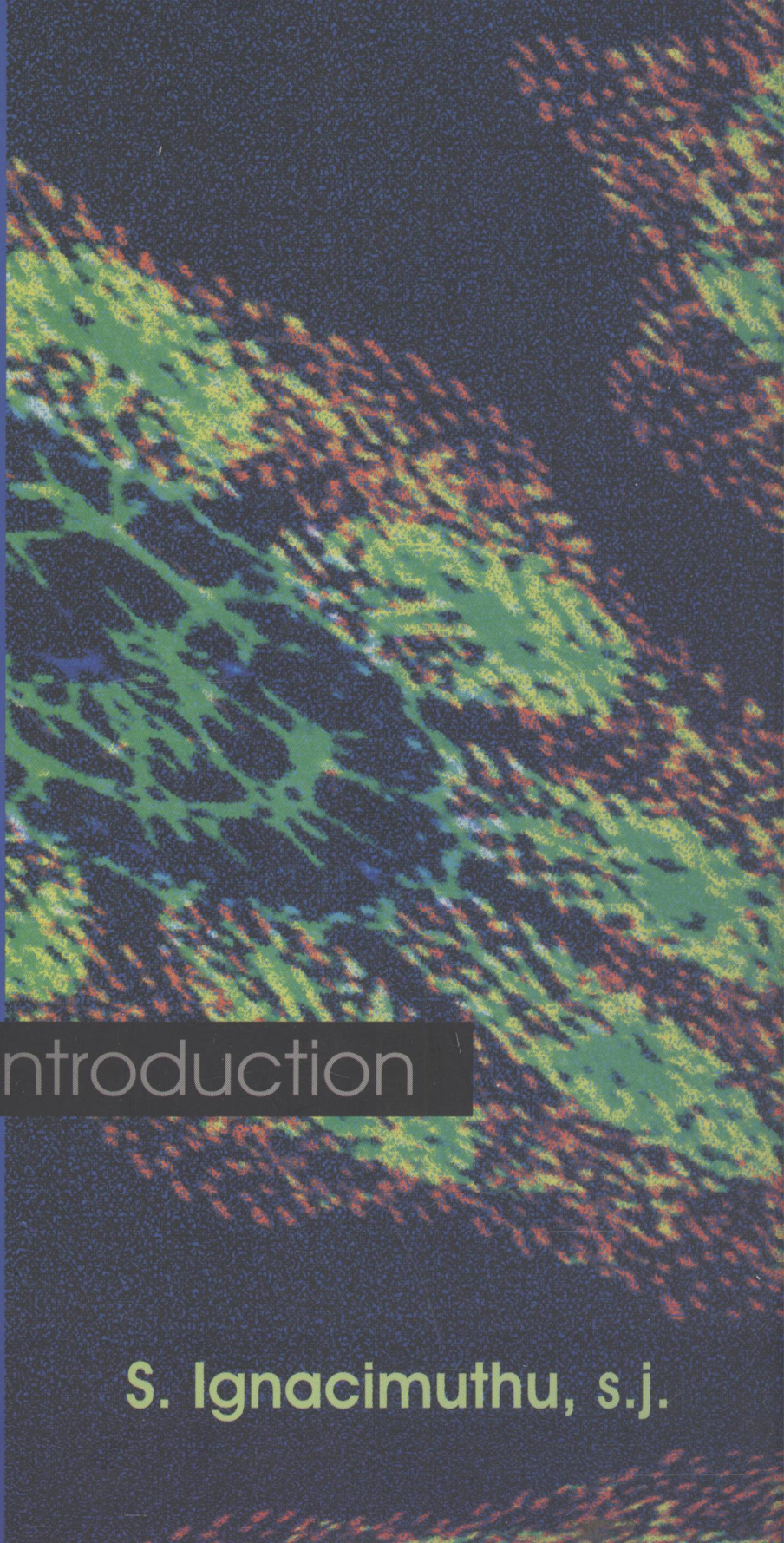
BIOTECHNOLOGY

An Introduction



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Alpha Science

BIOTECHNOLOGY

An Introduction

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Alpha Science International Ltd.
Oxford, U.K.

