Fungal Biology

Ajar Nath Yadav Sangram Singh Shashank Mishra Arti Gupta *Editors*

Recent Advancement in White Biotechnology Through Fungi

Volume 2: Perspective for Value-Added Products and Environments



Fungal Biology

Series Editors

Vijai Kumar Gupta Department of Chemistry and Biotechnolgy Tallinn University of Technology Akadeemia tee, Tallinn, Estonia

Maria G. Tuohy School of Natural Sciences National University of Ireland Galway Galway, Ireland

About the Series

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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Ajar Nath Yadav • Sangram Singh Shashank Mishra • Arti Gupta Editors

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Volume 2: Perspective for Value-Added Products and Environments



Editors Ajar Nath Yadav Department of Biotechnology Akal College of Agriculture Eternal University, Baru Sahib, Sirmour Himachal Pradesh, India

Shashank Mishra Biotechnology Biotech Park Lucknow, Uttar Pradesh, India Sangram Singh Dr. Ram Manohar Lohia Avadh University Faizabad, Uttar Pradesh, India

Arti Gupta Department of Zoology Dr. Ram Manohar Lohia Avadh University Gonda, Uttar Pradesh, India

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Foreword

White biotechnology is industrial biotechnology dealing with various biotech products through applications of microbes. The main application of white biotechnology is commercial production of various useful organic substances, such as acetic acid, citric acid, acetone, glycerine, etc., and antibiotics, like penicillin, streptomycin, mitomycin, etc., and value-added product through the use of microorganisms especially fungi and bacteria. The value-added products included bioactive compounds, secondary metabolites, pigments, and industrially important enzymes for potential applications in agriculture, pharmaceuticals, medicine, and allied sectors for human welfare. In the twenty-first century, humans acquired skills to harness fungi to protect human health (through antibiotics, antimicrobial, immunosuppressive agents, value-added products etc.), which led to industrial-scale production of enzymes, alkaloids, detergents, acids, and biosurfactants. The first large-scale industrial applications of modern biotechnology have been made in the areas of food and animal feed production (agricultural/green biotechnology) and pharmaceuticals (medical/ red biotechnology). In contrast, the production of bioactive compounds through fermentation or enzymatic conversion is known as industrial or white biotechnology. The beneficial fungal strains may play important role in agriculture, industry, and medical sectors. The beneficial fungi play a significant role in plant growth promotion and soil fertility using both direct (solubilization of phosphorus, potassium, and zinc; production of indole acetic acid, gibberellic acid, cytokinin, and siderophores) and indirect (production of hydrolytic enzymes, siderophores, ammonia, hydrogen cyanides, and antibiotics) mechanisms of plant growth promotion for sustainable agriculture. The fungal strains and their products (enzymes, bioactive compounds, and secondary metabolites) are very useful for industry. The discovery of antibiotics is a milestone in the development of white biotechnology. Since then, white biotechnology has steadily developed and now plays a key role in several industrial sectors, providing both high-valued nutraceuticals and pharmaceutical products. The fungal strains and bioactive compounds also play important role in the environmental cleaning.

