Editors

A.R. Alagawadi
P.U. Krishnaraj • K.S. Jagadeesh
J.H. Kulkarni • S. Kannaiyan

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Narosa

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Editors
A.R. Alagawadi
P.U. Krishnaraj
K.S. Jagadeesh
J.H. Kulkarni
S. Kannaiyan
Department of Agricultural Microbiology
University of Agricultural Sciences
Dharwad, India

N A R O S A P U B L I S H I N G H O U S E P V T. L T D.

22 Daryaganj, Delhi Medical Association Road, New Delhi 110 002
35-36 Greams Road, Thousand Lights, Chennai 600 006
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Microbial Biotechnology

FOREWORD

The contribution of microorganisms in human welfare in general and especially in agriculture and medicine was recognized long back. Also, in development of knowledge the microbes have been favourite material for researchers. The microbes are a rich source of useful genetic material and coupled with recent advances in biotechnology are proving to be a boon to mankind in increasing food production by developing "transgenic crops", harnessing solar energy to improve photosynthetic activity of plants, and in increasing Biological Nitrogen Fixation (BNF) by free living organisms, symbiotic *Rhizobium*, cyanobacteria and *Azolla–Anabaena* etc. There is a growing recognition of the potential of microorganisms to degrade herbicides, pesticides and oils in oil spills, the potential microorganism as food supplement, the exploitation of methanogenic bacteria to produce methane gas as energy sources of rural areas and potential of new therapeutic substances produced by microbes and genetically engineered organism by DNA modification in the production of interferon, human insulin and also to clean up toxic wastes.

The Association of Microbiologists of India, organized its 44th Annual conference at the University of Agricultural Sciences, Dharwad, Karnataka on "Microbes and Human Sustenance" and the Proceedings of the conference are being published in form of a volume entitled "Microbial Biotechnology". The publication includes valuable information on some important topics like Bio-fuels from organic wastes, Biodegradation of ramie fibres, Plant disease management, Microbes in IPM, N_2 fixation, organic farming, Phosphate solubilizing Bt-genes, Mushroom production, food processing medicine, diagnostic tools for Veterinary Sciences, etc.

I would like to congratulate the authors Prof. S. Kannaiyan, Prof. J.H. Kulkarni, Prof. A.R. Alagawadi, Dr. P.K. Krishnaraj, Dr. K.S. Jagadeesh for their efforts in bringing out this volume "Microbial Biotechnology" which will certainly be useful to the Teachers, Scientists, Scholars, industry and students.

(MANGALA RAI)
Secretary, Department of Agricultural Research & Education
and
Director General, Indian Council of Agricultural Research
Dr. Rajendra Prasad Road, Krishi Bhawan, New Delhi, India

PREFACE

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. In the ancient years, microbiologists used different useful microorganisms for the manufacture of a variety of useful materials. Microorganisms have been used to produce food and beverages such as beer, vinegar, wine, yoghurt and cheese for over eight millennia. Whatever by-products were obtained during normal cell growth were used by the people for their welfare. Normal growth of yeast cells play a significant useful role in grape juice; sucrose is converted to ethanol and the fermented juice containing alcohol is widely used as wine and wine is a part of life activities at every meal in the western world. Modern Biotechnology enables a microorganism to produce a totally new product, which it does not or cannot produce in its normal course of life. In today's scientific development, it is possible to engineer a new genetic potential in an organism and the technology is called genetic engineering.

The most dramatic current development in applied microbiology is due to the development of genetic engineering and recombinant DNA technology. By exploiting the modern techniques and current methodologies, microorganisms can be engineered through modification of its DNA to produce new substances such as human proteins and bacteria have been modified to produce human insulin and interferon.

Microbiology has become increasingly useful to our society now and it has strongly emerged as one of the most important branches of the life sciences. In today's scientific scenario, microbiology is the basic and fundamental foundation for the strong emergence of modern biotechnology. Many bacteria like *E. coli*, yeast and cyanobacteria were the model systems in demonstrating methodologies for product development and *Agrobacterium* - mediated transformation played a key role in developing transgenic crops.