Biotechnology
378 Pages/51 Tables/47 Figures

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Garsington Road, Oxford OX4 2JZ, U.K.

www.alphasci.com

ISBN 978-1-89265-451-4

Published by N.K. Mehra for Narosa Publishing House Pvt. Ltd.,
22 Delhi Medical Association Road, Daryaganj, New Delhi 110 002

Printed in India

BIOTECHNOLOGY

An Introduction

To
The Society of Jesus
for making me
what I am

Preface

Biotechnology refers to any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. Biotechnology is a very useful technology that is being exploited by many different sectors. The applications of biotechnology offer enormous potential for agricultural, pharmaceutical, environmental, healthcare and developmental purposes.

With the sequencing of the human genome, the researchers are moving to the next level, which involves understanding the genetic basis of diseases. Benefits for human development are already experienced. Breakthrough applications in medicine have huge potential for accelerating human development.

Apart from the usual topics related to biotechnology, aspects related to Polymerase Chain Reaction, Microarray, Gene targeting, Gene silencing, Animal cloning, Human Cloning, Stem cells, Genetically modified food, Fermentation technology, Enzyme technology, Bioinformatics, Drug Discovery, Nano-medicine, Biosensors and Bioremediation have been elaborated

I am confident that the students and teachers will benefit much from this book.

Rev. Fr. Dr. S. Ignacimuthu, s.j.

Acknowledgements

I am grateful to all those who have helped me to prepare this book. My failure to list their names individually does not detract from my indebtedness to them. I must thank Sisters Anne Xavier, Sarguna, and Regi, and Miss Parvathy, Holy Cross College (Autonomous), Tiruchirapalli for correcting the manuscript and giving the finishing touches. I would also like to specially thank Messrs Albert Rabara, D. Amalraj, and S. Arockiasamy, St. Joseph's College (Autonomous), Tiruchirapalli and Mr C. Muthu, Entomology Research Institute, Loyola College, Chennai, for their constant and generous help in typing the manuscript. I am grateful to Rex Johnson for embellishing the book with beautiful illustrations.

I thank my dear students for inspiring me to write this book and also for their constructive suggestions to improve it. I also thank Dr C.R. Babu, Department of Botany, University of Delhi, for his guidance and help.

My sincere thanks are due to the following publishers for granting me permission to use some of their diagrams, explanations, and tables:

Academic Press, New York
Blackwell Scientific Publications, Oxford
Cambridge University Press, Cambridge
Holt, Reinhart and Winston Inc., New York
John Wiley and Sons, New York
Jones and Bartlett Publishers, Massachusetts
Prentice-Hall, International, New Jersey
Springer Verlag, Berlin
W.H. Freeman and Company, New York
Wiley Eastern Limited, New Delhi

Let me thank and compliment the publishers for having published the book in a neat and excellent manner. Finally, I must acknowledge with deep appreciation the indispensable help and encouragement received from my dear friends.

Introduction

The most significant achievement of biologists is the rediscovery of biotechnology. As a subject, biotechnology is not a new area of scientific endeavour. Microorganisms have been used to produce food such as beer, vinegar, yoghurt, and cheese for over eight millennia. Certainly the ancient Sumerians were familiar with beer; alehouses were an established part of Roman civilization. Romans tried to introduce grapevines for wine-making. References to wine and vinegar are scattered throughout the Bible.

Biotechnology is an area of multidisciplinary science, involving a variety of distinct subjects, where living organisms or their useful parts are put into effective use to cater to the welfare of humanity. It can conveniently be grouped under two major headings, namely, 'conventional biotechnology' and 'modern biotechnology'.

