# APPLIED MICROBIOLOGY

Edited by

H. W. DOELLE

and

C.-G. HEDEN

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H. W. DOELLE

Department of Microbiology, University of Queensland, Brisbane, Australia

and

C.-G. HEDÉN

Microbiological Resources Centre (MIRCEN), Karolinska Institute, Stockholm, Sweden

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### APPLIED MICROBIOLOGY

#### Trends in Scientific Research 2

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#### Preface

Unesco, in cooperation with the International Council of Scientific Unions (ICSU), and a number of international and national institutions, such as the International Federation of Institutes for Advanced Study, is publishing a series of monographs 'Trends in Scientific Research'. Each monograph will be complete in itself, but will be linked to the others by a central theme — that of developments at the forefront of science of special interest to humankind.

The idea is far from new. Professor P. Auger published a major work in 1957, Trends in Scientific Research, which played a seminal role in stimulating the development of science in many countries and served for many years as a handbook for science policy makers. Since this book was published, there has been a considerable extension of our scientific knowledge and it is no longer possible to fit all of science into one book if the various disciplines are to be treated in some depth. It was therefore decided to publish a number of separate monographs written by specialists and linked together to provide an extensive and extending image of present and future trends in science.

The monographs will be interdisciplinary in character, bring together not only research in several disciplines but also the applied as well as the basic aspects of science, and in particular the uses of science in forecasting and in problem solving. Special attention will be paid to the use of science in the fulfilment of human needs.

The monographs are addressed to decision makers, to scientists interested in disciplines other than their own, the educated man in the street, and so on. It is intended that the series will provide interesting material that will influence young students to take up a career in the subjects treated.

Inasmuch as there will be a focus on applied aspects of the subjects treated, the monographs will also be of use in highlighting those types of scientific research most relevant to the requirements of developing countries and those fields of research and developments with the

greatest potential applications. Moreover, they will also try to highlight and foresee the most important aspects of the consequences on society of the introduction of new technologies.

The monographs will provide a series of interesting and stimulating publications which, it is hoped, will play an important role in shaping the development of scientific research and stimulating a greater interest in science in the world at large.

THE EDITORIAL BOARD

#### List of Contributors

- Chiao, J. S., Department of Microbiology, Shanghai Institute of Plant Physiology, Academia Sinica, Shanghai, China.
- DaSilva, E. J., Division of Scientific Research and Higher Education, Unesco, Place de Fontenoy, 75700 Paris, France.
- Doelle, H. W., Biotechnology Group, Department of Microbiology, University of Queensland, St. Lucia, Queensland 4067, Australia.
- Gyllenberg, H. G., Department of Microbiology, University of Helsinki, Helsinki 71, Finland.
- Hedén, C.-G., UNEP/Unesco/ICRO Microbiological Resources Centre (MIRCEN), Karolinska Institute, 19401 Stockholm, Sweden.
- Heinritz, B., Institut für Technische Chemie, Akademie der Wissenschaften der DDR, Leipzig, German Democratic Republic.
- Olguín, E. J., Instituto Mexicano de Tecnologías Apropriadas, Apartado Postal 63-254, 02000 México D.F.
- Ringpfeil, M., Institut für Technische Chemie, Akademie der Wissenschaften der DDR, Leipzig, German Democratic Republic.
- Sly, L. I., Culture Collection, Department of Microbiology, University of Queensland, Brisbane, Australia.
- Zavarzin, G. A., Institute of Microbiology, USSR Academy of Science, Puschino, USSR.

#### Introduction

Biotechnology has been defined as 'the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services' (Bull et al., 1982). This definition implies that applied microbiology is an integral part of biotechnology. The term 'scientific and engineering principles' further indicates that subjects such as microbial technology, biochemical engineering, microbiology, biochemistry and genetics form the core of biotechnology. 'Biological agents' can include microorganisms, plant cells, animal cells or enzymes, and similarly, 'materials' all organic and inorganic substances. There is no doubt that biotechnology is an area which cuts right across the traditional faculties of biology, engineering and medicine and thus is characterized by its interdisciplinary nature. It is true that industrial microbiology and food fermentation are as old as mankind, but both represent only a portion of what is now referred to as biotechnology, although both terms have been employed with different connotations by politicians and project planners (DaSilva, 1983). The introduction of economics and socio-economics as integral parts of biotechnology makes this field suitable for both rural and industrial applications (Rivière, 1983).

