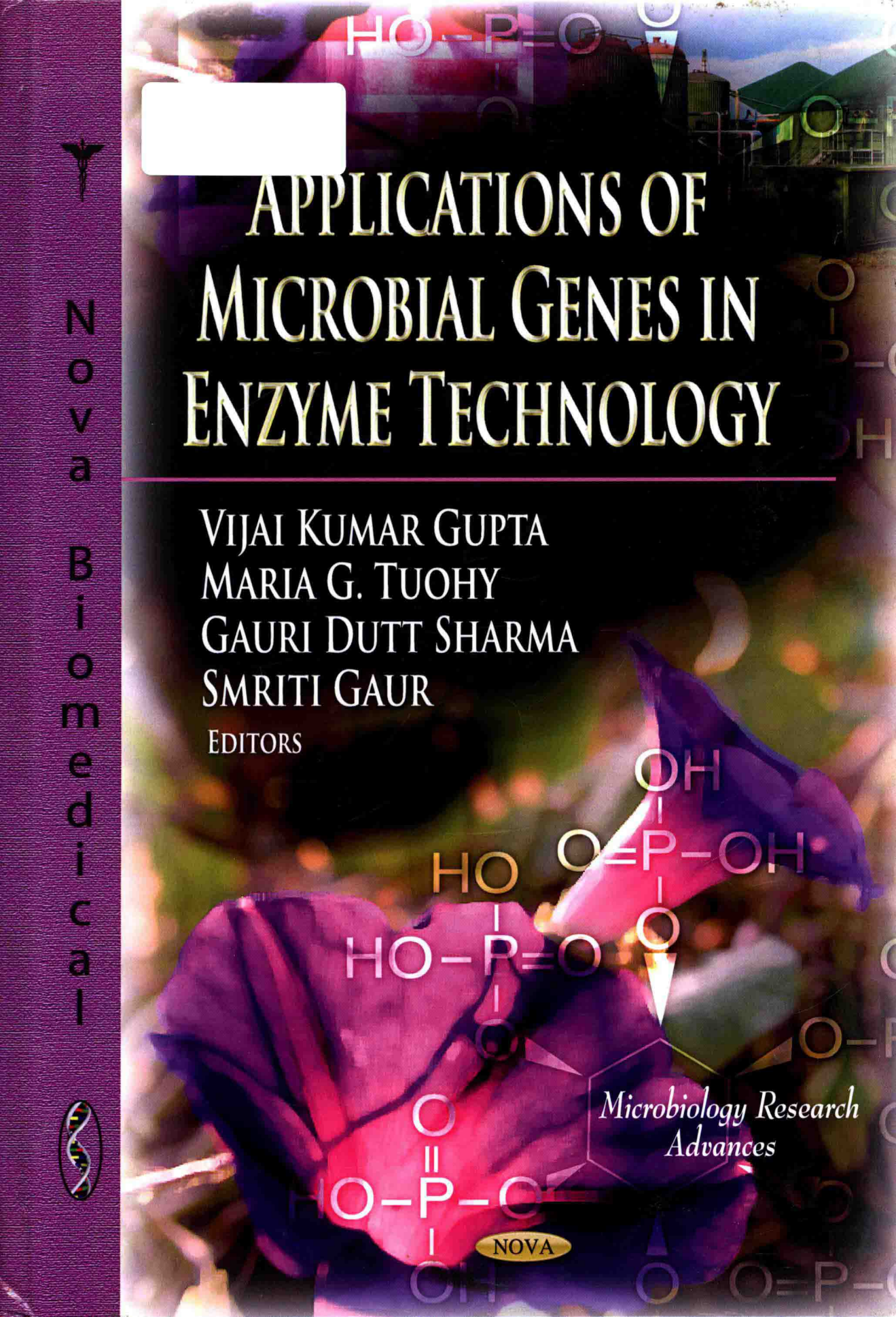
Nova Biomedical
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Microbiology Research Advances
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Foreword

The importance of sustainability in productive activities has been widely recognized, being required the replacement of chemical processes based on non-renewable inputs for chemical or biochemical processes that use renewable inputs. It is also recognized the need for replacing the multistep chemical process for biotechnological processes more efficient. This condition favors the use of renewable raw materials through biotransformation and biocatalysis. These technologies are already being used by industry, although there is a great interest in developing new processes. Its implementation results in higher quality products, obtained by means of lower energy consumption and lower environmental impact.

In function of these tendencies and needs, it is forecasted a significant increase in consumption of enzymes worldwide. This scenario is particularly attractive because the use of enzyme catalysis allies the technological development with the utilization of renewable raw materials as well as with environmental preservation. The enzymatic technologies found applications in agriculture as biocontrol agents, in food as additive or to its production and, more recently, in biofuels production due to great interest in development a technological and economical viable process for hydrolysis of lignocellulosic or starch-rich materials for second generation ethanol production.

