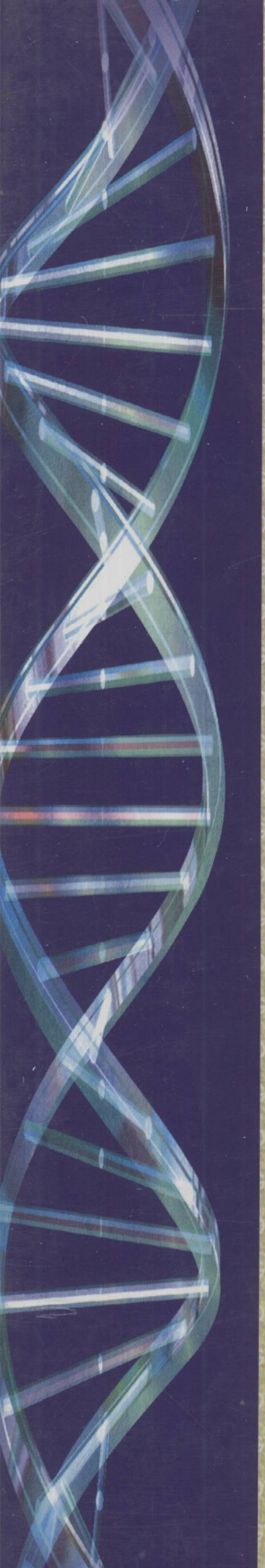

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MOLECULAR BIOTECHNOLOGY

Principles and Practices

Universities Press

CHANNARAYAPPA

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Universities Press

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC is an imprint of the Taylor & Francis Group,
an informa business

Universities Press (India) Private Limited

Registered Office
3-5-819 Hyderguda, Hyderabad 500 029 (A.P.) India
email: info@universitiespress.com

Distributed in India, Pakistan, Nepal, Myanmar (Burma), Bhutan, Bangladesh and Sri Lanka by
Orient Longman Private Limited
Registered Office
3-6-752 Himayatnagar, Hyderabad 500 029 (A.P.) India

Other Offices
Bangalore / Bhopal / Bhubaneshwar / Chennai
Ernakulam / Guwahati / Hyderabad / Jaipur / Kolkata
Lucknow / Mumbai / New Delhi / Patna

Distributed in the rest of the world by
CRC Press LLC, Taylor and Francis Group,
6000 Broken Sound Parkway, NW, Suite 300, Boca Raton, FL 33487, USA

First published in India by
Universities Press (India) Private Limited 2007

ISBN 13: 978 1 4200 5157 5
ISBN 10: 1 4200 5157 1

Set in Optima 10/13 by
OSDATA, Hyderabad

Printed in India by
Orion Printers Private Limited, Hyderabad 500 004

Published by
Universities Press (India) Private Limited
3-5-819 Hyderguda, Hyderabad 500 029 (A.P.) India.

MOLECULAR BIOTECHNOLOGY

To

The authors who provided me information and inspiration

Preface

Biotechnology is an important tool that can be applied to various economic sectors such as the production of food crops, livestock management, human health care, chemical industries, and environmental management. Many universities, understanding the importance of biotechnology research and the need for qualified manpower to exploit such technologies, have started undergraduate and master's degree programs. With the burgeoning number of such courses, experts have realized the urgent need for developing a suitable curriculum, possibly in the form of a model textbook aimed at providing undergraduate and postgraduate students with a strong base in this emerging and highly promising interdisciplinary area.

Molecular Biotechnology: Principles and Practices, is designed to balance between two important aspects of the science. The first aspect is the principles of molecular biology, which constitutes the theoretical knowledge pertaining to molecular biotechnology. The second aspect is practices in molecular biology, or the experimental approach to the study of biological processes. This book can serve as a textbook for both undergraduate and postgraduate students of molecular biology and biotechnology. It can also be used as a laboratory reference book in most research laboratories. The salient feature of this book is that it covers a wide range of molecular techniques in biotechnology and provides a source of information to readers at all levels. Concise and straightforward explanations of both theory and techniques associated with molecular/recombinant technologies are very few in literature. Most research articles in the field discuss either theory or techniques individually, but rarely explain both together and adequately. This makes life difficult for the student, teacher or researcher, who is new to the subject. Although several books on biotechnology have been published in the last decade, most are either very shallow or cover few areas in depth. Rarely do they cover the broad spectrum of topics which would provide enough information for understanding the subject or provide simple protocols for execution of molecular biology experiments. Realizing this deficiency, I have made an attempt to explain the basic concepts in biotechnology and the detailed steps of some important experiments in relatively simple terms. In my opinion, this book can help the reader to easily understand the subject and also execute the experiments very efficiently.