In the early days, biotechnologists used living organisms for the manufacture of a variety of useful materials. Whatever by-products were obtained during normal cell growth, were used by people. For example, during the normal growth of yeast cells in grape juice, sucrose is converted to ethanol and this fermented juice containing alcohol is used as wine. Similarly, *Penicillium notatum* and *P. chrysogenum* produce the antibiotic penicillin as a by-product of their secondary metabolism and this compound is used to fight microbial diseases. Microbial production of glycerol by yeast, acetone, and butanol fermentation using *Clostridium acetobutylicum,* citric acid production by *Aspergillus niger,* and streptomycin production using *Streptomyces griseus* are some of the fields developed under conventional biotechnology. Mutation, recombination and strain selection were employed to increase the yield of the product.

Modern biotechnology enables an organism to produce a totally new product, which the organism does not or cannot produce in its normal course of life. Since we are able to engineer a new genetic potential in an organism, this technology is also called genetic engineering. Basic techniques which stimulate the progress of modern biotechnology are: (1) recombinant DNA manipulation—genetic engineering, (2) plant and animal tissue culture, (3) protoplast fusion, (4) monoclonal antibodies, (5) protein engineering, (6) immobilized enzymes and cell catalysis, (7) biosensors, (8) computer-aided bioprocess, (9) new reactor design, (10) DNA transfer into living cells, (11) polymerase chain reaction, and (12) chromosome engineering. Without these techniques, the growth of biotechnology would have been impossible. All these techniques are used in most industries either to engineer a new capability or to reduce the cost of production or to introduce a safer industrial practice.

This book aims at providing some basic and relevant information on important applications of biotechnology, namely (1) fermentation industry, (2)

healthcare, (3) food processing industry, (4) agriculture, (5) chemical industry, (6) waste treatment, and (7) energy and environment. The major advancement in fermentation industry deals with reactor design and computer aided process control. This technology has its application in food, agriculture, alcoholic beverages, pharmaceuticals, and chemicals. New reactor designs such as air lift fermentors, fluid bed reactors and other systems designed to grow animal and plant cells in large quantities have revolutionised bio-technological applications.

In relation to healthcare, biotechnology has helped in the production of (a) small molecules such as antibiotics, hybrid antibiotics, amino acids, vitamins etc. (b) macromolecules such as interferons, insulin, hormones, vaccines, etc. (c) drug detecting agents, monoclonal antibodies, and magnetic microspheres, (d) purification agents such as immobilized monoclonal antibodies, (e) new catalysts, e.g. immobilized enzymes and cells, (f) diagnostic reagents like enzymes and monoclonals, (g) biomaterials like artificial skin, and (h) biomedical equipment like artificial kidney machines.

In the field of agriculture, biotechnology has contributed to crop improvement by developing salt and drought resistant crops, developing crop varieties which can fix atmospheric nitrogen, improving crop varieties for higher photosynthetic efficiency, improving the quantity and quality of storage proteins, and production of high yielding hybrid seeds. Further, it has contributed to crop protection by development of disease resistant varieties of transgenic plants which offer protection against virus, bacteria, fungi and insects, development of herbicide resistant plants and development of recombinant DNA based diagnostic reagents for early identification and treatment of viral, bacterial and fungal diseases. Tissue culture techniques have helped in the propagation of forest plants such as bamboo, teak, *Eucalyptus*, sandalwood, etc. and economically important plants such as cardamom, banana and brassica. Biotechnology has also helped in the production of single cell proteins and biofertilizers.

In the field of animal husbandry, biotechnology has helped to increase milk production in cattle, to achieve faster growth in farm animals and to produce disease resistant animals through transgenesis.

In the field of energy, biotechnology has helped in the conversion of cellulosic and agricultural waste to produce fuels such as ethanol and butanol by cloning active enzyme systems for their efficient degradation and subsequent fermentation. Biotechnology has helped in the production of biogas from agricultural and animal wastes by engineering methanogenic bacteria. It has also helped in the production of oil producing bacteria.

Biotechnology has also played an important role in waste treatment by developing high capacity bacteria to degrade cellulose and lignin, to degrade metals and cyanide, to clean up oil, to destroy TNT, to degrade chlorinated organic wastes, etc.