Microbial Biotechnology is aimed to utilize the current knowledge in microbiology for the welfare of the human beings. Several leading microbiologists of India and International Microbiologists have contributed papers on different current issues of microbiology. The book covers wide range of topics such as microbial diversity, anaerobic retting of fibres, transformation of rice against blast disease, induction of chitinase enzyme by PGPR, rhizobial denitrification, biodiversity of the algal symbiont, *Anabaena azollae*, molecular studies on *Yersinia enterocolitica*, *Deinoccus radiodurans*, endophytic N_2 fixation by *Azorhizobium caulinodans*, ammonia excreting mutants of *Azospirillum*, microbial mineral mineral phosphate solubilization, mycorrhizae, fluorescent pseudomonads, integrated pest management, integrated disease management, *Bacillus thuringiensis*, mushroom production, wine preparation and biofuels from organic wastes. The microbiologists have prepared these valuable scientific papers based on their presentation in the 44th Annual conference of the Association of

Microbiologists of India at the University of Agricultural Sciences, Dharwad. We are thankful to Dr. S.A. Patil, Vice-Chancellor, University of Agricultural Sciences, Dharwad for his full support in organizing the National level Microbiology conference by providing all the facilities which enabled us for the successful conduct of the conference. The book Microbial Biotechnology is the outcome of the National Microbiology conference. We appreciate the efforts of all Microbiologists who contributed the quality papers by sharing their current scientific knowledge and experience. The book will be useful to the scientists, teachers, scholars and P.G. students as a reference volume.

Dharwad

A.R. Alagawadi
P.U. Krishnaraj
K.S. Jagadeesh
J.H. Kulkarni
S. Kannaiyan

LIST OF CONTRIBUTORS

Abhay Kumar
Eureka Forbes Institute of Environment,
Bommasandra Industrial Area,
Hosur Road,
Bangalore

A.R. Alagawadi
Department of Agricultural Microbiology,
UAS, Dharwad – 580 005

S.C. Andras
Plant Sciences Division,
University of Nottingham,
Loughborough, Leicester LE12 5RD
United Kingdom

P. Anthony
Plant Sciences Division,
University of Nottingham,
Loughborough, Leicester LE12 5RD
United Kingdom

K. Arulmozhiselvan
Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University,
Coimbatore – 641 003

D. Balachandar
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

R.H. Balasubramanya
Central Institute for Research on
Cotton Technology (ICAR),
Adenwala Road, Matunga,
Mumbai – 400 019

M.R. Chandrasekhar
Department of Microbiology,
Karnataka Institute of Medical Science,
Hubli

M. Deiveekasundaram
Department of Microbiology,
Annamalai University,
Annamalainagar – 608 002

M.R. Davey
Plant Sciences Division,
University of Nottingham,
Loughborough, Leicester LE12 5RD
United Kingdom

I. Furtado
Department of Microbiology,
Goa University, Taleigao Plateau,
Goa – 403 203

Geeta Shirnalli
Bioconversion Technology Project,
UAS, Dharwad – 580 005

Gero Benckiser
Institute of Applied Microbiology,
Justus Liebig University,
Heinrich Buff Ring 26-32
35392, Giessen, Germany

G. Gopalaswamy
Centre for Plant Molecular Biology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

Heinz Schneider
Institute of Applied Microbiology,
Justus Liebig University,
Heinrich Buff Ring 26-32
35392 Giessen, Germany

K.S. Jagadeesh
Department of Agricultural Microbiology,
UAS, Dharwad – 580 005

P. Jones Nirmalnath
Department of Agricultural Microbiology,
College of Agriculture,
Bijapur – 586 101

S. Kannaiyan
Tamil Nadu Agricultural University,
Coimbatore – 641 003

D.N. Kambrekar
Department of Agricultural Entomology,
UAS, Dharwad – 580 005

N.P. Khairnar
Molecular Biology Division,
Bhabha Atomic Research Centre,
Mumbai – 400 085

P.U. Krishnaraj
Department of Biotechnology,
UAS, Dharwad – 580 005

K.A. Kulkarni
Department of Agricultural Entomology,
UAS, Dharwad – 580 005

K. Kumar
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

G. Krishnappa
Institute of Animal Health and Veterinary Biologicals,
Hebbal, Bangalore – 560 024

M.S. Kuruvinshetti
Department of Biotechnology,
UAS, Dharwad – 580 005

A. Lakshmanan
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

K. Lenin
Plant Sciences Division,
University of Nottingham,
Loughborough, Leicester LE12 5RD
United Kingdom

T. Marimuthu
Department of Plant Pathology
Tamil Nadu Agricultural University,
Coimbatore – 641 003

Hari S. Mishra
Molecular Biology Division,
Bhabha Atomic Research Centre,
Mumbai – 400 085