The book is divided into nine sections, containing 42 chapters and an appendix. In recent years, both the amount of molecular biology knowledge and its rate of growth have exploded. It is im possible to keep pace with this development and include references to all the work exhaustively. Therefore, in this book, only a few representative methods, which can provide standard protocols and general information on those techniques, are presented under each section. Alternative protocols provided for each technique make it more suitable for different laboratory conditions and systems used. Sources where additional information can be obtained are sufficiently quoted at the end of each chapter. Each chapter is adequately illustrated with computer graphics in an attempt to convey the practical as well as theoretical aspects of the techniques. It should, therefore be well suited for both the lecture room and the laboratory bench. The illustrations are adequately labeled and explains step-by-step, the procedure to be followed at each level of the process.

I dedicate this work to the authors of the books, which I have referred to as a source of information. They have given me the inspiration to write this book. Writing a book requires a lot of effort, patience and dedication without really gaining much benefit or appreciation. However, in my opinion, the benefits obtained by the readers is priceless. My personal experience of reading books is that it not only enriched my knowledge, but that it also helped me choose the right path in life. I hope this book will prove to be useful to the readers. I invite their valuable comments and suggestions for improvements in future editions.

I would like to thank many people for the encouragement and inspiration they have given me while I was writing this book. I especially thank the staff and management of Universities Press (India) Pvt. Ltd. for their editorial assistance and the fine production of this book. I want to thank Prof. M. Udaya Kumar, HOD, Crop Physiology, UAS; Mr. A. M. Veerabhadraiah, Associate Professor, Soil Chemistry, UAS; Dr. K. V. Devaraj, former Vice-Chancellor, UAS, Bangalore; Dr. Balakrishna Gowda, Associate Professor and Dr. R. Uma Shaanker, Associate Professor, UAS, Bangalore; Dr. K. V. Janardhan, HOD, Biotechnology, Vydehi Institute of Biotech Sciences, Bangalore; Dr. J. N. Vinay, Postdoctoral fellow, Arizona State University, USA, for their help and inspiration while writing this book.

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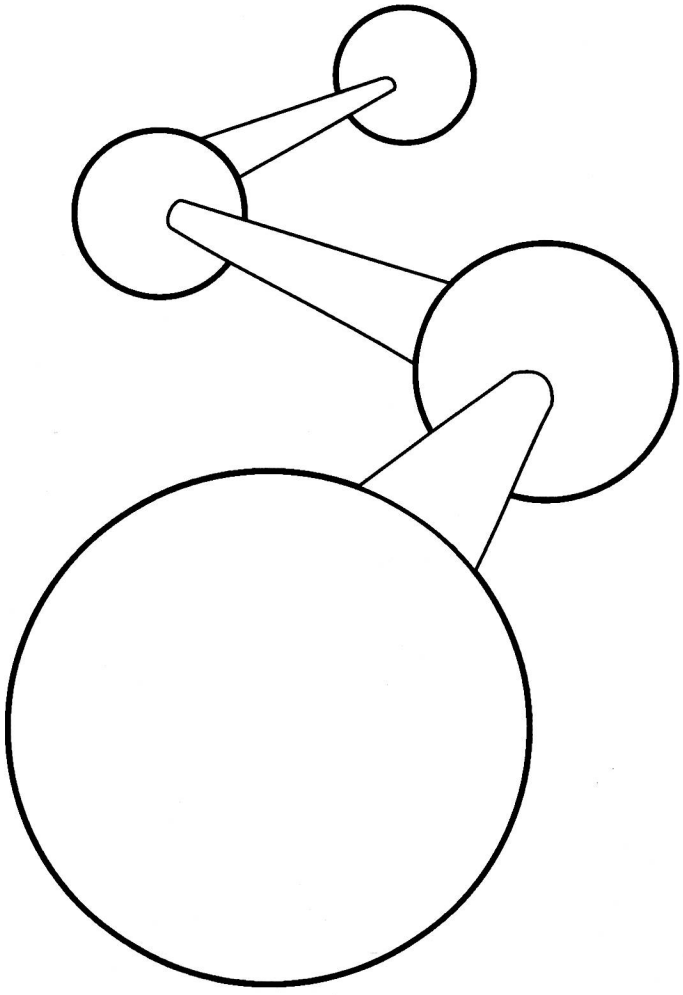
PART - I