The greatest challenge in the field of biotechnology has developed

The greatest challenge in the field of biotechnology has developed without any doubt over the past few decades. The realization of possible food shortages in the near future, disasters in climatic conditions leading to starvation in many areas of the world, the accumulation of industrial, human and animal wastes as a consequence of an ever-increasing population, together with an awareness of the changes in the environment that are increasing health hazards, have alarmed both scientific and governmental agencies. The energy crisis brought the realization that fossil fuels, up to now, have been channell d primarily towards meeting the population growth rate and its technological accomplishments (DaSilva and Doelle, 1980). This sudden realization of how much our lives depend upon fossil fuel energy and

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the fact that our natural fossil fuel resources will diminish with the ever-increasing demand by the growing population instigated a desperate search for resources that are renewable and technologies that could be used to replace the heavy energy-demanding industries. It became clear to the scientific and governmental communities that goods and services at present provided by the chemical industries must be partly or wholly replaced by those from biological industries, if mankind is to survive.

This realization immediately divided biotechnological development into two large camps, which coincide with development in the developed and the developing countries. Whereas the former is mainly concerned with maintaining the quality of life, the developing countries' main thrust is towards the improvement of the quality of life (Natl. Acad. Sciences, 1979; van Hennert et al., 1983). Starved of industrial development in the past, the developing world sees the chance of avoiding the establishment of energy-demanding industries and of promoting the use of renewable resources via biological industries (Campos-Lopez, 1980; King et al., 1980), of facilitating the evolution of relevant technologies that can serve as the base for industrial growth and employment, and also of seeing economic growth in urban and rural areas through the just distribution of food, fuel, and the like (DaSilva 1983). There is an important consideration here: that a certain development may be appropriate in one situation, but unsuitable to a variable degree in another. The reasons for this are manifold, including the availability of different renewable resources, a different socio-economic order, lack of infrastructure and like. A new term was created for this development: 'appropriate biotechnology'. In order to develop such appropriate biotechnology, careful consideration must be given to the resources available together with the existing social structure of the population (Doelle, 1982). The motivation for any developmental effort in this area should be either to satisfy basic needs, such as food, shelter and living conditions or to improve standards (Sørensen, 1979). The idea that improvement of the situation in the developing countries can be achieved through simple transfer of this 'appropriate biotechnology' or 'developed countries' technology' is therefore a naive and fruitless one. One needs to study the general technology available in other countries and to choose or select that process which could be best modified and with the available manpower to suit the local requirement. It is encouraging to see a definite trend towards such development by scientists of the developing countries. The best examples have been the attempts to integrate biotechnology with agricultural systems (Monroy and Viniegra, 1981; Olguin, 1982) in order to completely exploit and secure the renewable resource material, resulting not only in a range of different goods but at the same time in a cleaner and better environment.

The challenge lies in the difficulties which exist in biotechnological development. Taking into account that considerations are the existence of basic processes that can be geared towards the development as well as infrastructures of the particular country, or it developed or developing. Consequently, an awareness of the trends in the field of biotechnology is a great asset.

The two different directions of biotechnological development are reflected at the international level by the existence of the International Biotechnology Symposium devoted primarily or predominantly to the developed countries and the Global Impact of Applied Microbiology (GIAM) devoted to the developing countries. It is the function of the International Organization of Biochemical Engineering and Biotechnology (IOBB) in cooperation with the International Cell Research Organization (ICRO) to form a bridge between the two camps, and it was heartening to witness the fruitful interconnections as they occurred at the VIIth International Biotechnology Symposium in New Delhi in 1984.