The present book volume on "Recent Advancement in White Biotechnology Through Fungi" Volume 2: Perspective for Value-Added Products and Environments is a very timely publication, which provides state-of-the-art information in the area of white biotechnology, broadly involving fungal-based value-added products and applications of fungal communities for sustainable environments. The book volume comprises 16 chapters. Chapter 1 by Kour et al. describes agriculturally and industrially important fungi and their potential biotechnological applications. Chapter 2 presented by Dailin et al. highlights fungal phytases and their biotechnological applications in food and feed industries. Chapter 3 by Banik et al. describes about probiotic fungal strains, their mechanism of actions, health beneficial effects, and also their efficacy in the treatment of various diarrheal, skin, and vaginal complications. Chapter 4 by Challa et al. highlights the opportunities and challenges of fungal white biotechnology to meet food security. Roy and Banerjee describe the production of hydrocarbon and hydrocarbon-like compounds along with other quality volatile organics with high potential to be used as both "green chemicals" and fuels from endophytic fungi in Chap. 5. Chapter 6, by Parasuraman and Siddhardha, gives details of functional genomics and proteomics for the isolation and production of novel natural value-added metabolites from fungal community. Chapter 7 authored by Gholami-Shabani et al. highlights current knowledge about fungal natural products including primary and secondary metabolites, their biosynthetic pathways, and brief examples of each class of compounds including their bioactivities. In Chap. 8, Singh and colleagues describe in detail about the variety of secondary metabolite produced, its synthesis strategies via chemical and heterologous mode, as well as their biological applications. Enespa et al. highlight the fungal production of novel secondary metabolites with antimicrobial activity against plant and human pathogenic fungal and bacterial strains in Chap. 9. Kumar et al. explain about pigments produced by soil fungi and their potential applications in medical, textile coloring, food coloring, and cosmetics in Chap. 10. The endophytes that may contribute to their host plant and for the pharmaceutically important bioactive substances, as the search for better chemotherapeutic agents remains an important challenge, have been described by Carvalho et al. in Chap. 11. Chapter 12 by Sharma and Salwan describes the extracellular enzymes from Trichoderma and their role in the production of biofuel from nonedible biomass. Raven et al. highlight the third-generation biofuels generated with the assistance of fungi in Chap. 13. Diwan and Gupta discuss the advent of single cell oil and realization of its multiple prospects and possibilities in Chap. 14. Recent advancement and the way forward for Cordyceps have been discussed in Chap. 15 by Chaubey et al. Finally, Rashmi et al. describe about the status of synthetic biology for production of valueadded products and bioactive compounds from fungi for advancements of fungal white biotechnology in Chap. 16.

Overall, great efforts have been carried out by Dr. Ajar Nath Yadav, his editorial team, and scientists from different countries to compile this book as a highly unique, up-to-date source on fungal white biotechnology for the students, researchers, scientists, and academicians. I hope that the readers will find this book highly useful and interesting during their pursuit on fungal biotechnology.

H.s. Shaliwal Dr. H.S.Dhaliwal

Vice Chancellor Eternal University Baru Sahib, Himachal Pradesh, India



Dr. H. S. Dhaliwal is presently the Vice Chancellor of Eternal University, Baru Sahib, Himachal Pradesh, India, Dr. Dhaliwal holds Ph.D. in Genetics from the University of California, Riverside, USA (1975). He has 40 years of research, teaching, and administrative experience in various capacities. Dr. Dhaliwal is a Professor of Biotechnology at Eternal University, Baru Sahib, from 2011 until to date. He had worked as Professor of Biotechnology at Indian Institute of Technology (IIT), Roorkee (2003–2011); Founder Director of Biotechnology Centre, Punjab Agricultural University (PAU), Ludhiana (1992-2003); Senior Scientist and Wheat Breeder-cum-Director at PAU's Regional Research Station, Gurdaspur (1979–1990); Research Fellow at the Friedrich Miescher Institute (FMI), Basel, Switzerland (1976-1979); and D. F. Jones Postdoctoral Fellow at the University of California, Riverside, USA (1975–1976). Dr. Dhaliwal was elected as Fellow at National Academy of Agricultural Sciences, India (1992), and has worked as Visiting Professor in the Department of Plant Pathology, Kansas State University, Kansas, USA, (1989) and Senior Research Fellow at the International Maize and Wheat Improvement Center (CIMMYT), Mexico (1987). He has many national and international awards on his name such as Pesticide India Award from Mycology and Plant Pathology Society of India (1988) and cash award from the Federation of Indian Chambers of Commerce and Industry (FICCI) in 1985. He has to his credit more than 400 publications including 250 research papers, 12 reviews, 15 chapters contributed to books, 105 papers presented in meetings and conferences and abstracted, 18 popular articles, and 2 books/ bulletins/manuals. His important research contributions are identification of new species of wild diploid wheat Triticum urartu and gathered evidences to implicate T. *urartu* as one of the parents of polyploid wheat; Team Leader in the development of seven wheat varieties, namely, PBW 54, PBW 120, PBW 138, PBW 175, PBW 222, PBW 226, and PBW 299, approved for cultivation in Punjab and North Western Plain Zone of India; molecular marker-assisted pyramiding of bacterial blight resistance genes Xa21 and Xa13; and the green revolution semi-dwarfing gene *sd*1 in Dehraduni basmati and developed elite wheat lines biofortified for grain rich in iron and zinc through wide hybridization with related non-progenitor wild wheat species and molecular breeding. Dr. Dhaliwal made a significant contribution to the development of life and epidemiology life cycle of *Tilletia indica* fungus, the causal organism of Karnal bunt disease of wheat, and development of Karnal bunt-resistant wheat cultivar. Dr. Dhaliwal had the membership of several task forces and committees of the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, and is Chairman of Project Monitoring Committee for Wheat Quality Breeding, Department of Biotechnology, Ministry of Science & Technology, Government of India (2007–2010); Chairman of the Project Monitoring Committee in "Agri-biotechnology" of the Department of Biotechnology, Government of India, New Delhi (2014-2016); and presently, Member of newly constituted Expert Committee for DBT-UDSC Partnership Centre on Genetic Manipulation of Crop Plants at UDSC, New Delhi (2016 onward).