Introduction to Biotechnology



1

Biotechnology: Scope and Importance

I INTRODUCTION

The recent advances in science and technology have driven the biological sciences into a new era. All of this can probably be traced back to when Anton van Leeuwenhoek, a Dutch dry-goods dealer, ground the first microscope lens. Through his newly invented glass, he discovered a previously unseen cellular world. The second half of the 21st century was a truly exciting time for molecular biologists. Many inventions, including new methods for analyzing proteins, DNA and RNA, fueled an explosion of information and enabled scientists to understand cells and multicellular organisms at the molecular level. Now we have molecular blueprints (genomic sequences) for many organisms. The main objective of modern science, however, is to know the nature of genetic material and to find the answers to questions like: Which genes determine specific characters? How do they get switched on and off spatially and temporally? How do we correct genetic defects? How can we best manipulate genomes?

A. WHAT IS BIOTECHNOLOGY?

The field of biotechnology, which emerged as a new discipline, was a result of the fusion of biology and technology. Biology is the science of all living organisms or their components, whereas technology deals with the physical–chemical properties and techniques (Chapters 3–13) applied to the production of biological products/services. The emergence of biotechnology has been possible mainly due to the revolutionary discoveries made in these two areas. Biotechnology has been defined in many ways by many organizations. Biotechnology may be broadly defined as *"the controlled use of selected/manipulated biological systems or processes for the production of abundant/novel products or services"*. Therefore, the area covered under biotechnology is very vast and the techniques involved are widely divergent. Biotechnology can be applied in areas as diverse as agriculture, animal husbandry, medicine, environment, industry, and biological conservation.

Biotechnology is multidisciplinary by its very nature and encompasses several disciplines of basic sciences (e.g., genetics, biochemistry, molecular biology, chemistry, microbiology, immunology, cell and tissue culture, and physiology), engineering (processing technology,

biochemical engineering, electronics and physical sciences) and also other disciplines like sociology, economics, politics, law and ethics (Figure 1.1).

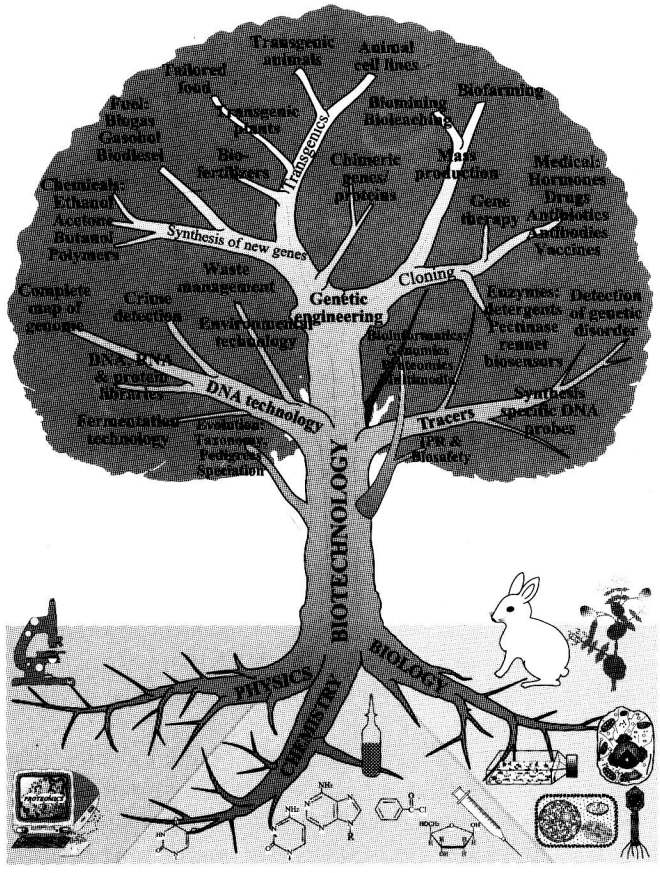


Figure 1.1 *Biotechnology tree: Evolution of the field of biotechnology and some important areas of biotechnology applications. The three main roots represent the importance of basic science knowledge*