S. Nakkeeran
Department of Plant Pathology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

Neeru Bhagat
Department of Microbiology,
University of Delhi South Campus,
New Delhi – 110 021

A.B. Patil
Department of Agricultural Microbiology,
College of Agriculture,
Bijapur – 586 101

Pooja Sachdeva
Department of Microbiology,
University of Delhi South Campus,
New Delhi – 110 021

J.B. Power
Plant Sciences Division,
University of Nottingham,
Loughborough, Leicester LE12 5RD
United Kingdom

D. Radhakrishna
Department of Agricultural Microbiology,
UAS, GKVK Campus,
Bangalore – 560 065

R. Radjacommare
Department of Plant Pathology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

T. Raghuchander
Sugarcane Research Station,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

Ch. Ramesh
Department of Botany,
Karnatak University,
Dharwad – 580 003

G.A. Ravishankar
Plant Cell Biotechnology Department,
Central Food Technological Research Institute,
Mysore – 570 020

Sachin Sharma
Department of Microbiology,
University of Delhi South Campus,
New Delhi – 110 021

M. Sahul Hameed
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

H. Sanath Kumar
Department of Fishery Microbiology,
College of Fisheries,
Mangalore – 575 002

B. Sandesh Kamath
Plant Cell Biotechnology Department,
Central Food Technological Research Institute,
Mysore – 570 020

R. Samiyappan
Department of Plant Pathology
Tamil Nadu Agricultural University,
Coimbatore – 641 003

R. Sarada
Plant Cell Biotechnology Department
Central Food Technological Research Institute,
Mysore – 570 020

T. Satyanarayana
Department of Microbiology,
University of Delhi South Campus,
New Delhi – 110 021

V.P. Savalgi
Department of Agricultural Microbiology,
UAS, Dharwad – 580 005

S. Shishupala
Department of Microbiology,
P.G.Centre, Kuvempu University,
Shivagangotri, Davangere – 577 002

M.N. Sreenivasa
Department of Agricultural Microbiology
College of Agriculture,
Raichur – 584 101

Srikant Kulkarni
Department of Plant Pathology,
UAS, Dharwad – 580 005

V. Sendhilvel
Department of Plant Pathology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

R. Subhashini
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University,
Coimbatore – 641 003

Vasanthakumar Athani
Department of Biotechnology,
UAS, Dharwad – 580 005

Veena Savalgi
Department of Agricultural Microbiology,
UAS, Dharwad – 580 005

B.M. Veeregowda
Institute of Animal Health and
Veterinary Biologicals,
Hebbal, Bangalore – 560 024

J.S. Viridi
Department of Microbiology
University of Delhi South Campus,
New Delhi – 110 021

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Editors: A.R. Alagawadi et al.

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S. Kannaiyan
President, AMI
Ex Vice Chancellor, Tamilnadu Agricultural University
Coimbatore - 641 003, Tamil Nadu

Introduction

Microbiology has become increasingly useful to our society now and it has strongly emerged as one of the most important branches of the life sciences. Microbiologists, have made substantial contributions to basic and fundamental biological sciences as well as in the applied areas of public health, medical, veterinary, agriculture, industry, pharmaceutical and environmental sciences. The most dramatic current development in applied microbiology is due to the development of genetic engineering and recombinant DNA technology. Using these current techniques and methodologies, microorganisms can be engineered through modification of their DNA to produce new substances such as human proteins. Bacteria have been modified to produce human insulin and interferon. Genetically engineered microorganisms hold greater promise and potentials for the production of drugs and vaccines, for improvement of agricultural crops and for other products and processes. There is a growing recognition of the potential of valuable microorganisms in many applied scientific areas. The ability of microorganisms as food supplement, the exploitation of microbial activity to produce methane gas as energy source for rural development and the potential of new therapeutic substances produced by microorganisms and the other uses of microorganisms are also becoming attractive. Bio-remediation processes use microorganisms to clean-up toxic wastes and toxic metabolites. Microorganisms are being used as biological control agents for pests and diseases. In gene therapy, viruses are used to carry replacements for defective or missing genes into human cells. Genetically engineered bacteria are used in agriculture to protect plants from frost and insects and to improve the shelf-life of produce.