Preface

White biotechnology is drawing much attention as a solution to produce valueadded product for human welfare. Fungi are used to synthesize functional bioactive compounds, hydrolytic enzymes, and compounds for plant growth promotion, biocontrol, and other processes for agriculture, medicine, industry, pharmaceuticals, and allied sectors. White fungal biotechnology is an emerging field in science arena that supports revealing of novel and vital biotechnological components. The fungi *Aspergillus, Bipolaris, Cordyceps, Fusarium, Gaeumannomyces, Myceliophthora, Penicillium, Phoma, Piriformospora, Pleurotus, Trichoderma*, and *Xylaria* are highly important fungal groups which can be utilized for production of different antibiotics, enzymes, pigments, and peptides useful in medical and industrial fields.

The present book on "Recent Advancement in White Biotechnology Through Fungi" Volume 2: Perspective for Value-Added Products and Environments covers agriculturally and industrially important fungi producing value-added products for agriculture, industry, and environments. The fungal community from different habitats such as from extreme habitats as well as plant associated having capability to produced extracellular enzymes, secondary metabolites and bio-active compounds are useful for diverse processes targeted at therapeutics, diagnostics, bioremediation, agriculture, industries and environments. This book volume will be immensely useful to biological sciences, especially to microbiologists, microbial biotechnologists, biochemists, researchers, and scientists of fungal biotechnology. We are honored that the leading scientists who have extensive, in-depth experience and expertise in fungal system and microbial biotechnology took the time and effort to develop these outstanding chapters. Each chapter is written by internationally recognized researchers/scientists, so the reader is given an up-to-date and detailed account of our knowledge of the white biotechnology and innumerable agricultural industrial and environmental applications of fungi.

We are grateful to the many people who helped to bring this book to light. Editors wish to thank Dr. Eric Stannard, senior editor, Botany, Springer; Dr. Vijai Kumar Gupta and Maria G. Tuohy, series editors, Fungal Biology, Springer; and Mr. Rahul Sharma, project coordinator, Springer, for generous assistance, constant support, and patience in initializing the volume. Dr. Ajar Nath Yadav gives special thanks to

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Baru Sahib, Himachal Pradesh, India Lucknow, Uttar Pradesh, India Faizabad, Uttar Pradesh, India Gonda, Uttar Pradesh, India Ajar Nath Yadav Shashank Mishra Sangram Singh Arti Gupta

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Contributors

Soraya Sander Amorim Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Nur Izyan Wan Azelee Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

Mohammed Muzeruddin Baig Central Tasar Research and Training Institute, Nagri, Ranchi, Jharkhand, India

Debdulal Banerjee Microbiology and Microbial Biotechnology Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal, India

Abhijit Banik Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India

Surekha Challa Department of Biochemistry and Bioinformatics, GITAM Institute of Science, Gandhi Institute of Technology and Management (GITAM Deemed-to-be-University), Visakhapatnam, AP, India

Prem Chandra Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, Uttar Pradesh, India

Rahul Chaubey Kala Azar Medical Research Center, Rambag Road, Muzaffarpur, Bihar, India

Daniel Joe Dailin Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor, Malaysia

Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia **Raissa Hellen da Silva Florindo** Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Núcleo de Pesquisas em Ciências Biológicas, Federal University of Ouro Preto, Ouro Preto, MG, Brazil

Jéssica Catarine Silva de Assis Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Camila Rodrigues de Carvalho Química de Produtos Naturais Bioativos, Rene Rachou Institute, Fiocruz, Belo Horizonte, MG, Brazil