B. WHEN DID BIOTECHNOLOGY BEGIN?

Although the term biotechnology is a recent development, its origin can be traced back to prehistoric times. Humans have been altering the genetic composition of plants for millennia – retaining seeds from the best crops and planting them in the following years, breeding and cross- breeding varieties to make them taste sweeter, grow bigger, last longer, etc. In this way, early agriculturists transformed the wild tomato (*Lycopersicon*), from a fruit the size of a peanut to today's giant, juicy and fleshy tomato. From a weedy plant called *teosinte* with an "ear" barely an inch long has emerged our foot-long ears of sweet, nutrient-rich, yellow corn. Man has also selected hundreds and thousands of new crop varieties by selection and hybridization. In ancient scripts, it has been documented that humans employed microorganisms as early as 5000 BC for making wine, vinegar, yogurt, leavened bread, etc. The discovery that fermentation

converted fruit juice into wine, milk into cheese and yogurt, and solutions of malt and hops into beer seems to have set in motion the study of biotechnology. The early animal breeders soon realized that different physical traits could be either magnified or lost by mating the appropriate pairs of animals, thereby engaging in the traditional manipulations of biotechnology. However, the use of microorganisms for the production of chemicals on a commercial scale begun during the First World War, and has recently been more fully exploited due to the advancement of modern biotechnology.

C. MODERN BIOTECHNOLOGY

Modern biotechnology is innovative and quite different from the conventional practices. Traditional breeders made crosses only between related organisms whose genetic composition was compatible (genetically closer). Doing it this way involved the transfer of tens of thousands of genes (many genes were not required) after years of long selection procedure. By contrast, today's genetic engineers can transfer just a few desirable genes at a time, between species that are distantly related or not related at all. In other words, scientists can extract a desirable gene from virtually any living organism and insert it into virtually any other organism. They can put human gene into a plant or microorganism or in any other combination. For example, they can put a rat gene into lettuce to make a plant that produces vitamin C or insert a microbial toxin gene into cotton plants to make it insect-resistant. All this genetic manipulation became possible by the discovery of techniques of gene splicing and recombinant DNA technology (see Chapters 7–19). The engineered organisms which scientists produce by transforming genes between species are called "transgenic" organisms. Transgenic animals and several dozen transgenic food crops are currently in the market. Most of these crops are engineered to help farmers deal with age-old agriculture problems: good seeds, insects, diseases, nutrient composition, stress tolerance, etc (see Chapters 20–22).

The beginning of modern biotechnology can be traced back to 1865, when Gregor Mendel published the results from his experiments conducted on the garden pea on the inheritance of seven different physical traits. This and many other studies eventually led to the concept of the gene as the basic unit of heredity. Over the next century, many other researchers with sophisticated techniques and instruments contributed to the growth of modern biotechnology (Table 1.1).

Table 1.1 *Chronology of some major developments in biotechnology*[*]

Before 6000 BC	Yeasts used to make wine and beer
About 4000 BC	Yeasts were used for making leavened bread
1866	Mendel published his research findings, experiments conducted on the garden pea, which led to the concept of the gene as the basic unit of heredity.
1869	Friedrich Miescher isolated nuclein, later shown to be DNA, from the nuclei of white blood cells.
1885	*E. coli* bacterial cells are identified and grown under controlled conditions.
1902	Archibald Garrod's report that the human disease "Alkaptonuria" behaves as a Mendelian recessive trait led to the suggestion that enzymes are encoded by genes.

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Continued. . .

Table 1.1 *Chronology of some major developments in biotechnology*[*]