Biotechnology

Microbiology is the basic and fundamental foundation for the emergence of Modern Biotechnology. Many bacteria including *E. coli* and other microorganisms like yeast, actinomycetes and cyanobacteria were the model systems in demonstrating methodologies and product development in Biotechnology. The *Agrobacterium* – mediated transformation played a key role in developing transgenic crops. Information Technology has taken a center stage and Biotechnology Revolution or BT Revolution is going to change particularly in Life Sciences. BT would be a major player in Technology Development for human welfare, which would reflect on economic growth of the country.

The history of Biotechnology dates back to the time when microorganisms were used to produce yoghurt, vinegar, beer, wine, cheese etc. The biotechnological expertise, with the growing awareness, spread and employed for the manufacture of products like ethanol, butanol, glycerol etc. The discovery of Penicillin by Alexander Flemming and Streptomycin by Selman Waksman during 1930 and 1940's augmented the prospects of biotechnological approaches and this was later culminated to the implication in the fields of amino acids, enzymes and detergents. The concept of new Biotechnology came into existence in 1940's. The European Federation of Biotechnology (FEB) defined Biotechnology as "The integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological application of the capabilities of microorganisms, cell culture and their components". The role of modern Biotechnology includes, in the area of pharmaceutical industry, the production of antibiotics, enzymes, vaccines, hormones, etc., in the field of biomass and bio-energy, it includes biogas, bio-fuel, alcohol, production of hydrogen and hydrocarbon, etc., in the field of food industry, it includes mass production of yeasts, mushroom and single cell proteins, etc., enhanced competition in the international market.

Biotechnology refers to any technological application that uses biological systems, of living organisms or derivatives thereof to make or modify products or processes for specific use. BT is of course, a new label for a process that humans have used for thousands of years to ferment foods such as beer, wine, bread and cheese through microbiological process. Yeast has been a component of baking and fermenting throughout recorded history.

Vaccination against the small pox virus was introduced in the 18^{th} century; long before the details of cell structure and action were known. Biotechnology also covers a range of different technologies such as gene manipulation and gene transfer, DNA typing and cloning of plants and animals.

Modern era of Biotechnology was started in 1953 when James Watson and Francis Crick discovered the structure of DNA molecule that carries genetic information. Since then the science of genetics and its technological application have advanced rapidly.

- In 1961, the first bio-pesticide was developed to protect important agricultural crops.
- In 1973, came the first alteration of DNA molecule, the biotech process currently referred to as recombinant DNA technology.
- In 1982, the US Food and Drug Administration approved the first drug developed by Biotechnology i.e., human insulin produced in genetically modified bacteria.
- In1989, cotton crop was genetically modified as transgenic BT cotton to protect against bollworms and in 1990 transgenic maize was developed in USA and it is an outstanding scientific contribution in crop science by using the scientific knowledge of Genetic Engineering.
- In 1997, the first animal was cloned from an adult cell, Dolly Sheep.

- Advances in Biotechnology are accelerating and the scope of Biotechnology's applications is widening very much at international scene for the human World. It is a modern scientific tool for boosting the economy of any Nation and it could be exploited in India, and Biotechnology Revolution is going to change the modern world.

The application of Biotechnology offers enormous potential for agricultural, pharmaceutical and environmental purposes. Benefits for human development are just beginning through Biotechnology. Breakthrough by Biotechnological applications in Medicine, Agriculture, Veterinary, Food processing, Forestry, Fisheries have huge potentials for accelerating human development. But this potential could be truly tapped only if Biotechnology is used to address the key challenge in health and agriculture sectors of our Nation and also for all the developing countries across the world.