Batul Diwan Department of Biotechnology, National Institute of Technology, Raipur, India

Titash Dutta Department of Biochemistry and Bioinformatics, GITAM Institute of Science, Gandhi Institute of Technology and Management (GITAM Deemed-tobe-University), Visakhapatnam, AP, India

Hesham El Enshasy Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor, Malaysia

Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

City of Scientific Research and Technological Applications (SRTA-City), New Borg El Arab, Alexandria, Egypt

Elsayed Ahmed Elsayed Department of Zoology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia

Department of Chemistry Natural and Microbial Products, Pharmaceutical Industries Division, National Research Centre, Gizza, Egypt

Enespa Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, Uttar Pradesh, India

Jennifer Eyahmalay Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor, Malaysia

Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

SBG Agrifeed (M) Sdn Bhd, Pasir Gudang, Johor, Malaysia

Mariana Costa Ferreira Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Aditya Francis Vaugh Institute of Agricultural Engineering and Technology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India

Mohammadhassan Gholami-Shabani Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Ministry of Industry, Mine and Trade of Iran, Tehran, Iran

Chandradipa Ghosh Department of Human Physiology with Community Health, Vidyasagar University, Midnapore, West Bengal, India

Pratima Gupta Department of Biotechnology, National Institute of Technology, Raipur, India

Suman Kumar Halder Department of Microbiology, Vidyasagar University, Midnapore, West Bengal, India

Siti Zulaiha Hanapi Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Skudai, Johor, Malaysia

Sezotalu Kezo Department of Horticulture, School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Lumami, Nagaland, India

Divjot Kour Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Amit Kumar Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India

Ashok Kumar Department of Genetics and Plant Breeding (Plant Biotechnology), Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India

Upendra Kumar Department of Molecular Biology, Biotechnology & Bioinformatics, College of Basic Sciences & Humanities, CCS Haryana Agricultural University, Hisar, Haryana, India

Poonam Maan Department of Agriculture Biotechnology, College of Agriculture, SVBP University of Agriculture & Technology, Meerut, U.P, India

Keshab Chandra Mondal Department of Microbiology, Vidyasagar University, Midnapore, West Bengal, India

Smriti Nandan Department of Genetics and Plant Breeding (Plant Biotechnology), Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India

Nageswara Rao Reddy Neelapu Department of Biochemistry and Bioinformatics, GITAM Institute of Science, Gandhi Institute of Technology and Management (GITAM Deemed-to-be-University), Visakhapatnam, AP, India

Trisha Guha Neogi Department of Genetics and Plant Breeding (Plant Biotechnology), Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India

Nikhil Department of Genetics and Plant Breeding (Plant Biotechnology), Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India

Abhay K. Pandey Department of Biochemistry, University of Allahabad, Allahabad, India

Paramanantham Parasuraman Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India

Srishti Prajapati Department of Genetics and Plant Breeding (Plant Biotechnology), Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, India

Priyanka Department of Botany, Government Girls Degree College, Meerut, U.P, India

Harvesh Kumar Rana Department of Biochemistry, University of Allahabad, Allahabad, India

Kusam Lata Rana Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Rashmi Department of Genetics & Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Ali A. Rastegari Department of Molecular and Cell Biochemistry, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

Spriha Raven Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

Mehdi Razzaghi-Abyaneh Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Luiz Henrique Rosa Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Sudipta Roy Microbiology and Microbial Biotechnology Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal, India

Richa Salwan Department of Social Science and Basic Sciences, College of Horticulture and Forestry, Hamirpur, India

Anil Kumar Saxena ICAR-National Bureau of Agriculturally Important Microorganisms, Kusmaur, Mau, India

Masoomeh Shams-Ghahfarokhi Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Vivek Sharma University Centre for Research and Development, Chandigarh University, Gharuan, India

Busi Siddhardha Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry, India

Amit Kumar Singh Department of Biochemistry, University of Allahabad, Allahabad, India

Jitendra Singh Central Tasar Research and Training Institute, Nagri, Ranchi, Jharkhand, India

Joginder Singh Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

Vickpasubathysiwa Siwapiragam SBG Agrifeed (M) Sdn Bhd, Pasir Gudang, Johor, Malaysia

Chitra Srivastava Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

Dalia Sukmawati Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta, Indonesia