In this scenario, the use of microbial genes in enzyme technology is fundamental to obtain success during the developing and implementation of enzymatic process, because the use of genetic engineering employs tools that allow the selection/obtainment of more specific enzymes or to improve the its affinity to a specific substrate. These possibilities will lead to an improvement on process productivities, making it technologically and economically viable. In this sense, the book "Applications of Microbial Genes in Enzyme Technology" reports a theme in the frontier of knowledge and brings the readers with new development on genes manipulation to improve enzyme production/application.

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Preface

Microbes are excellent models for understanding biological interactions and evolutionary biology due to their large ecological and genetic diversity. With the advancement of biotechnology, increasing numbers of enzymes have been identified and produced before being used in various industries including medicine, agro-industry, commodity production biofuel and modern biotechnology. Microbes are ubiquitous in all ecosystems and vital for its functions. The Enzyme Technology work as to establish to identify and characterize novel enzymes with desirable characteristics by taking advantage of biodiversity, especially the hugely diverse variety of microorganisms. Microbial enzymes are economical and can be produced on large scale within the limited space and time. The amount produced depends on size of fermenter, type of microbial strain and growth conditions. It can be easily extracted and purified. Microbial diversity is one of the important resource for development of new micro-organisms and strain improvements, for several important genes and production of enzymes having high value to food and pharmaceutical and biotechnology industry. The identification of microbial resources allows the heterologous high-level overexpression of the corresponding enzymes. They used to produce broad range of hydrolytic enzymes that can break down complex biopolymers and produce chemically and structurally complex compounds with high industrial interest. A better understanding of microbial ecology may lead to the identification of novel species, functions and biomolecules for a variety of biotechnological applications. Exploitation of the valuable genetic resources that microbial diversity comprises, most often requires modern biotechnological methodology. Identifying species of organisms by short sequences of DNA has been at the center of current research. The microorganisms have specific genes introduced into their DNA through genetic engineering, so that they produce enzymes naturally made by other micro-organisms or newly developed strain of particular microbe. The research includes all aspects of enzyme biotechnology from screening of enzymes from microbial isolates and from metagenomic libraries, gene isolation, enzyme production in wild-type microbes and recombinant systems to development of enzymatic processes in industry. It means that due to presence of high genetic flexibility they can be genetically manipulated to increase the yield of enzymes within very short generation times. Since genes encode enzymes, the changes in gene certainly bring about alteration in enzyme structure, so using enzyme engineering systems and their modification of enzyme structure by alteration of gene/ modified gene it seems to be a promising technology for the production of stable enzymes by genetically engineered microbial cells in pilot scale.

This book provides an extensive survey of applications of important microbial genes and their functions in enzymes production for several industrial processes. The chapters presented in the book will cater the need of students of undergraduate, postgraduate courses and researchers across disciplines and sectors where microbial diversity and enzyme research and experimentation are undertaken. Moreover, this book covers the recent updates on important genes, their functions in microbial systems and their applications in enzyme technology. Therefore, this publication will be very useful not only to experienced researchers but also for the beginners.

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04th May, 2012

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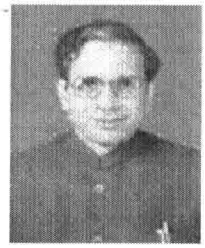
ARI, Pune, India. Also, his group in NUI Galway is under process to submit 02 inventions for European patent. He is the editor and member of 9 International and 2 National journals with 38 International/National research publication and 23 book chapters in books published from highly recognized International/National publishers in his hand. He is the editor/author/internal editorial board member of books/book series from reputed publishers of International fame viz. Elsevier, The Netherlands; Science Publisher, New Hampshire, USA; Taylor and Francis, USA; Springer, USA and LAP Lambert Academic Publishing, Germany.



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Professor G. D. Sharma has about 40 years of experience in the field of teaching and research in various central universities of India. He is Pro Vice-Chancellor and Dean School of Life Sciences, Assam University, Silchar, India. He has been Vice Chancellor of Nagaland University, India. He has supervised 30 Ph.D. and M. Phil students. He has authored/edited 10 books and published 250 research papers in journals of repute. For his research contributions certain prestigious Awards like Dr Narsimhan Medal by Indian Phytopathology Society, Birbal Sahni Award by Indian Botanical Society, Rashtriya Ratan Award by International Study center, DEED Award by Confederation of Indian Universities affiliated to UN-ECOSOC, International CCLP Honor, Vice President of Indian Mycology Society etc.Dr Sharma has visited several countries for various academic assignments. His current area of interest includes Microbial diversity, Microbial enzymes, Biocontrol, Food microbiology medicinal plants and health.