Thus, biotechnology has contributed to the welfare of humanity in many and varied ways. Biotechnology has become an important and integral part of any life-science today. This book has been written in as concise and simple a manner

as possible with a view to help the students and teachers in many universities and colleges. It is hoped that undergraduate and postgraduate students would find it useful as a textbook as well as for research purposes.

Suggestions for further improvement of the book are welcome.

Rev. Fr. Dr. S. Ignacimuthu, S.J.

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1
Biotechnology—An Overview

Introduction

Biotechnology has been defined in various ways. In simple terms, biotechnology refers to the use of living organisms or their products for the welfare of humanity. One rather vague definition says that biotechnology is the application of biological organisms, systems or processes to manufacturing and service industries. In other words it simply means biology applied for use.

According to the definition adopted by the European Federation of Biotechnology, created in 1978, 'biotechnology makes it possible, through an integrated application of knowledge and techniques of biochemistry, microbiology, genetics and chemical engineering, to draw benefit, at the technological level, from the properties and capacities of microorganisms and cell cultures. Biotechnology offers the possibility of producing, from widely available renewable resources, substances and compounds essential to life and the greater well-being of human beings.' In short, biotechnology comprises technical processes that enable us not only to manipulate the DNA, but also to use in other ways living organisms for specific purposes. Biotechnology is defined as the application of current scientific methods and techniques to improve the biological systems, be they plants, animals, microorganisms, for the betterment of human beings.

The term biotechnology was brought into popular use in the mid-1970s as a result of the increased potential for the application of the emerging techniques of molecular biology. The word itself seems to have been first employed by the Leeds City Council in the United Kingdom in the early 1920s, when they set up an Institute of Biotechnology. In fact, biotechnological processes are nearly 5000 years old. It began with the discovery of fermentation and the consequent production of alcoholic beverages. Present interest in biotechnology has been stimulated by the potential that can result from the marriage of biological processes and techniques—some old, some new—with production engineering, electronics and bioprocessing.

1.1 History

Probably the oldest biotechnological processes are found in microbial fermentations, as borne out by a Babylonian tablet dated around 6000 B.C., unearthed in 1981, depicting the preparation of beer. The Sumerians were able to brew as many as 20 different varieties of beer in the third millennium B.C. The perfecting of fermentation processes, their increased efficiency, and the

discovery of a large number of microbial bioconversions, along with the isolation of substances of bacterial and fungal origin to replace synthetic products has led to the discovery of more effective drugs and medicines. Recent biotechnological processes rely on genetic recombination techniques as well as the use of immobilized enzymes, cells or cell organelles.

At this stage, a brief history of various events that led to the present-day knowledge of biotechnology will be useful. The foundation of modern biotechnological applications can be traced to 1866 when Czech monk Gregor Mendel published the results of his experiments on garden pea. He suggested the involvement of some factors in the transfer of traits from one generation to another. Later this factor was determined as the gene. After the rediscovery of Mendelism in 1900, biologists were concerned mainly with the inheritance of structural or other visible variations. Later, A.E. Garrod (1902) recognized a class of defects in human beings such as diabetes, phenylketonuria, tyrosinosis cretinism, albinism etc., which were caused by a fault in the metabolism within the body.

O.T. Avery, C.M. MacLeod and M. McCarty (1940) were the pioneers in studying the chemical nature of the substance that was responsible for bacterial transformation. G.W. Beadle and E.C. Tatum (1941) carried out genetic experiments with the breadmould *Neurospora crassa* with a biochemical slant, and established that genes worked through biochemical pathways. They also postulated that each gene was responsible for the synthesis of one particular enzyme.