There is no doubt that the social and economic pressures stemming from an increasing demand for better health and nutritional standards, environmental concerns for waste management, and the increasing cost of oil-based feedstocks, will ensure that biotechnology has a major impact in the world (Bull et al., 1982; Healy, 1979). Some of these pressures are important in the development of appropriate socio-economic bioindustries (DaSilva, 1980). DaSilva (1981) used the terms high-capital, intermediate-capital and low-capital or village-type technology. Some village-type technology is, of course, as old as industrial microbiology. Here we are referring to the solid substrate or food fermentation industry (Djien, 1982). Whereas industrial microbiology has made spectacular advances, the food fermentation industry has not developed as quickly. The reasons are obvious if one concerns oneself with the process technologies. It is much easier to develop processes with a single pure culture than with a mixed culture population.

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This brings us to the heart of the problems and the processes and major concern in the development of biotechnology: the catalyst or biological agent itself. If we restrict ourselves to the microorganism as the biological agent, the enormous potential of this biological agent has been stressed many times in the past (Porter, 1980; DaSilva 1981; Bull et al., 1982). The problem is, however, that the number of microbiologists who have the understanding and vision to convince the public that the beneficial activities of the microbial world can be exploited for the human good, is still very small. Process-oriented basic research is still relatively weak, as it requires an acknowledgement by the public and research bodies that such a direction is at least as useful as basic research for the advancement of pure science (Bull et al., 1979). One has only to mention the developments in plant cell and animal cell cultivation, hybridoma technology, genetical engineering, immobilization and the like, to visualize the benefits coming from biotechnology.

This volume on trends in applied microbiology and biotechnology has as its aim to show the achievements, trends and future scope of biotechnology in developing countries. The enormous range of R&D work undertaken in the world at present forces the editors to be selective in their approach and it is hoped that a future volume in the series might cover other areas as they develop further and show definite trends.

Microbial cultures or their enzyme components are the basic elements in applied microbiology and biotechnological processes, as they represent the catalysts for these processes. Most of us have used cultures from culture collections, but are unaware of their everincreasing importance in preserving the microbial culture heritage. The first chapter discusses the World Data Centre and Microbial Resource Centres (MIRCENs) established by Unesco and UNEP for the purpose of providing the manpower skilled in the preservation of new cultures and acting as the data source for the location and supply of particular cultures. It should be realized that maintenance and preservation depend on the type of organism and become almost an art if they concern the viability of the microbe and its capability in a microbial process. Many cultures have been lost in the past owing to mismanagement and we cannot afford the enormous efforts required for the development of new strains (Rivière, 1975), and particularly patented strains. There is no doubt that development in biotechnology

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depends very much on future trends in microbial process development, which involves isolation, optimization and preservation of our cultures. It is very unfortunate that many government agencies often overlook the importance of culture collections and it is hoped that the MIRCEN network together with the new MIRCEN Journal of Applied Microbiology and Biotechnology will rectify the situation in the near future and safeguard new biotechnological development.

Biotechnology is a field that relies very heavily on the information content of the microbe (Bull et al., 1982), which has a vast potential to be tapped (DaSilva and Doelle, 1980). The exploration and search for this information and its exploitation for the development of a microbial process is outlined in the second chapter. Very often overlooked by many researchers is the potential of the right microbe. If this is the case for the so-called wild-type strains, where are the limits if one considers the opportunities of improvement and extension created through genetical engineering in conjunction with the new cultivation techniques developing through the immobilization technology?

The greatest benefit to the whole of microbial technology comes from the development of the single-cell protein (SCP) process (Bull et al., 1979; Rose, 1979). Technological innovations in the handling of water-insoluble substrates, pressure-cycle fermenter design, the first large-scale chemostats, and tower fermenter desgin are a few examples of the initial concepts coming from the biochemical engineers that have stimulated interest and activity in this field. It is often forgotten that the initial work with n-alkanes as substrate was carried out because n-alkanes were unwanted or waste products of oil refineries, as were also in many cases methanol and methane. In order to bring unit costs down, the oil industry had to find new products from such wastes. The SCP process was certainly the first attempt to produce proteinaceous food and feedstuffs without the aid of agriculture and independent of climatic conditions. M. Ringpfeil and B. Heinritz describe trends in single cell protein technology as SCP is an everincreasing source of animal feed and can now be produced using a large variety of waste products.