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Dr. Maria G. Tuohy is the Head of the Molecular Glycobiotechnology Research Group, Department of Biochemistry, School of Natural Sciences, NUI Galway which has developed a strong track record in Glycobiotechnology and Enzyme Biotechnology. She has more than 20 years experience in the molecular biochemistry, genetics and biotechnology of fungi, with a special interest in thermophilic ascomycetes and the characterization of these fungi as cell factories for protein production, including novel thermostable enzymes/enzyme systems. Dr. Tuohy and her group have developed patented enzyme-based technologies for key bioenergy and biorefinery applications from terrestrial and marine biomass and wastes, including 3rd generation feedstocks. The group also investigates the use of enzymes for the recovery and selective modification of high-value biochemicals and plant carbohydrate-derived bioactives ('Glycobioengineering'). Dr. Tuohy is a PI in the Energy Research Centre, NUI, Galway and the recently funded national Bioenergy and Biorefinery Competence Centre, is a member of the EU FP7 Biofuels Platform and a national research PhytoNetwork. Dr. Tuohy has been a visiting researcher in RUGhent, Belgium and BSH Institut fur Holzchemie, Hamburg. Dr. Tuohy is author of ~132 research publications, including refereed publications, book chapters, conference papers poster/short communications. She is also a reviewer for international journals and funding agencies and several books as co-editor- Elsevier, The Netherlands, Springer Science Publisher, USA; CRC Press, Taylor and Francis, USA; Lambert, Germany; Nova Science Publisher, USA and Elsevier Press, USA (under Progress) with Dr. V. K. Gupta.

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University, Gwalior. She has done her PhD in Biotechnology in 2010, where she isolated, characterized and purified proteases from plant and microbial sources. She has been Secured second position in her graduation and master courses. She has attended many international and national conferences and presented her research work both in India and abroad. She has been awarded travel grant from Society of Industrial Microbiology and Biotechnology, USA and Department of Biotechnology, Ministry of Science and Technology, Govt of India. She is presently focused in the area of Microbial biotechnology and enzyme technology. She has submitted one bacterial strain sequences to NCBI, USA. She has 2 international research publication and 2 international book chapters in her hand.

Contents

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Chapter 1

Use of Metagenomics for the Production of Novel Enzymes

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Abstract

This chapter is an overview of the recent advances in metagenomics, which is a novel method of utilizing the unravelled genetic information of microorganisms which are uncuturable using classical microbial methods. With the understanding that only a small fraction (less than 1%) of infinite microbial diversity can be grown in nutrient media, the culture-independent approach of metagenomics seems to have considerable potential. The possibility of recombining the genes of useful enzymes retrieved from these "unculturable" microorganisms into the organisms, which we know to culture and genetically modify, opens up the possibility of producing a large number of enzymes with novel properties making them useful in a number of biotechnological applications. This chapter includes an example of the possibility of producing new cellulase enzymes from the metagenome of microbes which grow symbiotically in rumens of herbivores creating

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breakdown of the cellulosic feed they consume. The array of methods that are used in genetic screening for novel genes, introduction of microorganisms in the phylogenetic tree, etc. are discussed.

Introduction

Enumeration of microbes in environmental samples can be done either by direct count using a microscope, or by viable count by growing diluted-samples in nutrient media. The disparity between the two was not given much importance for a long time. But recent studies have shown that we are not yet able to cultivate 99% of the microorganisms available in nature (Amann *et al.*, 1995; Streit and Schmitz, 2004), as we are not providing them the appropriate conditions for them to grow (Bull *et al.*, 2000). Many studies which explore the diversity and potential of microorganisms using classical microbial culture methods are thus certainly limited.

With the advent of genetic engineering and molecular biological methods, the possibility of using the wealth of genetic information contained in the microorganisms which we are not able to cultivate, now called unculturable microorganisms, is now promising. It is possible to mine for genetic information in these microorganisms, capable of carrying out a specific bioconversion, and reproduce them using a culturable host microorganism, such as *E. coli*. This method of using the genetic information of unculturable microorganisms is known as metagenomics.

The overall technique would then be to release all DNA from a mixture of microorganisms (e.g. from rumen fluid, or a soil sample), breakdown of the DNA into manageable fractions and then clone them into a well-studied host. Then an analysis based on functional genomics may be able to identify new catalysts, or metabolites, with characteristics better than those produced by known culturable microorganisms. Besides its practical and possible use in industrial applications, metagenomics can play a vital role in studies related to ecological diversity, phylogeny, understanding symbiosis, enriching gene families, etc.

There are many various stages in developing a metagenome. The first step is certainly sampling, like in traditional microbiology, from a source at which there is the maximum likelihood of getting the catalyst or metabolite, one is aiming to produce. It should be a representative sample from the source. The total number of microorganisms present is difficult to access as they cannot be seen. Some previous experience on the numbers involved in the sample will be helpful in this decision. Utilization of the whole sample or filtration to screen out undesirable microorganisms (e.g. viral particles from eukaryotes) is the next step. This is followed by cell lysis, extraction and shearing of metagenomic DNA. The DNA fragments are then cloned into plasmids and libraries are created. Sequencing of the clones then follows and finally alignment of the sequences is done using bioinformatics. A typical sequence procedure is to use the shotgun method, where random fragments of genetic material are cloned into plasmid vectors and amplified in quantity before being sequenced. However, more rapid and powerful methods are currently being developed and used. Assembly of the sequence information to complete DNA forms is indeed very difficult and its use in metagenomic studies to carry out partial assembly to the level of whole domains or multi-domain genes. For practical application, the most important part is then to identify useful genes. The incomplete nature of metagenomic data makes this difficult. The commonly