The whole structure of a protein, insulin, was established by Sanger (1953). Crick and Watson (1953) showed that deoxyribonucleic acid (DNA) had a double-stranded structure. Nirenberg (1963) deciphered the genetic code that was applicable from bacteria to man. Merrifield (1963) manufactured and marketed the first automatic polypeptide synthesizing machines. Edman and Begg (1967) developed methods for protein degradation. Arber, Smith and Nathans (1972) discovered the restriction enzymes which cut out DNA at specific points. Gilbert, Maxam and Sanger (1976) developed rapid methods for chemical analysis of DNA. Itakura and his co-workers (1977) synthesized the genes of human somatostatin and insulin. N. Goodon and M.D. Chilton (1977) proved that the transfer of genes was possible using the bacterium *Agrobacterium tumefaciens* as a carrier H.G. Khorana (1979) succeeded in synthesizing, for the first time, an entirely artificial gene capable of functioning within a living cell. Itakura (1980) also constructed the first gene assembler. Hood (1981), who invented the protein micro-analyser, built another automated machine for the same purpose.

Kary Mullis (1983) invented Polymerase Chain Reaction (PCR) which revolutionized biotechnological applications. Alec Jeffrey (1984) developed genetic fingerprinting technique which can be used to identify individuals by analysing the varying sequences (polymorphisms) in the DNA. The human genome project was initiated in 1986. In 1995 M. Sehena developed complementary DNA microarray system to monitor gene expression; also the

Institute for Genomic Research reported the first complete DNA sequence of the genome of a free-living organism. In 1997 Dolly the sheep was cloned using somatic cell by Ian Wilmut and his colleagues. Also the complete sequence of the genome of the yeast *Saccharomyces cerevisiae* was reported. In 1999 human chromosome 22 was sequenced. In 2000, the genome sequence of *Adabidopsis thaliana* was completed. In 2001, complete map of the genome of rice was reported. Also annotations and analysis of human genome was published. In 2002, cloned pigs were reported. In 2003 human stem cells were used to treat diseases. Mouse genome was also sequenced.

In the field of tissue culture, isolation of protoplast was carried out by Klercker (1892). Haberlandt (1902) demonstrated the totipotency of cells. White (1934) showed the possibility of growing excised tomato root tips *in vitro* for an indefinite period. Gauthert (1937) succeeded in cultivating undifferentiated carrot tissues. Van Overbeek and his group (1941) isolated embryos of *Datura* which could grow and develop on a chemical medium supplemented with coconut milk. The possibility of regenerating plants *in vitro* from the shoot apex of certain angiosperms was first demonstrated by Ball (1946). Skoog and Tsui (1948) showed that shoot initiation, also termed as caulogenesis, in tobacco stem segments and callus could be chemically regulated by manipulating the nutrient medium. La Rue (1949) managed to grow immature maize endosperm in culture.

Morel and Wetmore (1951) were the first to achieve success with monocot cultures. Tulecke (1953) obtained the first haploid callus from the pollen grains of *Ginkgo biloba*. Munir, Hildebrandt and Riker, (1954) reported the growth of isolated cell cultures in a liquid medium. Skoog's group (1955) identified 6-furfurylaminopurine, a degraded product of herring sperm DNA, as a chemical capable of stimulating cell divisions. Skoog and Miller (1957) showed that shoot and root initiation in tobacco callus cultures could be regulated by maintaining a subtle ratio between auxin and cytokinin in the medium, paving the way for the chemical regulation of organogenesis. Carew and Schwarting (1958) were the first to get callus cultures from rye, a monocot. Steward (1958) discovered the differentiation of somatic bipolar embryos in carrot using cell suspension techniques. Reinert (1959) reported the use of a nutrient medium solidified with agar for embryogenesis.

Cocking (1960) isolated plant protoplasts enzymatically. Morel (1960) developed the techniques of shoot apex culture of orchids for clonal propagation. Guha and Maheshwari (1964) obtained direct embryos from cultured anthers of *Datura* which led to the development of haploid plants. Nishi and his colleagues (1968) were the first to induce differentiation in monocot callus culture of rice.

Binding and his colleagues (1970) isolated streptomycin resistant callus of *Petunia hybrida*. Carlson and his group (1972) were the first to fuse the protoplast of *Nicotiana glauca and Nicotiana langsdoiffti,* two sexually compatible species of tobacco, and regenerated a parasexual hybrid.