Industrial Exploitation of Microorganisms

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I.K. International Publishing House Pvt. Ltd.

NEW DELHI • BANGALORE

Published by

I.K. International Publishing House Pvt. Ltd.
S-25, Green Park Extension
Uphaar Cinema Market
New Delhi – 110 016 (India)
E-mail: info@ikinternational.com

ISBN 978-93-80026-53-4

Published by Krishan Makhijani for I.K. International Publishing House Pvt. Ltd., S-25, Green Park Extension, Uphaar Cinema Market, New Delhi – 110 016 and Printed by Rekha Printers Pvt. Ltd., Okhla Industrial Area, Phase-II, New Delhi – 110 020.

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PREFACE

Microorganisms have been exploited in multifarious ways since the dawn of human civilization. The ancient humans developed techniques of producing alcohols, acids, beverages, fermented foods, etc. in different countries of the world. For example, yeasts have been exploited throughout the world in several countries for production of different kinds of preparation from kitchen to industry. Many yeast plasmid- and chromosome-based vectors have been constructed for biotechnological applications in industry. Still it is also a major tool for biotechnological applications. Considering the importance of microorganisms, demand of industrial application of microorganisms got increased gradually. Nowadays thousands of antibiotics have been produced through microorganisms during the last 80 years from the discovery of the first antibiotic and many more have been commercialized. In recent years the researchers have developed the techniques to produce products through introducing the desired genes of interest in fungal/bacterial/plant/animal cells. Therefore, prokaryotic and eukaryotic cells are being used as hosts for introduction of recombinant vector(s) containing genes of desired functions for production of valuable products.

Though the direct use of microorganisms is known since the beginning of 16th century but much emphasis has been given on industrial microbiology in recent years for improvement of human and animal health. Among these the lactic acid bacteria are considered and selected as 'Probiotics'. Some non-lactic acid bacteria are also considered as Probiotics. In addition, different species of *Spirulina* have also been exploited in food industry and *Spirulina*-based products have now been commercialized.

However, due to expanding population day-by-day global food shortage has alarmed the world population. Many varieties of mushrooms have been explored and utilized in multifarious ways since vedic period. Recently, mushrooms are looked up on for the presence of industrially important bioactive molecules, for example anticancerous compound in Shitaki mushroom. In addition, certain higher fungi hold much promise due to the presence of medicinal principles like digestive enzymes, stress reliever, antiviral effects, anti-tumour activity, strengthening health resisting disease, etc. Certain mushrooms like *Cordyceps sinensis* has traditionally been used for treatment of human diseases like chronic bronchitis, insomnia, hypertension, pneumonia, tuberculosis, anaemia, etc.

There is resurgence in the search of new antibiotics in clinically important bacteria, especially in actinomycetes. There are many companies throughout the world that are devoted to commercialization of bioactive compounds from actinomycetes. Therefore, prospects of bioactive compounds in Pharma industries are also increasing day-by-day for large-scale production and commercialization of bioactive compounds. Use of bacterial consortium with or without yeast as Probiotics has revolutionized the area of industrial microbiology because of combating the health-related problems of humans. Several bacterial, fungal and viral preparations in different forms are being formulated and marketed because of being eco-friendly and non-hazardous to plants and animals.

Commercial production of highly valued microbial enzymes is gaining attention in recent years due to its great role in many areas of industry, for example, microbial proteases, lipases, cellulases, laccases, phytases, etc. Besides, production of 'nanoparticles' through microorganisms has attracted the attention of researchers throughout the world. The nanoparticles can play a key role in many technologies of the future. One of the key aspects is the development of reliable experimental protocols for the synthesis of nanoparticles over a range of chemical compositions, sizes and high nanodispersity. Several microorganisms are known to produce inorganic materials intra- or extracellularly, for example, nanoparticles of silver, gold, magnetite, siliceous materials, calcium carbonate, metal sulphides, etc. Such microorganisms can be a boon for industries in future. The ability of pharmaco-kinetic behaviour, of different molecules, their toxicity in mammals and the possibility for their future application in many areas of medical sciences need to be explored in future.

All the articles included in this book are devoted to industrial exploitation of potential microorganisms which would be very helpful to the undergraduate and postgraduate students, researchers and teachers of microbiology and biotechnology in India and abroad.