1910	Thomas Hunt Morgan showed the first evidence of the presence of genes in chromosomes. He used microorganisms to treat sewage.
1912–14	Large-scale production of acetone, butanol and glycerol using bacteria.
1917	Karl Ereky coined the term "biotechnology".
1940	George Beadle and Edward Tatum hypothesized the concept of "one-gene-one-enzyme".
1943	Penicillin was produced on an industrial scale.
1944	Avery, Macleod and McCarty demonstrated that DNA, not protein, carries hereditary information. Penicillin was produced on a large scale for the first time.
1952	Alfred Hershey and Martha Chase demonstrated that the genes of a bacteriophage are made of DNA and are capable of directing the synthesis of new bacteriophage proteins.
1953	Watson and Crick determined the structure of the DNA double helix. They drew from the work of other scientists to propose that the molecule is an alpha double helix structure in which the two strands are both complementary and antiparallel to one another.
1955–65	The role of tRNA, mRNA, and rRNA as well as of DNA and RNA polymerases in gene function was elucidated.
1957	The Central Dogma, which states that hereditary information flows from DNA to RNA to protein, was put forth by Francis Crick and George Gamov.
1961	Marshall Nirenberg and Har Gobind Khorana correctly translated the genetic code.
1962	Uranium was mined with the aid of microbes (Canada).
1967	DNA ligase was isolated and identified.
1970	Stewart Lin and Werner Arber identified the first restriction endonucleases. In the same year researchers discovered the enzyme reverse transcriptase, which catalyzes the reaction in which DNA is transcribed from an RNA template.
1972	Khorana and co-workers synthesized an entire tRNA gene. Paul Berg created the first recombinant DNA molecule.
1973	Stanley Cohen, Herbert Boyer and colleagues constructed a functioning plasmid, containing genes, which confer resistance to both tetracycline and streptomycin; i.e., the establishment of recombinant DNA technology.
1976	Techniques were developed to determine the sequence of DNA.
1977	Somatostatin became the first human hormone to be synthesized by a bacterial cell as a result of transformation with human DNA.
1981	The use of monoclonal antibodies for diagnosis was approved in the USA.
1983	Approval was granted for the use of insulin produced by genetically-engineered microbes (GEMs).
1984	Animal interferons, produced by GEMs, were approved of for the protection of cattle against diseases.
1988	The polymerase chain reaction (PCR) method was published.
1990	Approval was granted in the USA for a trial of human somatic cell gene therapy.
To date	The field of biotechnological research enormously expanded and is still expanding exponentially.

[*] *Collected from different books and journals.*

D. GENE TECHNOLOGY IS A BASIC TOOL FOR BIOTECHNOLOGY

Scientists continue to find new ways to insert desirable genes for specific traits into the DNA of different biological systems (plants, animals and microorganisms). A field of promise and a subject of debate, genetic engineering is changing the food we eat and the world we live in. Many scientists envision a cornucopia (similar to the "*kalparuksha*" or "*Kamadenu*"): the mass production of rare plants; highly variegated and long shelf-life flowers; tomatoes and broccoli produced with pharmaceutical compounds and industrial chemicals; vaccine-producing bananas; vitamin-enriched rice, sweet potatoes, and cassava to help the malnourished poor and vegetable oils so loaded with therapeutic ingredients that doctors "prescribe" them for patients at risk of cancer, heart disease, diabetes, etc; cheaper and safer fuel; clean environment, etc. The possibilities are endless.

Overall, gene manipulation has provided novel solutions to experimental problems in biology; these solutions, in turn, have led to novel products. Most biotechnology companies and research institutes make use of gene technology or genetic engineering, which mainly involves recombinant DNA and gene cloning. This technology allows the splicing of a DNA molecule at desired places to isolate a specific DNA segment and then inserting it into another DNA molecule at a desired position (Chapter 15). The resultant product is called "recombinant DNA" and the technique often called "genetic engineering". Using molecular techniques, we can isolate and clone a single copy of a gene or a DNA segment into an indefinite number of copies with similar properties. This became possible due to the identification and modification of different kinds of vectors (a self-replicating DNA molecule is called "vector", e.g., plasmid, phage or virus) with suitable properties (Chapter 16), and which can be mobilized to a suitable host, where they reproduce along with the host. The vector carrying the inserted DNA will also replicate faithfully through the vector DNA. This technique is called "gene cloning" (Chapter 14). A gene is a part of a chromosome and is responsible for a specific character or trait of an organism/cell. Genes produce their phenotypic effects by specifying the amino acid sequences of specific proteins. There are also various biological tools that are used to carry out manipulation of genetic material and cells.

The gene cloning technique has had a tremendous impact on all areas of molecular biology and, consequently, on biotechnology. Recombinant DNA technology broadly involves: (1) the isolation of a specific DNA (Chapter 7), (2) the selection of vectors (Chapter 16), (3) the preparation of a chimeric DNA (Chapter 14), (5) cloning of the chimeric DNA, and (6) the screening of recombinants (Chapter 19).

II WHAT MAKES BIOTECHNOLOGY A POWERFUL TECHNOLOGY?

Biotechnology is a unique and powerful technology, since it helps humans in many ways, for example, (i) mass production, (ii) the generation of novelty, and (iii) better service.