Biological fertilizers

Scientific discoveries over the past 30 years have revolutionized our knowledge on the basis of legume nodulation and biological nitrogen fixation (BNF). Some 3000 scientific papers have been published on *Rhizobium* since 1994. However, very little of this knowledge is being used in farmers fields in developing countries to enhance production, reduce costs and increase farm profitability. Although FAO and other International organizations have established inoculants production facilities in some developing countries, there are still many parts of the world where quality inoculants are not available to farmers. In developing countries, the most important challenge is to produce sufficient food for the growing population from inelastic land area. Products of biological origin could be advantageously blended to replace a part of the energy – intensive inputs. It is in this context, biofertilizers can provide to the small and marginal farmers, and biofertilizers are economically viable biological system for realizing the ultimate goal of increasing productivity. The microbial system siphon out appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil with this important and key nutrient element Nitrogen, that is vital for crop growth and productivity. The crop – microbial – soil ecosystem can, therefore, be energized in sustainable agriculture with considerable ecological stability and environmental quality. Although the potential of biofertilizers in crop production systems has been well documented and substantiated, the major reason for the shifting fortunes of these biological inputs lies in the lack of an organized industrial back up, an effective quality control mechanism, powerful extension machinery and a broad and effective research system.

Biofertilizers have for long been witnessed shifting fortunes in agriculture. While the technology of using them has been successfully exploited in may developed countries, competent exploitation in developing countries has been hampered by several factors, including lack of trained manpower, lack of appreciation of the benefits of inoculation and absence of suitable industrial support. A major problem, especially relevant to our biofertilizers programme has to do with relation between research institutions and industries. It must be promoted as effectively as possible through cooperation between institutional researches, which is usually public. At the same time, we should not overlook the significance of ensuring and maintaining a high quality standard of the product. The limited shelf-life, particularly of bacterial biofertilizers dictates that product streams must be handled with a quick delivery system at low temperatures. A strong extension and training programme actively supported by research and industry is the need of the hour. We should recognize that in adopting a rational approach to the use and management of natural resources in sustainable agriculture, the microbial fertilizers hold vast potential for the future and not only that, the biological system is a renewable one, environmentally safe and economically viable and suitable in sustainable agriculture.

Biofertilizers are apparently environment and farmers friendly, renewable source of non-bulky, low cost organic input. While blue green algae (BGA) and *Azolla* are crop specific, bioinoculants like *Azotobacter, Azospirillum*, phosphorus solubilizing bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) etc., could be regarded as broad spectrum biofertilizers. Their use has so far not received desired attention. The crop specific strains of rhizobia and efficient strains of blue green algae are now available to our farmers as easily usable Bio-inoculants. It has been shown that through the use of appropriate strains of rhizobia, the yield of crops like chickpea and soybean could be increased by 15-30%. The use of blue green algae like *Nostoc* and *Tolypothrix* and *Anabaena* in association with *Azolla* have been found to improve yields of wetland rice by 15-20%. Our knowledge of other free-living organisms like *Azospirillum* and *Azotobacter* is still limited. Both these N_2 fixing bacteria have the potential to fix atmospheric nitrogen up to the level of 15-20 kg per hectare, per season which can be utilized by all the crops and thus will have a much wider applicability for increasing the yield in a sustainable agricultural system and improving the long term soil fertility.

Endophytic nitrogen fixation

Preliminary studies on the feasibility of extending N_2 fixation by rhizobia to non-legumes revealed the formation of nodule-like structures in the roots of rice, maize and sorghum when inoculated with *Azorhizobium caulinodans* together with 2, 4-D at 0.5 and 3.0 ppm. Further research revealed the stimulation of lateral root formation in rice, maize, pearlmillet (*Pennisetum typhoides*), sugarcane and grasses due to the inoculation of *A. caulinodans* with growth hormones. Recently, it has been found that inoculation of *A. caulinodans* alone could induce nodule-like structures in rice, maize and baby corn.

Studies of plant-microbe interactions have benefited from the use of genetically modified microorganims harbouring reporter genes under the control of constitutively expressed promoters. Stimulation of lateral roots formation by naringenin in rice has been reported. By using the *A. caulinodans* strains carrying a marker gene, it is now possible to detect both visually and enzymatically the internal colonization of inoculated diazotrophs. Recently, it has been clearly established that the diazotroph, *A. caulinodans*-ORS 571 colonized the xylem, which ultimately might be capable of fixing nitrogen in a systemic manner in cereal crops.

During the few years, explanation for large-scale contribution of biological nitrogen fixation in crops like sugarcane and non-legumes has become much easier by description of newer obligate endophytic bacteria, *Gluconacetobacter diazotrophicus*, which get colonized within the roots, stems and leaves of certain plant genotypes. In addition to *Gluconacetabacter diazotrophicus*, *Herbaspirillum seropedicae*, *Herbaspirillum rubrisubalbicans*