D.K. Maheshwari
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EDITORS

D.K. Maheshwari started his teaching carrier as a lecturer in the department of Botany, D.A.V (PG) College, Muzaffarnagar and thereafter served as Reader in the Department of Microbiology, Barkatullah University, Bhopal. He joined Gurukul Kangri University, Haridwar as Professor in the year 1990 and served as Dean, Faculty of Life Science (2004-2006). Prof. Maheshwari is an active member of several scientific bodies of international repute and in the board of panels of various academic and administrative bodies including UPSC and NAAC of Government of India. He has been elected as Editor of the *Journal of Indian Botanical Society* (1999-2002) and he was awardee of young scientist "Prof. Y.S. Murthy Medal" for his outstanding contribution.

As a young scientist, he was selected under UNESCO programmes and worked in Biological Research Center. Szeged (Hungary) in the year 1983-84 and also visited Czechoslovakia, Belgium, Holland, Germany, Japan and S. Korea. He was visiting Professor in Science University of Tokyo, in 1993 and 1998, and Guest Professor, in the University of Ulm (Germany). Prof. Maheshwari visited S. Korea in the year 2000, 2003 and 2006 under bilateral scientific exchange programmes sponsored by Indian National Science Academy (INSA), New Delhi.

Prof. Maheshwari has many research papers in leading peer-reviewed journals both Indian as well as foreign. More than 30 candidates have been awarded Doctorate (Ph.D.) degree under him. He has completed 10 major research projects sponsored by various funding agencies. Prof. Maheshwari is the editor of *Microbes: Agriculture, Industry and Environment* (2000) and *Innovative Approaches in Microbiology* (2002). He is co-author of *Practical Microbiology* (2001) and *A Textbook* of Microbiology and member Editorial Board, Korean J. Agric, Chem. and Biotechnology.

He is a Fellow of the Indian Botanical Society and Indian Phytopathological Society and Life Member of Indian Science Congress, Phytopathological Society of India, Botanical Society and Association of Microbiologists in India. He is nominated as Convenor "Science forum" from Uttarakhand Council for Science and Technology, Govt. of India, Dehradun.

His field of interest is soil microbiology and microbial biotechnology having expertise in biological control of soil-borne plant pathogens, mycorrhizae, Rhizosphere microbiology and botanical pesticides, besides enzymology and heavy metal toxicity. He has published more than 75 research and review papers in the national and international journals of repute. He has produced 14 Ph.Ds and is supervising several research students on varied aspects of microbiology. He has handled research projects sponsored by UGC, UCOST and CSIR.

He has authored three books viz., *Practical Microbiology* (2004), *A Text Book of Biotechnology* (4th edn., 2006) and *A Text Book of Microbiology* (2nd edn., 2005). He is also co-editor of *Himalayan Microbial Diversity* (1997); *Microbes: Agriculture, Industry and Environment* (2000) and *Innovative Approaches in Microbiology* (2002).

He was the Organizing Secretary of the National Seminar on Bioinoculants for Holistic Sustainable Rural Development jointly organized by the Department of Botany & Microbiology and DDU state Institute for Rural Development (U.P. Govt.) (1998). He was the *Co-coordinator* of the Workshop on *Molecular Biology and Applied Microbiology*, and Hands-on-Training on *Microbial Fermentation and Inoculants Production* (2008).

He is the Life Member and Fellow of the Indian Botanical Society; Indian Phytopathological Society and the International Society for Conservation of Natural Resources. He was Associate Editor of the *Journal of the Indian Botanical Society* (2000-2004), and at present he is in the Editorial Board of the *Journal of Environmental Biology and Conservation.*

R. Saravanamuthu did his postgraduate degree at the St. Joseph's College, Tiruchirappalli (TN), M. Phil. at the Centre of Advanced Study in Botany, University of Madras (Chennai) and Ph.D. at the Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi. He has more than thirty years of teaching and research experience in the Department of Botany, A.V.C. College (Autonomous), Mannampandal, Tamil Nadu.

Dr. Saravanamuthu has successfully guided eight Ph.D. scholars and published number of research articles in the reputed national and international journals. He has contributed about ten chapters of research interest in different edited books, and also has been the editor of books, journals and proceedings.

Dr. Saravanamuthu is the Life Member and Fellow of the Indian Botanical Society and member of several professional bodies and academic societies. He has served as the member of the Board of Management of the Tamil Nadu Agricultural University.

