroid hormones, as well as the mass production of many enzymes. The diverse catalytic activities of microorganisms are being used more and more widely to perform specific chemical reactions in the industrial production processes.

Microbial biotechnology was pushed to a new height in the eighties when continuous fermentation and airlift fermentation processes were developed for the production of food and feed grade microbial protein

from industrial by-products, such as methanol and alkanes. These processes lead to considerable savings in capital, energy and labor costs.

Modern techniques of gene manipulation, and advanced bioinformatics and biocomputing are powerful tools for genomic and proteomic research. The scientific breakthroughs that ensued have made feasible the industrial manufacturing of non-microbial products, such as human growth hormone, interferon and viral vaccines.

It is the aim of Part I of this book to provide the readers with in-depth and comprehensive scientific knowledge in the areas as listed below, so as to facilitate the understanding of the various applications of micro-organisms and the production of their bioactive molecules in the biotechnological systems:

- Screening for microbial products
- Bioprocess technology
- Enzymology
- Manipulation of genes
- Application of bioinformatics and biocomputing.

Chapter 1

Screening for Microbial Products

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1.1. Introduction

If the discovery of penicillin marked the beginning of the antibiotics era, then the development of its fermentation has ushered in what might be called the golden age of industrial microbiology. The onset of this has resulted in the production of a large number of plant or microbial primary and secondary metabolites that are of commercial importance. Whereas primary metabolism is universal among living systems, secondary metabolism is mainly carried out by plants and microbes and is usually strain specific. The primary metabolites that are important in the bio-industry are the organic acids, vitamins, amino acids and purine nucleotides. However, of all the traditional products made by bioprocess, it is the secondary metabolites that are of great importance and value to the human health. Secondary metabolites, particularly from microbial sources, are selective in their actions on pathogenic bacteria and fungi. The success rate was so impressive that the pharmaceutical industry screened secondary metabolites almost exclusively for antibacterial, antifungal and antitumor chemotherapy as well as against diseases not caused by bacteria, fungi or tumors.

1.2. Screening for New Antibiotics

The main approach in which new antibiotics have been discovered has been by screening of groups of microorganisms such as *Streptomyces*, *Penicillium*, and *Bacillus*. In the screening approach, a large number of possible antibiotic-producing microorganisms are obtained from nature and pure isolates tested for antibiotic production by observing for diffusible materials that are inhibitory to the test or indicator bacteria. The classical method for testing potential antibiotic-producing microbial isolates is the cross-streak method, used by Fleming in his studies on penicillin. Isolates that demonstrate evidence of antibiotic production are then subjected to further studies to determine if the antibiotics they produce are new. When an organism producing a new antibiotic is discovered, it is produced in large quantity, purified and tested for cytotoxicity and therapeutic activity in infected animals. Most new antibiotics will fail the *in vivo* testings but a few of these new antibiotics that prove useful medically are then produced commercially. Since antibiotic-producing strains isolated from nature rarely produce the desired antibiotic at sufficiently high concentration, it is necessary to isolate new high yielding strains. In the commercial production of penicillin, the yield of this antibiotic was increased by 50,000 times using strain selection and appropriate medium development. Strain selection involves mutagenesis of the wild type culture, screening for mutants and testing of these mutants for enhanced antibiotic production.

1.3. Screening for Beneficial Cultures

Crude lactic cultures are known to inhibit some psychrotrophs in milk and ground beef. A large number of lactic acid bacteria, singly or in combination, have been shown to display varying degrees of antimicrobial activity against pathogenic microorganisms. In addition, viable cultures or components of lactic acid bacteria are useful in the treatment of displaced endogenous intestinal microflora, which are characteristic of many intestinal disorders and enhanced gut permeability of the host. Such bacteria are able to survive gastric conditions to colonize the intestine, at least temporarily, by adhering to the epithelium. They have been reported