Archana Tiwari Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

Ajar Nath Yadav Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Neelam Yadav Gopi Nath P.G. College, Veer Bahadur Singh Purvanchal University, Deoli-Salamatpur, Ghazipur, Uttar Pradesh, India

Carlos Leomar Zani Química de Produtos Naturais Bioativos, Rene Rachou Institute, Fiocruz, Belo Horizonte, MG, Brazil

About the Editors



Ajar Nath Yadav is an Assistant Professor in Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India. He has 3 years of teaching and 9 years of research experiences in the field of industrial biotechnology, microbial biotechnology, microbial diversity, and plant-microbe interactions. Dr. Yadav obtained doctorate degree in Microbial Biotechnology, jointly from Indian Agricultural Research Institute, New Delhi, and Birla Institute of Technology, Mesra, Ranchi, India, M.Sc. (Biotechnology) from Bundelkhand University, and B.Sc. (CBZ) from the University of Allahabad, India. Dr. Yadav has 101 publications, which include 37 research papers, 15 review articles, 3 books, 1 book manual, 31 book chapters, 8 popular articles, 7 editorials, 2 technical reports, and 1 patent with h-index 23, i10-index 49, and 1551 citations (Google Scholar). Dr. Yadav has published 105 research communications in different international and national conferences. Dr. Yadav has got ten Best Paper Presentation Awards, one Young Scientist Award (NASI-Swarna Jyanti Purskar) and three certificate of excellence in reviewing awards. Dr. Yadav received "Outstanding Teacher Award" in 6th Annual Convocation 2018 by Eternal University, Baru Sahib, Himachal Pradesh. Dr. Yadav has a long standing interest in teaching at the UG, PG and PhD level and is involved in taking courses in agriculture microbiology, bacteriology, bioprocess engineering and technology, environmental microbiology, industrial microbiology, and microbial biotechnology. Dr. Yadav is currently

handling two projects one funded by Department of Environments, Science & Technology (DEST), Shimla entitled "Development of Microbial Consortium as Bio-inoculants for Drought and Low Temperature Growing Crops for Organic Farming in Himachal Pradesh" as Principal Investigator and another funded by HP Council for Science, Technology & Environment (HIMCOSTE) on "Value-added products" as Co-PI. He also worked as an Organizing Committee Member for seven international conferences/symposia in the related field. Presently, he is guiding two scholars for Ph.D. and one for M.Sc. dissertation. In his credit ~6700 microbes (Archaea, bacteria, and fungi) isolated from diverse sources and ~550 potential and efficient microbes deposited at culture collection of National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, India. He has deposited 2386 nucleotide sequences and 3 whole-genome sequences (Bacillus thuringiensis AKS47, Arthrobacter agilis L77 and Halolamina pelagica CDK2) and 2 transcriptome to NCBI GenBank databases: in public domain. Dr. Yadav and his group have developed method for screening of archaea for phosphorus solubilization for the first time. He has been serving as an Editor/Editorial Board Member and Reviewer for more than 35 national and international peer-reviewed journals. He has lifetime membership of the Association of Microbiologists of India; Indian Science Congress Council, India; and National Academy of Sciences, India. Please visit https://sites.google.com/site/ajarbiotech/ for more details.



Sangram Singh is an Associate Professor in the Department of Biochemistry, Dr. Ram Manohar Lohia Avadh University, Faizabad, India, and has 11 years of teaching and 14 years of research experiences in the field of applied biochemistry. Dr. Singh obtained his Ph.D. and M.Sc. in Biochemistry from Dr. Ram Manohar Lohia Avadh University, Faizabad, India. Dr. Singh has published 34 national and international research papers and 2 book chapters. He has presented nine papers in different national and international symposia/seminars/conferences/workshops.