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CONTENTS

Chapter 1

Biotechnological Potential and Industrial Applications of Yeast

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Yeast is one of the eukaryotic microorganisms, which has wide applications in various spheres and is being used since very long time. Its application in the brewries and bakeries is well known. The history of application of yeast in industries is very long. This organism is exploited in the production of ethanol, beers, wine and bread. Besides, it is also a source of microbial protein (SCP), and directly used as food, feed and food supplements. Hence, it is also referred as 'nutritional yeast'. Its use as probiotics is not uncommon. Yeast is also a major source of enzymes such as invertase, β-galactosidase, alcohol dehydrogenase, glyceraldehydes 3-phosphate dehydrogenase, hexokinase, etc. The processed yeast products (yeast extract) are used as food additives or flavours. Some strains of yeast find place in bioremediation. Yeast was the first choice among eukaryotic microorganisms for the application of recombinant DNA technology. It is the best organism suited for gene manipulation and transformation experiment. Thus, yeast has been used as industrial process organism, as protein source and also as tool in gene manipulation and transformation technologies.

INTRODUCTION

Yeasts are a group of eukaryotic microorganisms classified in the kingdom of fungi. Approximately, 1,500 species of yeasts have been described (Kurtzman, 2006), most of which reproduce asexually by budding, although a few cases also reproduce by binary fission. Yeasts are cosmopolitan and widely distributed in almost all natural habitats. The most well-known and commercially significant yeasts are the related species and strains of *Saccharomyces cerevisiae*. These organisms have long been utilized to ferment the sugars of rice, wheat, barley and corn to produce alcoholic

beverages, and in the baking industry to expand or raise the dough. *S. cerevisiae* is commonly used as baker's yeast and also for some types of fermentation. Yeast is often taken as a vitamin supplement because it has 50% protein and also a rich source of B vitamins, niacin and folic acid. During the last two decades, *S. cerevisiae* has been the model system of the molecular genetic research because of the basic cellular mechanisms of replication, recombination and cellular metabolism, which are generally considered between yeasts and larger eukaryotes, including mammals. Yeasts have recently been used to generate electricity in microbial fuel cells (Walker, 1998) and to produce ethanol for the biofuel industry.

HISTORY

The word "yeasts" comes from the old English language "gist"/"gyst" ultimately from the Indo-European root "yes" meaning boil, form or bobble. Yeasts are microbes, which are probably one of the earliest domesticated organisms. People have used yeasts for fermentation and baking since a long time. Archaeologist digging in Egyptian ruins found early girding stones and baking chambers for yeast mediated manufacturing bread as well as drawings depict of the 4,000 years old bakeries and breweries. In 1680, the Dutch naturalist Antony Van Leeuwenhoek was the first person to observe the yeast microscopically, but at that time he did not consider them to be living organisms but rather described as globular structure. In 1857, French microbiologist Louis Pasteur proved and reported in his paper entitled memorie *sur la fermentation alcoholique* "that alcoholic fermentation was conducted by living yeast and not by a chemical catalyst". Pasteur showed that by bubbling oxygen into the yeast broth, cell growth could be increased, but the fermentation inhibited an observation later called the "Pasteur effect". Thus, yeast is one of the earliest or the first known microorganisms.

TAXONOMY

Yeasts do not form a specific taxonomic or phylogenetic grouping. At present it is estimated that only 1% of all the yeast species have been described (Kurtzman and Piskur 2006). The term "yeast" is often taken as a synonym for *S. cerevisiae* (Kurtzman 1994), however, the phylogenetic diversity of yeast is shown by their placement in both divisions, Ascomycota and Basidiomycota. The budding yeast (true yeast) is classified in the Saccharomycetales.

YEASTS AS A MODEL EUKARYOTE

The yeasts species *S. cerevisiae* has been a very important model organism in modern cell biology research and is the most thoroughly researched eukaryotic microorganism. Although yeast has greater genetic complexity than bacteria, containing 3.5 times more than *Escherichia coli* cells, they share many of the technical advantages that permitted rapid progress in the molecular genetics of prokaryotes and viruses. Some of the properties that make yeast particularly suitable for biological studies include rapid growth, dispersed cells, the ease of replica plating and mutant isolation, a well-defined genetic system, and the most important aspect is a highly versatile DNA transformation system. Unlike many other microorganisms, *S. cerevisiae* is visible with

Production of Ethanol

S. cerevisiae is the main producer of ethanol. It is used as a major tool for the production of ethanol since the discovery of fermentation process by Louis Pasteur. During 1980, fermentation of broth was discovered in sugar solution by the addition of yeast extracts obtained by its grinding. The ability of yeast to convert sugar into ethanol has been harnessed by the biotechnological industries, which has various uses. The process starts with milling sugar cane, sweet corn or cheap cereal grains and then adding dilute sulphuric acid, and fungal alpha amylase enzymes, which enhances the breakdown of the starch into simple sugars. A glucoamylase is then added to simple sugars. After this, yeasts are added to convert the simple sugar to ethanol, which is then distilled off to obtain ethanol up to 96% in concentration. After the products are harvested, these yeasts are used as microbial source of protein and animal feed supplements. Molasses, cheese, whey, sulphite liquor and gas oil fractions are used as main fermentation media components. But these media usually need some growth stimulating nutrients such as biotin, ammonium ions, sulphate, etc.