A. OVERPRODUCTION OF CELLULAR COMPONENTS

For commercial use or in academic studies, the determination of the structure, function or utility of a protein demands that adequate amounts of purified material are available. This is not always an easy task, particularly when the protein is normally present in very low levels in the cell mass. Genetic engineering provides a means of generating sufficient material. For example, 5 mg of somatostatin was first isolated from half a million sheep brains and a small amount of epidermal growth factor from 40,000 gallons of human urine. After the advent of gene cloning, the same amount of material was obtained from a few liters of bacterial culture. This principle has been applied to a wide range of cellular proteins and was the basis for many of the biotechnology start-up companies in the world. Over-production need not be restricted to proteins. It is possible to raise the levels of most intracellular components, provided that they are not toxic to the producing organism. This can be done by cloning all the genes for a particular biosynthetic pathway and over-expressing them. Alternatively, it is possible to shut down particular metabolic pathways and thus re-direct particular intermediates towards the desired end product.

B. GENERATION OF NOVELTY

By and large, gene manipulation has provided novel solutions to experimental problems in biology. These solutions have led to creation of novel products. Gene manipulation has been used to permanently modify the germ cells of animals ("transgenesis"), e.g., the production of 'supermice' which are extra-large as a result of the over-production of the human growth hormone. Transgenic animals that over-express foreign proteins and secrete them in milk have been developed. Novel therapies for various human diseases have been also created. Transgenic plants carrying genes resistant to various stresses have also been produced. However, the development of these products has also raised some novel problems. Some of these benefits have been discussed in the following sections.

C. BETTER SERVICES

Biotechnology can also be of better service to human beings, other organisms and to the ecosystem by providing solutions to many natural and man-made problems. For example, biotechnology can provide solutions to environmental pollution, it can help to monitor and conserve biodiversity, and reduce the rapid loss of natural resources by providing alternative solutions. It can also aid in the overall improvement of biological safety and in maintaining the ecological balance (see the following sections for more details).

III APPLICATIONS OF BIOTECHNOLOGY

Biotechnology has rapidly emerged as an area of activity that will have a potential impact on virtually all domains of human welfare – food processing, human health, agriculture animal improvement, and environmental protection (Table 1.2). As a result, it plays a very important role in employment, production, trade, economics and economy, human health, conservation

of biodiversity and even in the socio-economical and political status of a nation. This is clearly reflected in the emergence of numerous biotechnology companies throughout the world.

Table 1.2 *A list of areas in which biotechnology is making significant contributions*

Pharmaceutical industries	Agriculture
Human health care products	Horticulture and floriculture
Animal improvement	Environment
Dairy and animal husbandry	Renewable energy and biofuels
Secondary metabolites	Population control
Food processing and beverage	Microbial-mining or leaching
Crime detection and parentage disputes	Fisheries and aquaculture
Embryo-cloning and gene therapy	Forestry
Intellectual property rights and trade	Biodiversity conservation

The importance of biotechnology to human welfare is becoming increasingly more important. The products of DNA research, ranging from proteins to engineering organisms, have a wide range of applications. The following are some of the contributions of biotechnology to overall development of human welfare.

A. MEDICAL BIOTECHNOLOGY

1. Diagnosis of biological disorders

The production of biological reagents for the diagnosis of biological disorders and infectious diseases is a major industry. On a global scale, the revenue from sales is estimated at many billions of dollars. There are three types of diagnostic reagents: biochemical reagents for assaying specific enzymes, antibodies for detecting specific proteins, and a recent development, nucleic probes (Chapters 12–13).

Molecular analysis of genetic disorders: There are several hundred genetic diseases in man, which are the result of mutations. For many of these genetic diseases there is no definitive treatment, although in some cases human gene therapy may become possible. DNA research helps in understanding many diseases or genetic disorders at the molecular level, such as sickle cell anemia, thalassemias, familial hypercholesterolemia, etc.

Laboratory diagnostic applications: By using recombinant DNA techniques, many diseases can be diagnosed, e.g., AIDS, hepatitis B, etc. In the case of most diseases, diagnosis is the detection of a specific microorganism. For example, a patient with a disorder of the gastrointestinal tract could be infected with more than 15 types of microorganisms. By using the right probe, a particular infectious microbe can be accurately detected from stool samples without any cultivation and cytological work.

Prenatal diagnosis of diseases: The DNA collected from the amniotic fluid can be used to predict the risk of developing genetic diseases (e.g., sickle cell anemia and many other genetic defects) and this can be done by using DNA probes.

2. Hybridoma technology

This produces large quantities of monoclonal antibodies, which are used for the diagnosis of various diseases, e.g., venereal diseases, hepatitis B, viral diseases, cancer, etc (Chapter 26).