About the Editors



Shashank Mishra is presently working as Scientist "C," Biotech Park, Lucknow, Uttar Pradesh, India. He obtained his doctorate degree in Science "Industrial Biotechnology," from Birla Institute of Technology, Mesra, Ranchi, India; M.Sc. (Biotechnology) from Barkatullah University, Bhopal; and B.Sc. (CBZ) from Dr. Ram Manohar Lohia Avadh University, Faizabad, India. He has made pioneering contributions in the area of microbial biotechnology, natural product synthesis, and environmental microbiology for food, pharmaceutical, and human health. To his credit, he has 19 publications [6 research papers, 2 review articles, 1 book, and 10 book chapters] in different reputed international and national journals and publishers. He has published 16 abstracts in different conferences and got 1 Best Poster Presentation Award. He has a long-standing interest in teaching at the UG, PG, and Ph.D. level and is involved in taking courses in industrial biotechnology, bioprocess engineering and technology, environmental biotechnology, environmental microbiology, industrial microbiology, microbial biotechnology, and techniques in microbiology and biotechnology. He is Reviewer in international journals including BMC Microbiology, Indian Phytopathology, PLOS One, Scientific Reports, and Archive of Phytopathology and Plant Protection. He has the lifetime membership of the Association of microbiologists of India.



Arti Gupta is an Assistant Professor in the Department of Biology, Sri Avadh Raj Singh Smarak Degree College, Bishunpur Bairiya, Gonda, India. Dr. Arti received her B.Sc. (CBZ) and M.Sc. in Biotechnology from Chaudhary Charan Singh University, Meerut, India. Dr. Gupta obtained her Ph.D. from Mahatma Jyotiba Phule Rohilkhand University, Bareilly, India. Dr. Gupta's current research interests are animal biotechnology, molecular plant biotechnology, molecular animal biotechnology, bioprocess technology, and microbiology. Dr. Gupta has published 1 monograph, 1 book, and 21 national and international research papers and has attended 36 national and international conferences. Dr. Gupta has been awarded with Gold Medal, M.Sc. (Biotech), Young Scientist Award, two Poster Presenter Awards, Fellowship Award awarded by International Consortium of Contemporary Biologist, and Dr. V. P. Agarwal Gold Medal. Dr. Arti has lifetime membership of Indian Science Congress Association, Biotech Research Society of India, Zoological Society of India, and International Consortium of Contemporary Biologist.

Chapter 1 Agriculturally and Industrially Important Fungi: Current Developments and Potential Biotechnological Applications



Divjot Kour, Kusam Lata Rana, Neelam Yadav, Ajar Nath Yadav, Joginder Singh, Ali A. Rastegari, and Anil Kumar Saxena

1.1 Introduction

Fungi are chemoheterotrophic organisms and are known to be present in subaerial and subsoil environments. They are known to play a major role as decomposers, simultaneously being important animal and plant mutualistic symbionts as well as pathogens, further being the spoilage organisms of natural as well as manufactured materials (Burford et al. 2003; Gadd 1999, 2006, 2007). They also play a chief role in maintaining soil structure, due to their filamentous branching growth and frequent production of the exopolymer. Most of the fungi possess a filamentous growth habit and some are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells (Gadd 2007; Gorbushina et al. 2002, 2003). The filamentous mode of growth provides them the capability to adapt to both exploitative or explorative growth strategies, and the formation of linear organs of

D. Kour · K. L. Rana · A. N. Yadav (🖂)

N. Yadav

Gopi Nath P.G. College, Veer Bahadur Singh Purvanchal University, Deoli-Salamatpur, Ghazipur, Uttar Pradesh, India

J. Singh

Department of Microbiology, Lovely Professional University, Phagwara, Punjab, India

A. A. Rastegari

A. K. Saxena ICAR-National Bureau of Agriculturally Important Microorganisms, Kusmaur, Mau, India

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Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India e-mail: ajar@eternaluniversity.edu.in

Department of Molecular and Cell Biochemistry, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

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aggregated hyphae for protected fungal translocation (Fomina et al. 2005; Gadd 2007). Interestingly, the earliest fossil of filamentous fungal remains appear to be from the mid- to late Precambrian (Gadd 2007) and have been revealed to be extremely diverse by Devonian, times, when forms belonging to major groups and even some genera present today are found (Gadd 2007; Heckman et al. 2001). Since that time fungi have been known to be the components of the microbial communities of any terrestrial environment (Hawksworth 2001) which may either be hostile habitats such as the Arctic and Antarctic and hot deserts or may be metal-rich and hypersaline soils (Burford et al. 2003). The majority of fungi have been demonstrated to inhabit soil environments, which are known to be apparently much more hospitable as compared to the bare rock surfaces. Fungal communities in soil are diverse, where they may occur as plant and animal pathogens, free-living or in symbiotic associations, as well as unicellular yeasts (Gadd 2007). They may be present inside the plants which are known as the endophytic fungi where they may reside without causing any harmful effect to the host plant. Plants are in fact considered to be a major reservoir of abundance of endophytes (Rana et al. 2016a, b, 2017). It has been estimated that more than one million fungal species inhabit different genera of the plant which reflects on hyperdiversity of endophytic fungi (Bilal et al. 2018; Strobel and Daisy 2003). Endophytic fungi are known to be one of the best sources of natural bioactive compounds which have potential applications in diverse fields such as food industry, medicine, and agriculture (Bilal et al. 2018; Strobel et al. 2004; Verma et al. 2009; Yadav et al. 2015b).