S. cerevisiae have been genetically engineered to ferment xylose-one of the major fermented sugars present in cellulosic biomasses, such as crop residues, paper wastes, and wood chips (Dujon, 1996). This development proves that ethanol can be potentially and efficiently produced from inexpensive agricultural waste. This become an alternative source of ethanol fuel which could be competitively produced as a low priced fuel alternative to gasoline fuels.

Beer

Dan Emil Christian Hansen classifies beer brewers yeasts into top fermenting yeast and bottom fermenting yeast. Top fermenting yeasts are so called because they form foam at the top of the wort during fermentation. They can produce high quantity of alcohol concentration and prefer relatively high temperatures producing frutifier, sweetener and ale type beers, e.g., *S. cerevisiae*. Bottom fermenting yeasts are used to produce lager type beers. Bottom fermenting yeasts ferment more sugars, leaving a crisper taste, and grow well at low temperature, e.g., *S. pastorianus*. In industrial brewing, to ensure purity of strain, a clean sample of yeast is stored at refrigeration temperature. After a certain number of fermentative cycles, full-scale propagation is made from this laboratory sample. Typically, it is grown up in about 3 or 4 stages using sterile brewing wort and oxygen.

Root Beer and Sodas

Root beer is also produced by the methods used for the production of beer with only variation in carbonation process—the active phase of yeast is stopped sooner producing only trace amount of alcohol (consumed by all ages), and a significant amount of sugar is left in the drink. Low calorie or nearly sugar free root beer and soda can be produced in such a way by using small amount of sugar and also replacing the majority with artificial sweeteners.

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Distilled Beverages

It is a beverage that contains ethanol, which has been purified by distillation. Carbohydrate containing plant material is fermented by yeast, producing a dilute solution of ethanol. Alcoholic beverages such as whiskey and rum are prepared by distilling this dilute solution of ethanol. Components other than ethanol are collected in the condensate including water, esters and other alcohols, which account for the flavour of the beverages.

Wine

Yeast is used in wine making where it converts the sugars present in grape juice or *must* into alcohol. Yeast is normally already present on the grapes, often visible as a powdery film (also known as bloom or blush) on their exterior. The fermentation can be done with this indigenous (or wild) yeast, however, this may lead to unpredictable results depending on the exact type of yeast species that is present. For this reason a pure culture is generally added to the *must*, which predominantly enhances the process of fermentation as it proceeds. Most of the widely used wine yeast include *S. cerevisiae*, however, not all strains of the species are suitable for wine-making (Gonzalez *et al.*, 2001). Different strains of *S. cerevisiae* differ in their physiological and fermentative properties. Therefore, the actual strain of yeast should be selected that exhibits a direct impact on the finished wine (Dunn *et al.*, 2005). Significant researches have been undertaken for the development of novel wine yeast strains that produce atypical flavours, profiles or increased complexity in wines (McBryde *et al.*, 2006). The growth of yeast species such as *Zygosaccharomyces* and *Brettanomyces* in wine can result in wine faults and subsequent spoilage (Loureiro and Malfeito Ferreria, 2003). *Brettanomyces* produces an array of metabolites when growing in wine, some of which are volatile phenolic compounds. Altogether these compounds are often referred as "*Brettanomyces character*" and are often described as antiseptic or "barnyard" type aromas. *Brettanomyces* is a significant contributor to wine faults in the wine industry.

Baking

S. cerevisiae is used in baking as a leavening agent, where it converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or raise the carbon dioxide formed as pockets or air bubbles. When the dough is baked and carbon dioxide pockets remain, the baked product becomes soft and spongy in texture. The use of potatoes, water from potato boiling, eggs or sugars in bread dough accelerates the growth of yeast. Salt fats and butter slow down the growth of the yeast. The majority of the yeasts used in baking are the same species, which are commonly used in alcoholic fermentation. *Saccharomyces exiguus* (also known as *S. minor*) is a wild yeast found on plants, fruits and grains that is occasionally used for baking. Today there are several retailers of baker's yeasts; one of the best known is Fleishmann's yeast, which was developed in 1868. During World War II, Fleischmann's developed granulated active dry yeasts, which did not require refrigeration and had a longer shelf life than fresh yeast.