Apart from food and feed, many areas in the world are still without energy and electricity for cooking and industrial development, and others feel the high prices of oil imports. The oldest and most advanced technology can be found in the conversion of domestic and xvi Introduction

agricultural wastes into methane (Hobson et al., 1981). Although the basic foundations for biogas reactor development were laid in India, the most spectacular advance in their exploitation have occurred in the People's Republic of China. This enormous development is outlined in the chapter by J.S. Chiao.

E. J. Olguin then gives an example of the future trends in socioeconomic or appropriate biotechnology. This integrated system combines the potential of algae, yeast and bacteria with agricultural systems and waste treatment technology to raise not only the economic, but also the health and nutritional standard of the people in a small community using typical village-type technology. Such integrated systems can lead to food, feed and fertilizer supply, can take care of environmental problems and certainly raise living standards in arid zones. Many of these systems have been proposed not only in Latin America, but also in Kuwait and southeast Asia.

The following two chapters by Zavarzin and Heden - are significant in that they outline two important aspects that are apt to be overlooked in biotechnological approaches to development. Whereas the former outlines some of the biochemical and ecological aspects that may impir ge on new bio-industries such as ore-leaching, the latter focusses on the paradoxical scale-factor in biotechnology. In this chapter attention is also given to the important aspect of biotechnology transfer.

The final chapter deals with a number of biotechnological considerations that interrelate with world development. In a discussion on policies, potentials and prospects a number of cases are examined in the light of biotechnology being deployed for goods and services. In agreeing that "known" goods and services can be produced more beneficially than before, the conclusion dervied at, is that real and farreaching progress is bound actually to *new* products, and completely *new* goods and services. These, hardly foreseen, are not outside the cope and reach of biotechnology.

It is the aim of this volume to indicate trends in applied microbiology using the most established systems as examples whether complete or still in the process of development. There is no doubt that much exciting developmental work is in progress in many other areas, but it is a long way from an idea, via development, to established reality. If this volume succeeds in passing on some of the excitement about the work that has already been done using the vast potential of

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the biological agent, indicates what could be done if this potential is harnessed further, and stimulates even stronger development, then its purpose will have been achieved to a great extent.

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# Culture Collection Technologies and the Conservation of Our Microbial Heritage

#### INTRODUCTION

Microbial cultures or their enzyme components are the basic elements in applied microbiology and biotechnological processes. The diversity of the microbiological community offers a seemingly limitless pool of metabolic information for the production of useful products or the conversion of others for the benefit of mankind. Technological development in the science of genetic engineering has brought a new perspective to our microbial heritage and brought many processes within the practical reach of the biotechnologist.

If Antonie van Leeuwenhoek was the first microbiologist, then Louis Pasteur should surely be regarded as the first applied microbiologist. It was he who played the important fundamental role in recognizing the link between microorganisms and the processes now regarded as applied microbiology and biotechnology. Pasteur observed that microorganisms had a specific role in human and animal diseases, in the production of foods and drinks such as cheese, beer, wine and vinegar, and in production difficulties such as those in the silk industry.

The early observations by Pasteur and other microbiologists confirmed the close association between microorganisms and mankind and showed that these associations could be either beneficial or harmful to man. The subsequent isolation of pure cultures by Koch in 1880 was soon followed by others and the concept of the collection of cultures was born. Over the last 100 years microbiologists have been active in isolating pure culture strains of microorganisms which comprise the species and genera of the microbial world. This task is not complete by any means and perusal of the scientific literature shows that rany new taxa are described each year.

The isolation of pure cultures is an essential part of the identification of microorganisms in nature and the selection of microorganisms which exhibit rare or unusual capabilities of degradation or production of compounds which could be exploited by man.