Numerous endophytes have been examined for their capability to produce metabolites which promote the growth of the plants (Verma et al. 2013, 2014). Further, there is rhizospheric fungi which also play an important role in plant growth promotion by different mechanisms such as production of diverse plant growth regulators; making availability of various nutrients to the plants such as phosphorus, potassium, zinc etc.; and production of the siderophores and diverse hydrolytic enzymes; furthermore, these also help the plants to overcome abiotic stress conditions such as salinity, drought, and high or low temperature, and all these characteristics make them good source to be used as biofertilizers (Saxena et al. 2015a; Suman et al. 2016; Verma et al. 2016b; Yadav and Yadav 2018b). Furthermore, they have also proved to be good biocontrol agents. Thus, these could be potent and novel alternatives to synthetic pesticides and chemical fertilizers, and such beneficial microbes are perfect candidates for sustainable agricultural production (Kumar et al. 2018; Palaniyandi et al. 2013).

Thus, from past few decades, there has been a strong upsurge of fungal community whether it may be in the agricultural sector or in the spheres of food, feed, and therapeutics. Adding more, their biocatalytic potential has been utilized for centuries for production of bread, wine, vinegar, and many more products (Fig. 1.1). Even the first report of commercial application of yeast for production of alcoholic beverages from barley was by the Babylonians and Sumerians as early as 6000 BC (Biswas et al. 2018; Singh et al. 2016a; Yadav et al. 2018c). Adding more, microbes are favored sources for industrial enzymes among which fungi are in fact attractive producers of diverse enzymes as they are easily available and due to their fast

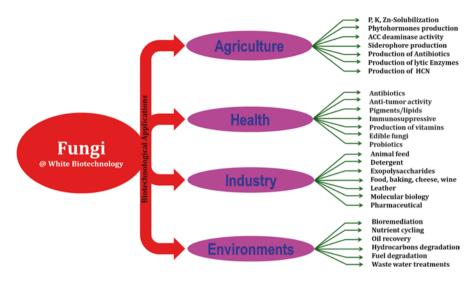


Fig. 1.1 Biotechnological applications of fungi and their value-added products in agriculture, health, industry, and environments

growth rate. Furthermore, genetic changes in microbial cells for elevated production of enzymes using recombinant DNA technology are also easy (Illanes et al. 2012; Singh et al. 2016a). Therefore, keeping in view the importance of the fungal communities in the agriculture, industry and allied sectors, the present chapter deals with the impact of fungi in agriculture and fungal enzymes utilized in diverse industrial and allied sectors.

1.2 Beneficial Impact of Fungal Communities in Agriculture

Plant growth-promoting fungi are gaining significant interest to be used as bioinoculants as they possess manifold benefits on the quantity as well as quality of the plants and because of the positive relation they exhibit with the ecological environment. Though most of the work on plant growth-promoting microbes (Verma et al. 2015a, b, c, 2016a) is focused on bacteria as well as mycorrhizal fungi (Johansson et al. 2004; Kumar et al. 2018), fungi still possess certain characteristic features which are far superior to bacteria, for example, fungi are able to tolerate acidic conditions better and they are in fact far better in mobilizing bound phosphates over bacteria (Kumar et al. 2018; Wahid and Mehana 2000). Furthermore, fungi have been demonstrated to produce phytohormones including indole-3-acetic acid (IAA), gibberellins (Kumar et al. 2018), and siderophores (Kumar et al. 2018; Milagres et al. 1999) (Fig. 1.2). Thus, this section deals with the role of fungi in plant growth promotion.